GASTRULATION IN THE MARINE HYDROID *DYNAMENA PUMILA*: AN EXAMPLE OF EVOLUTIONARY ANTICIPATION BASED ON DEVELOPMENTAL SELF-ORGANIZATION

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ABSTRACT: The morphology of gastrulation in the hydroid *Dynamena pumila* is subject to variation from non-genetic causes; nevertheless, the individual variations prove to be identical to morphologies that in more derived Coelenterata become invariant taxonomic features. The variability of gastrulatlon in *Dynamena* is shown to come from developmental aelf-organization, which means that the local cooperation of cells in the process of their epithelization is independent of their initial position in the embryo. Thus, small random fluctuations in the arrangement of cells emerge on the macroscopic scale, giving rise to different types of gastrulation. All that is needed from selection here is to provide these types with the appropriate (genetically determined) initial conditions, i.e., to change the earlier developmental stages, prior to the beginning of gastrulation. Thus, although in the ontogeny of multicellular organisms the type of gastrulation is determined by the pattern of blastulation, the converse should be true when we consider the evolution of multicellularity.

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In order to claim that evolutionary new spatial patterns arise from developmental self-organization, one needs to show that, at the moment of their origination, these patterns have no definite biological function (Cherdantsev and Scobeyeva, 1994). When one is dealing with a definitive (adult) morphology one can hardly present conclusive evidence that it has no function of its own. However, developing systems provide an opportunity to show that the origination of a new pattern can precede the acquisition of functionality. To show this, the following conditions should be satisfied. First, the new pattern should arise as a degenerate developmental variation which vanishes at later developmental stages with no effect on further development. Second, this variation should anticipate something which is fixed in further evolution, i.e., in more derived taxa.

Gastrulation of *Dynamena*

The early development of a marine hydroid, *Dynamena pumila* L. (Thecaphora, Thecata), provides such an example (for details see Krauss and Cherdantsev, 1995). The early cleavage varies from pseudospiral to anarchic: in both of these cases a loose aggregate of cells arises with no morphological distinction between the outer and inner cells. This

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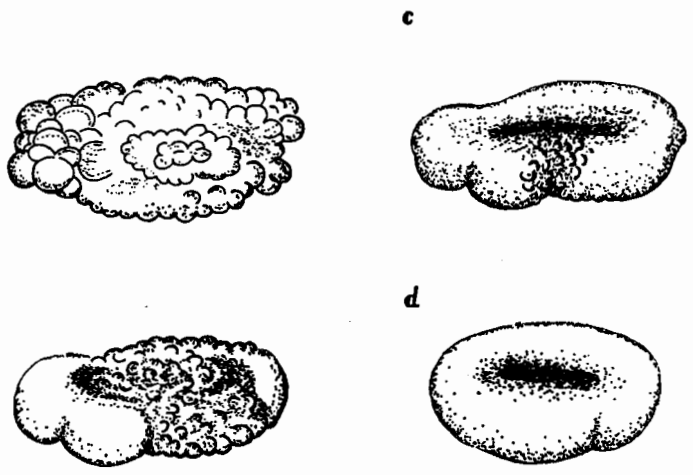

Figure 1. Gastrulation of *Dynamena*. See text.

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aggregate cannot maintain a spherical form, and the embryo acquires the shape of a flat disc (Figure 1a). Gastrulation begins with a smoothing and swelling of the circumference of this disc, while in its central (flat) region deep wrinkles appear (Figure 1b). The circumference continues to swell, this leading to a decrease in the surface area of the central region (Figure 1c), and the wrinkles fuse into a crater which perforates the embryo (Figure 1d). Thus, the embryo acquires a toroidal shape.

Histological analysis shows that epithelization of the outer cells lies at the heart of gastrulation. The epithelization process begins just at the circumference of the embryonic disc (Figure 2a, in the zone marked by the asterisk) and, at the next stage, spreads into the central region (Figure 2b). This process includes an increase in apicobasal cell length, and an increase in the ratio of the apical (outer) to the basal (inner) cell surfaces. This is shown in Figures 2c and 2d. In these figures the mean values of measurements (in arbitrary units) which

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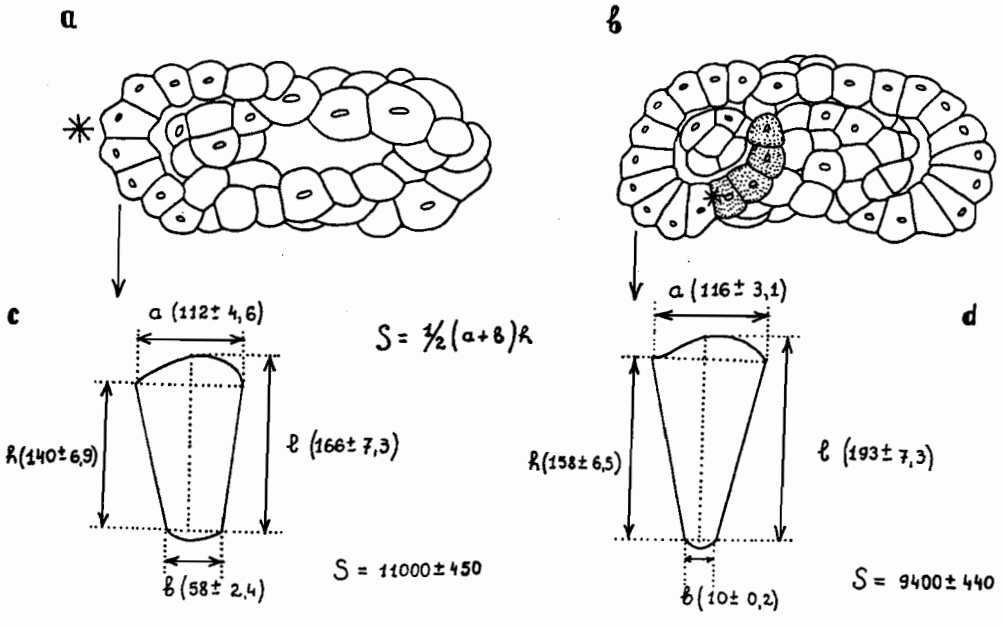

Figure 2. Epithelization of *Dynamena*. See text.

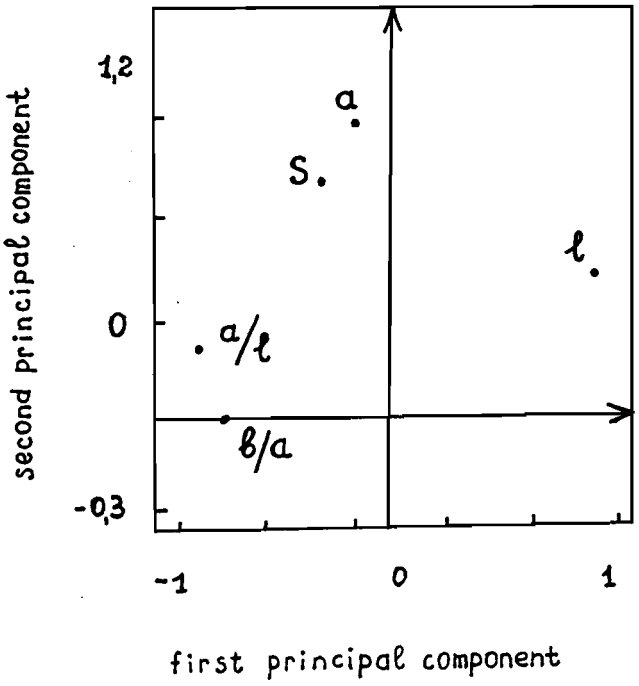

Figure 3. Principal-component analysis of epithelial cell dimensions. See text.

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describe the cell shape are given in parentheses, the sample size being 100 cells screened from a particular region at a particular developmental stage. Note that the process is initiated in a region (see the asterisk in Figure 2a) corresponding to a bending point in the curvature of the outer surface.

As epithelization spreads from one region (circumferential) to another (central), the outer cells in these regions differ in the same way as the cells of the same region at different stages of the epithelizatlon process. This permits us to recognize details of this process by analysing its regional variation. In fact, all (or almost all) superficial cells should become epithelialized in their normal developmental course. The regional difference among the cells is thereby subject to variation from non-genic causes, this being explained by random fluctuation in the rate, but not in the direction, of the change in cell shape. Further analysis by principal components provides a means to interpret this variation in dynamical terms (for the detailed explanation see Cherdantsev and Scobeyeva, 1994).

The distribution of metric characters (for which the symbols are the same as in Figures 2c and 2d) in the space of the first two principal components is given in Fig.3. The sample consisted of half a hundred superficial cells from different regions of the embryo; we screened all the cells in which we could measure all the characters under consideration. The frequency distributions of these characters were shown to be normal.

As shown in Figure 3, a positive contribution to the first principal component is introduced only by an increase in cell apicobasal length, while the ratio between the basal and apical cell surfaces, and also the cell surface area, make a negative contribution to the apicobasal elongation. It follows that the strongest process contributing to epithelization can be defined as follows: The lateral walls of the cell elongate as a result of shrinkage of the basal cell surface, and this leads to a decrease in the surface area of the cell in lateral view. The

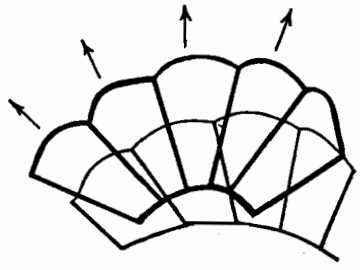

Figure 4. Movement of cells during epithelization. See text.

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primary contribution to the second principal component comes from the cell surface area and the length of the apical surface. Consequently, the second process, which is partly independent of the first one, consists of an enlargement of the apex of the cell, which thereby increases the cell surface area (or retards the overall decrease). Note in Figures 2c and 2d that the second process, in contrast to the first, does not change the mean values of corresponding characters, the small ostensible increase in the apical surface length given in Figure 2 being statistically insignificant. This means that the apical enlargement proceeds at a slower rate than the basal shrinkage does.

The epithelization process

From this perspective we can interpret the process of epithelization as a whole. The apical end of the cell appears to be homologous to the leading (anterior) edge of a moving individual cell (individual fibroblasts or the individual cells of slime molds provide well-known examples), while the basal end is homologous to the trailing (posterior) end of such a cell. In fact, the vector of the cell displacement during epithelization is indeed apically directed. In Figure 4, the more advanced stage of epithelization is shown by the heavier lines, and the arrows show the direction of cell displacement. The apical end corresponds to a "source", and the basal end corresponds to a "sink", of the cell surface. It follows that the epithelization process is inherently associated with an increase in positive curvature of the epithelial sheet. A corollary is that there is a positive feedback between the cellular and supracellular levels of epithelization: the more compact a particular part of an epithelial sheet is, the more pronounced is the epithelial shape of its cells, and vice versa.

The wrinkles which form in the central part of the embryonic disc appear to be regions of non-epithelialized (or weakly epithelialized) cells. These regions are manifested as invaginations only because of the increase in curvature of the surrounding regions. We should emphasize that these regions completely lack bottle-like cells, which should have been observed if they were real invaginations. In addition, in the wrinkle regions the superficial epithelial-sheet fragments are flanked by inner cells (see the asterisk in Figure 2b); this is the way in which the inner cells are recruited into epithelization (see the shaded cells in Figure 2b). This account portrays epithelization as a cooperative process capable of active transmission from one cell to another. Such a conclusion could have been drawn, *a priori*, from the fact that there is a cooperation between the cellular and supracellular scales of the epithelization process.

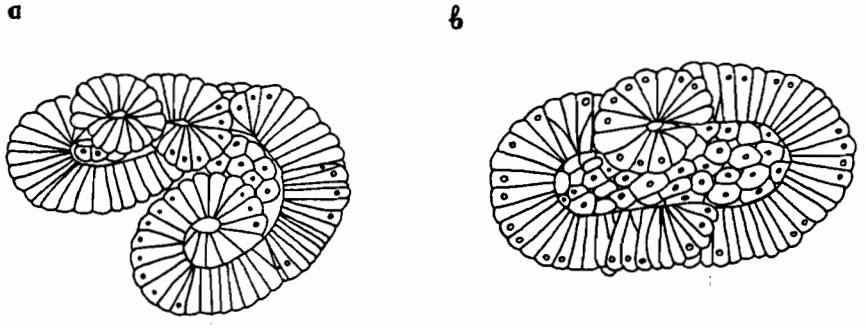

Figure 5. Variation in the amount of epithelization in *Dynamena*.

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There are no reasons to suppose that the recruitment of the inner cells into epithelization is genetically programmed, because the extent of this process is a matter of random variation. In fact, in some embryos all cells of the initial aggregate become epithelialized (Figure 5a), while in other embryos epithelization is predominantly concerned with superficial cells (Figure 5b).

To the preplanula

The formation of the preplanula proceeds with closure of craters on the outer surface of the embryo, caused by a further increase in the curvature of this surface and decrease in its area. This process is related to further columnarization (i.e., to a further increase in the ratio between the apicobasal length and lateral width) of the superficial cells. In most embryos this leads to the formation of a continuous spherical layer of superficial epithelial cells. In this case the inner cells lose their epithelial shape, and the gastral cavity arises by a secondary epithelization of the non-epithelialized inner cell mass (Figure 6a). However, in other embryos the initial crater cavity persists even after the closure of its margins (Figure 6b, the crater cavity being indicated by the central asterisk). In the latter case, where morphological continuity between the outer and inner cell layers is preserved and the inner cells keep their epithelial shape continuously and unchanged, it is the crater cavity which turns into the gastral cavity.

The variability of embryonic structure during the formation of the preplanula should be attributed to developmental self-organisation. Self-organization here is based on cooperation between neighbouring cells during the process of epithelization. Since the apicobasal polarization is homologous to the anteroposterior polarization of a single cell, this is a property which is inherent to the cell itself. On the other hand, it is this very property which is capable of cell-to-cell spreading (cf. Freeman, 1981). Thus, the cellular polarization acquires a new macroscopic (supracellular) scale, and this change of scale lies at the heart of epithelization itself.

Self-organization

In fact, epithelization demonstrates all the general properties which are inherent to self-organization. Note, first, that the symmetry order is decreased, as the apical and basal surfaces of the epithelial sheet become different from each other. Second, epithelization arises from non-specific causes: in fact, it is triggered at the bending points of the curvature of the outer surface, and the reason for bending is simply that

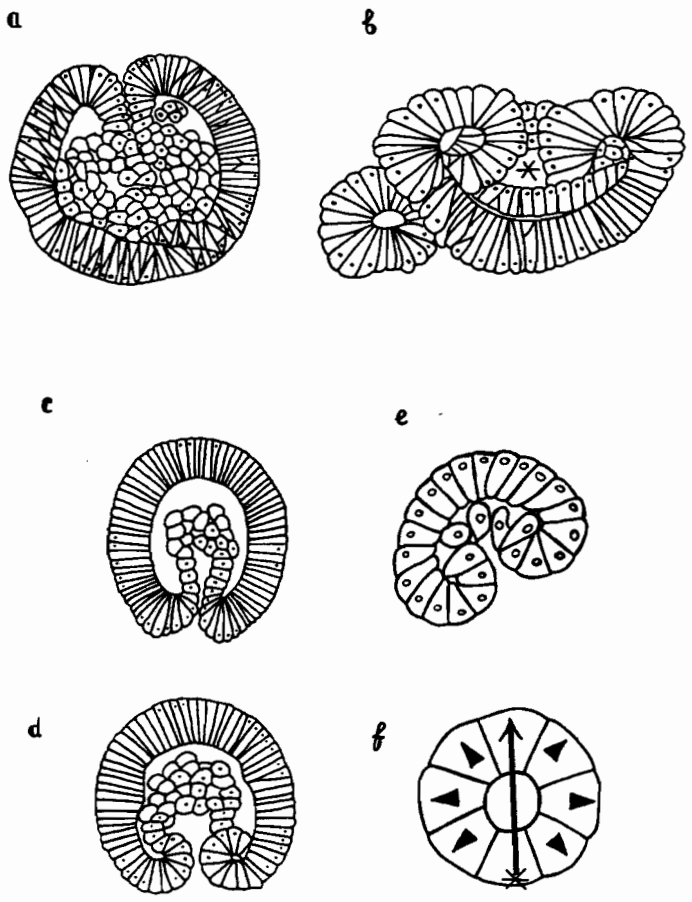

Figure 6. Processes of early development. See text.

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the early (non-epithelialized) embryo is unable to maintain a regular shape. It follows that epithelization does not require any pre-existing positional information. In other words, it implies that there are no constraints with respect to the type of cleavage. And finally, important details of the process of epithelization (how many cells will be recruited into epithelisation) are matters of random variation. In fact, the differences described among the modes of gastrulation may result from small random fuctuations in the disposition of cells inside the aggregate just before the beginning of epithelization.

Furthermore, one of this modes (in which the inner cells preserve their epithelial structure up to the formation of the preplanula) is almost identical to those gastrulation modes which are common for more derived taxa of Coelenterata, in particular for the gastrula of *Scyphozoa* (Figure 6c shows the gastrulation of a scyphozoan, *Lynerges mercurius*, redrawn from Conklin, 1908) and *Anthozoa* (Figure 6d shows the gastrulation of a sea anemone, *Metridium dianthus*, redrawn from Gemmill, 1920). Thus, some morphological patterns which initially arose from small random fluctuations have become adopted in the course of further evolution. All that is needed to adopt them, is to fix the course of the earlier developmental stages.

Of course, this does not mean that *Dynamena* is phylogenetically related to the taxa mentioned. On the contrary, there is good reason to suppose that the early development of this hydroid has been secondarily modified during evolution. In fact, the embryo develops inside a

sporosac: the blastula has no need to swim, and so there is no functional requirement to make a normal blastula (for the details see Buss, 1987). Thus, in the phylogenetic sense the development of the *Dynamena* embryo can hardly be considered as primitive. However, this does not contradict the claim that it may be considered as primitive in the evolutionary sense. The reason is that the removal of a well-defined blastula stage eliminates developmental constraints imposed on the developing system at the supracellular level, and this may revive mechanisms of cell integration that, it is plausible to suggest, could have worked during the origin and early evolution of multicellularity.

Evolutionary origin of the blastula

The data discussed here seem to be related to one of the main problems touching on the origination of multicellularity in the animal kingdom. The question is why the blastula (i.e., the hollow sphere consisting of epithelialized cells) is always present, as a transient stage, in the life cycles of these organisms. That this question is of crucial importance was recognized initially by Zakhvatkin (1949) and then, independently, by Buss (1987).

The answer seems to lie in our inference as to the identity between apicobasal and anteroposterior polarity of animal embryonic cells. The point is that this identity introduces a basic connection between the formation of a sheet of epithelial cells and the increase of its positive curvature. In fact, this means that the apical movement of cells in the course of epithelization is connected with the decrease in their basal surface area, so that epithelization automatically leads to a compaction (spherization) of the cell aggregate, whatever its initial structure. Thus, the epithelization itself provides a mechanism for the aggregation of cells into a compact spherical mass.

What merits consideration in this context is a striking resemblance that exists between the compactization of the *Dynamena* embryo and the behaviour of isolated (dissected) epithelial fragments in the early embryos of higher multicellular organisms, for example, in *Echinodermata* (Beloussov and Bogdanovsky, 1980) or *Amphibia* (Beloussov et.al., 1974). In all these cases the removal of a fragment by dissection triggers (in a few minutes) columnarization of cells. This is associated with a decrease in their basal surface area, so that the fragment turns into a compact spherical aggregate of cells (Figure 6e, redrawn with some modifications from Beloussov et al., 1974). Thus, the primary evolutionary connection between epithelization and aggregation proves to be evolutionarily stable. This seems to mean that the described mechanism of epithelization had arisen before it came to participate in the aggregation of cells (as the apicobasal polarity of a cell comes from the anteroposterior polarity). Since, however, this mechanism happened to be preadapted for aggregation, it was not subject to change in the course of further evolution.

On the other hand, the involvement of this mechanism in the organization of cells to make up an aggregate introduces a new developmental connection that arises between the polarity of individual cells and that of the aggregate as a whole. All that is needed is that the aggregate have no free margins. Recall that the apical end of the cell corresponds to a "source", and the basal end to a "sink", of the cell surface, so that each cell involved in epithelisation moves in the apical direction. Then, the pole of the aggregate at which epithelization begins (in the initial evolutionary phase the location of this pole is a matter

of random fluctuations) will act as an anterior source, while the opposite pole will act as a posterior sink of the outer aggregate surface. If the outer surface is closed, then this posterior pole corresponds to a place in which **the anteroposterior (apicobasal) polarity of cells is reversed with respect to the anteroposterior polarity of the cell aggregate.** This is shown in Figure 6f: the isolated arrowheads show the directions of the anteroposterior polarity of the cells, the arrow shows the anteroposterior polarity of the closed aggregate of cells (for simplicity this aggregate is portrayed as a monolayered epithelial sheet), and the asterisk shows the point of reversal.

Thus, in order to match the direction of the polarity of the aggregate, some cells should have reversed their own polarity. However, reviewing the morphology of gastrulation in the lower *Metazoa* (i.e., in *Spongia* and *Coelenterata*) makes it clear that in these taxa, in contrast to the *Chordata* and, probably, to the higher invertebrates, a reversal of cell polarity is not compatible with the integrity of an epithelial sheet of cells. In fact, in all the groups of lower invertebrates, the origination of regions with negative curvature is always associated with a breach of epithelization in the outer cell layer. It follows from the proposed model of epithelization that such a breach will automatically lead to the internalization of the corresponding cells, in the manner in which this occurs in the development of *Dynamena*. The fact that in *Dynamena* the inner cells may preserve their epithelial structure does not contradict this statement: recall that this occurs only in the case in which these cells have initially occupied an internal position.

Internalization of cells is known to afford a natural opportunity to make a distinction between ectoblast and entoblast cells at the very origin of multicellularity. On the other hand, we have shown internalization to be a corollary of a purely morphogenetic fact, that the same polarization mechanism (based on a distinction between the "source" and the "sink" of the cell surface) works both at the cellular and supracellular levels. This means that there are no reasons to regard selection as actively favouring this differentiation. In other words, our claim is that in the primitive multicellular organism the robust (i.e., structurally stable) topological form is not the sphere, but the sphere with a hole.

From this perspective, the true (monolayered) epithelial blastula should be considered as a secondary derivative of the primary bilayered form. This kind of blastula seems to have arisen in the same way as we have proposed for the evolutionary fixation of different morphological types of gastrulation which occur as random intraspecific variation in hydroid embryos. The characteristic feature of development preceding a true blastula is a regular cleavage, this helping epithelization to be shifted to an earlier developmental stage than it was initially.

Origin of multicellularity as an episelective process

Thus, we portray the origination of multicellularity as a process which, at the moment of its initiation, is not subject to direct selection. On the contrary, it is the origination of a new developmental connection that opens the way to a new selective trend. In this sense our idea of the origin of multicellularity differs from that recently formulated by Buss (1987). However, we come to a conclusion which

converges with an idea of Buss's in that the primary distinction between the germ layers (i.e., between the ectoblast and entoblast cells) cannot be considered as resulting from a differentiation of cell morphological types. The difference between the ectoblast and entoblast cells consists not in morphological states that they acquire, but rather in the ways in which they acquire the same (epithelial) state. In fact, this difference can be defined as a difference between the primary (ectoblast) and secondary (entoblast) epithelization.

The point is that the cells of the primary germ layers differ in their degree of specialization. The inner cells prove to be less specialized than the outer cells even provided that both types of cells have had the same number of the cell divisions. Thus, at the heart of differentiation lies a distinction between the "mature" (ectoblast) and "immature" (entoblast) cells. This suggests (see also Buss, 1987; Cherdantsev et al., 1996) that multicellularity should be considered as primarily a new mode of phenotypic self-reproduction, the essential feature of which is that some cells are forced to refrain from taking part in the reproduction of the multicellular organism; it is this loss of reproductive capacity itself which turns the reproduction of cells into the reproduction of multicellularity. In the simplest case (which is provided by the slime molds) this involves a distinction between the reproductive and nonreproductive cells, and with true multicellularity the difference among cell types becomes a matter of their capacity for expressing their phenotypes on the supracellular scale. Therefore, the new mode of reproduction is inherently connected with the origin of a new scale of differentiation.

Literature cited

Beloussov, L.V., and Bogdanovsky, S.V. 1980. Cellular mechanisms of embryonic regulation in sea urchins. Ontogenez (Soviet Journal of Developmental Biology) 11: 467-475 (in Russian).

Beloussov, L.V., Dorfman, J.G., and Cherdantsev, V.G. 1974. Rapid changes in form and cell architecture in isolated fragments of embryonic amphibian tissues as an experimental model of morphogenesis. Ontogenez (Soviet Journal of Developmental Biology) 5: 323-333 (in Russian).

Buss, L. 1987. The Evolution of Individuality. Princeton University Press. 320 pp.

Cherdantsev, V.G., and Scobeyeva, V.A. 1994. The morphological basis of self-organization: developmental and evolutionary aspects. Rivista di Biologia (Biology Forum) 87: 57-85.

Cherdantsev, V.G., Kreslavsky, A.G., and Severtsov, A.S. 1996. Episelective evolution. Evolutionary Theory 11: 69-87.

Conklin, E.G. 1908. The habits and early development of *Lynerges mercurius*. Papers from the Tortugas Laboratory, Carnegie Institution of Washington, 2: 153-170.

Freeman, G. 1981. The role of polarity in the development of the hydrozoan planula larva. W. Roux's Archives of Developmental Biology 190: 168-184.

Gemmill, J.F. 1920. The development of the sea anemones *Metridium dianthus* (Ellis) and *Adamsia palliata* (Bohad). Philosophical Transactions of the Royal Society of London (B) 209: 351-375.

Krauss, Y.A., and Cherdantsev, V.G. 1995. The primary epithelization of cells in the early ontogeny of a marine hydroid, *Dynamena pumila* L. Ontogenez (Russian Journal of Developmental Biology) 25 (2) (in press).

Zakhvatkin, A.A. 1949. Sravnitel'naya Embriologiya Nizshykh Bespozvonochnykh. Moscow: Nauka. 211 pp.