
REVIEWS

Evolutionary Reorganizations of Ontogenesis in Related Species of Coenobial Volvocine Algae

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Abstract—The evolutionary aspects of ontogenesis in green volvocine algae have been considered on the basis of the author's and published data, as well as the information on taxonomy, phylogeny, and ecology of this group. Analysis of the rate, diurnal rhythm, and light/dark control of cell divisions in various species, as well as experiments with the nucleic acid and protein synthesis inhibitors made it possible to elucidate cellular mechanisms underlying evolutionary rearrangements of asexual development in the genus *Volvox*.

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Analysis of reorganizations of ontogenesis in evolution of closely related species is an actual trend in the modern evolutionary developmental biology (Raff and Kaufman, 1986; Wilkins, 2001; Raff and Love, 2004). It is essential that the corresponding model system for analysis of development reorganizations consists of two or more related taxa with distinct ontogenetic differences. Also, phylogenetic relations within a given group should be sufficiently well known. Cellular and molecular mechanisms of the evolution of development have been most comprehensively studied on model systems, such as two recently diverged species of sea urchins of the genus *Heliocidaris* (Raff, 1987, 1999) and several species of ascidians of the genus *Molgula* (Jeffery, 1997; Jeffery et al., 1999). Interesting model systems also include starfishes of the genus *Patiriella* (Byrne and Cerra, 1996; Cerra and Byrne, 2004), some representatives of anuran amphibians (Callery et al., 2001; del Pino and Elinson, 2003), insects (Tikhomirova, 1991; Grbič, 2003), and other groups of multicellular animals.

Studies of evolutionary reorganizations of ontogenesis are also possible in coenobial (colonial) algae of the family Volvocaceae (division Chlorophyta, order Volvocales). For example, green algae of the genus *Volvox* enable us to analyze development in a system having only two cell types: somatic and reproductive. Despite a relative simple organization, the eukaryotic microorganism *Volvox* became a very popular model for studying a number of interesting phenomena and processes: growth and subsequent series of divisions of the reproductive cells, which proceed differently in various species; formation of cell lineages; morphogenesis; control of cell differentiation by sex pheromones, etc. (for reviews see Starr, 1970a; Kochert, 1975;

Desnitskiy, 1980, 1991, 1996a; Kirk and Harper, 1986; Schmitt et al., 1992; Kirk, 1998, 2001; Hallmann, 2003; Nedelcu and Michod, 2004). The studies were mostly carried on *Volvox carteri* and, in rare cases, on other species.

Meanwhile algae of the monophyletic family Volvocaceae sensu Nozaki including the genera *Pandorina*, *Volvulina*, *Yamagishiella*, *Eudorina*, *Platydorina*, *Pleodorina*, and *Volvox* (Table 1), are of traditional interest for evolutionists. The phylogeny of this group comprising approximately 40 species is now analyzed from the viewpoint of classical morphology (Nozaki and Kuroiwa, 1992; Hoops et al., 1994; Nozaki and Itoh, 1994; Nozaki, 1996), as well as with the use of molecular methods (Larson et al., 1992; Liss et al., 1997; Kirk, 1998; Coleman, 1999; Nozaki et al., 2000, 2002; Nozaki, 2003). The results of molecular-phylogenetic studies suggest the origin of coenobial Volvocales from unicellular ancestors about 50 million years ago.

In this review, evolutionary reorganizations of ontogenesis in coenobial volvocine algae are analyzed on the basis of the author's and published data. We studied physiological and ecological aspects of development in six species of Volvocales: *Volvox aureus*, *V. carteri*, *V. nagariensis*, *V. globator*, *V. spermatochaera*, *V. tertius*, and *Pleodorina californica*, since these species differ markedly from each other by important developmental features, such as size of mature gonidia, presence or absence of cell growth during a series of divisions, peculiarities of differentiation into two cell types, etc. The rate, diurnal rhythms, and light/dark control of the division of gonidia were determined and the effects of inhibitors of synthesis of various macromolecules on cell divisions during asexual development were studied. The results obtained made it possible to clarify the

Table 1. Characteristics of the family Volvocaceae sensu Nozaki

Genera	Number of cells in an asexual coenobium	Cell types	Sexual reproduction
<i>Pandorina</i>	8 or 16	All cells reproductive	Isogamous
<i>Volvulina</i>	8 or 16	The same	The same
<i>Yamagishiella</i>	16 or 32	"	"
<i>Eudorina</i>	16 or 32	All cells reproductive, except <i>E. illinoisensis</i> , in which four anterior cells are facultative somatic	Anisogamous, with antheridial packets
<i>Platydorina</i>	16 or 32	All cells reproductive	The same
<i>Pleodorina</i>	32, 64 or 128	In the anterior part of coenobium obligate somatic cells and in the posterior part, reproductive	"
<i>Volvox</i>	200, 500 and more (up to 50000)	The overwhelming majority of cells obligate somatic; a small number of reproductive cells in the posterior (and equatorial) part of coenobium	Oogamous, with antheridial packets; in some species, sexual development is controlled by pheromones

patterns and mechanisms of the evolution of ontogenesis in the family Volvocaceae and discuss them in the general context of data on evolutionary reorganizations of development in related species of sea urchins, anuran amphibians, and some other animals.

RATE, DIURNAL RHYTHMS, AND LIGHT/DARK CONTROL OF GONIDIAL DIVISIONS IN *VOLVOX* AND SOME OTHER VOLVOACEAE

Asexual cycles of development of *Volvox*, *Pleodorina*, and other algae of the family Volvocaceae include growth and divisions of gonidia (asexual reproductive cells), inversion (turning inside out) of young coenobia, their growth inside the parental organism, liberation from the parent, etc.

Two main types of asexual development were traditionally distinguished within the genus *Volvox* comprising 18 species distributed into four taxonomic sections (Starr, 1970a, 1970b; Kochert, 1975; Desnitskiy, 1980). In *V. africanus*, *V. carteri*, *V. gigas*, *V. obversus*, *V. powersii*, *V. spermatosphaera*, *V. tertius*, i.e., in all species of the section *Merillosphaera*, and *V. pocockiae*, one of two species of section *Janetosphaera*, gonidia reach very large sizes as result of long-term growth before the beginning of divisions and exceed somatic cells in diameter at least six- to eightfold; cell growth is absent during the period of divisions. On the other hand, in *V. aureus* (the second species of section *Janetosphaera*), *V. dissipatrix* (the only species of section *Copelandosphaera*), *V. amboensis*, *V. barberi*, *V. capensis*, *V. globator*, *V. merrillii*, *V. perglobator*, *V. prolificus*, and *V. rousseletii*, i.e., all species of section *Euvolvox*, mature gonidia are relatively small and exceed somatic cells in diameter no more than three- to fourfold, since the period of growth is rather short and cells grow during intervals between consecutive divisions.

Biochemical studies on two *Volvox* species have shown that in *V. carteri*, RNA and proteins are most

actively synthesized during the growth of gonidia, while during gonidial divisions, the rates of transcription and translation markedly decrease (Kochert, 1975). On the contrary, in *V. aureus*, RNA is synthesized at a maximum rate in the gonidia undergoing a series of divisions (Tucker and Darden, 1972). In both species, nuclear DNA is replicated only during intervals between consecutive divisions and duplication of nuclear DNA precedes every mitosis (Coleman and Maguire, 1982; Kobl et al., 1998). Unfortunately, the method ³H-thymidine autoradiography cannot be used for analysis of cell cycle structure in dividing gonidia, since this labeled nucleoside is not specifically incorporated into the nuclear DNA in *Volvox* (Desnitskiy, 1982a, 1982b). Exogenous ³H-thymidine appears to be predominantly incorporated into the chloroplast DNA, as well as in other cell components.

The cycle of asexual development of *V. carteri* can be considered a palintomic process: the prolonged hypertrophied growth of reproductive cells is accompanied by a series of fast divisions without cell growth. The term "palintomy" is borrowed from manuals on comparative embryology (Ivanova-Kazas, 1975) and protozoology (Sleigh, 1989). It can be stated that within the genus *Volvox*, palintomy occurs not only in *V. carteri*, but also in *V. pocockiae* (Starr, 1970b), *V. obversus* (Karn et al., 1974), *V. gigas*, and *V. powersii* (Vande Berg and Starr, 1971). These *Volvox* species with large gonidia dividing without cell growth are also characterized by a high division rate. However, analysis of the published data (Darden, 1966; McCracken and Starr, 1970; Starr, 1970a; Desnitskiy, 1980) suggests that in *V. aureus* and other species with small gonidia, there is no typical palintomy during asexual reproduction. According to our data, the cell diameter during a series of division of the gonidia in *V. aureus* gradually decreases from 20–25 to 4–6 μm (Desnitskiy, 1981a). The ratio of these values suggests that the cell volume decreases as a result of 10 divisions approximately

100-fold, rather than 1000-fold, which should have happened in the absence of growth. Thus, cell growth between consecutive divisions is undoubtedly present, but it does not compensate fully a twofold reduction of cell volume during each division.

At the illumination ca. 2000 lx, temperature 22–24°C, and diurnal light/dark regime 16/8 h, divisions of gonidia in the cultures of *V. carteri* f. *nagariensis* begin practically always in the second half of the light period, between 9 and 12 h after switching the light on. The duration of cell cycle determined as an interval between two consecutive divisions amounts to ca. 1 h (Desnitskiy, 1984a). Observations on asexual development in the cultures of *V. aureus* and *V. tertius* (Desnitskiy, 1984a, 1985a) have shown that the durations of cell cycle in these species amount to ca. 4 and 3 h, respectively and that divisions begin in the first half of the light period. Under natural conditions, cell divisions begin in *V. tertius*, like in the culture, in the morning (Desnitskiy, 1985b).

The direct effect of light on cell divisions has been studied in the cultures of different *Volvox* species (Desnitskiy, 1984a, 1985c). The transfer of *V. carteri* f. *nagariensis* coenobia with 2–4-cell gonidia into darkness does not affect subsequent divisions and inversion. On the contrary, the division of gonidia in *V. aureus* and *V. tertius* is a light-dependent process and has been arrested after transfer of the cultures into darkness. In *V. aureus* cultures, 0.40–0.61 cell divisions proceed within 12–24 h in the dark, while in *V. tertius*, 0.97–1.13 cell divisions within 20–24 h.

The rate and light/dark control of divisions have also been studied in *V. globator* and *V. spermatozphaera* (Desnitskiy, 1995a). During asexual development of *V. globator*, the gonidia divide at a low rate (the interval between two consecutive divisions is ca. 3 h) and light is essential for divisions (1.29 divisions proceed, on average, within 16 h in darkness). On the contrary, the *V. spermatozphaera* gonidia divided at a high rate (the interval between divisions is ca. 1 h) and irrespective of light.

V. spermatozphaera and *V. tertius* may be considered sibling species, using the term of Mayr (1970), since asexual coenobia of these representatives of section *Merrillosphaera* are rather similar in general morphology and are distinguished only according to the number of gonidia (Smith, 1944). A very close evolutionary affiliation between *V. spermatozphaera* and *V. tertius* has been shown with the use of molecular-phylogenetic analysis (Liss et al., 1997), but, according to our results, these two species differ distinctly by ontogenetic and ecophysiological features: rate and light/dark control of gonidial divisions.

In *Pleodorina californica*, gonidia begin to divide in the second half of the light period, the interval between two consecutive divisions amounts to 50–60 min, and, once the 2-cell stage is reached in the light, further divi-

sions can proceed at the normal rate in darkness (Desnitskiy, 2003a).

Note that, as concerns the light/dark regulation of asexual development, *V. aureus*, *V. globator*, and *V. tertius* differ not only from *V. carteri*, *V. spermatozphaera*, and *P. californica*, but also from many other volvocine algae, such as unicellular *Chlamydomonas reinhardtii* (Spudich and Sager, 1980) and coenobial *Eudorina elegans* (Luntz, 1968), in which series of rapid divisions can proceed in darkness. The occurrence of divisions during the dark phase, both in culture and nature, is characteristic for diverse groups of photoautotrophic phytoflagellates: euglenoids, dinoflagellates, coccolithophorids, etc. (for reviews see Desnitskiy, 1984b; Chisholm et al., 1984). Why have some *Volvox* species specific features in the “schedule” of gonidial divisions during the diurnal light/dark cycle? Since cell divisions of *V. aureus*, *V. globator*, and *V. tertius* stop in darkness, these species would not be able to sustain a competition with related palintomic species of coenobial Volvocales in their natural habitats, if they have no the above mentioned differences in the timing of gonidial divisions.

But since the light day duration and its seasonal changes markedly vary at different latitudes, the data on geographical distribution of coenobial Volvocales seem quite relevant. Such detailed information has been collected for *Volvox*, *Pleodorina*, and other representatives of the family Volvocaceae (Desnitskiy, 1996b, 1997, 2003a): a total of 36 species from seven genera, except several poorly studied or dubious *Pandorina* and *Eudorina* species.

Note that Skripchinsky (1975) proposed that the geographical (latitudinal) distribution of plants and animals is in some cases determined by the natural day length, but the data on photoperiodic reaction in Volvocales were very scarce at that time. It is of interest that an attempt has been undertaken to trace a correlation of the latitudinal distribution of some Volvocaceae with the peculiarities of light/dark control of their asexual reproductive cycles (Desnitskiy, 2003b). Among palintomic *Volvox* species, in which fast gonidial divisions can proceed in darkness, *V. carteri* and *V. spermatozphaera* are characterized by the northernmost distribution (up to 48–57 degrees North). The palintomic *Pleodorina californica* and other *Pleodorina* species, like palintomic *Volvox* species have never been recorded to the north of 50–57 degrees.

V. aureus, *V. globator*, and *V. tertius* occur in high latitudes of the Northern hemisphere (to the north of 50–57 degrees), i.e., three nonpalintomic species of coenobial Volvocales, as well as palintomic species without stable differentiation of coenobia into somatic and reproductive cells: *Pandorina charkowiensis*, *P. morum*, *Volvulina steinii*, *Eudorina cylindrica*, *E. elegans*, and *E. illinoisensis*. All these species also occur in lower latitudes.

An advantage of development of *Volvox* species with light-dependent cell division appears to consist in

a significant autonomy of divisions and DNA replication, irrespective of temperature fluctuations, which is very important under the conditions of cool northern climate and long summer day. This suggestion is favored by cytophysiological studies on cultures of *Chlamydomonas reinhardtii* (Donnan and John, 1983) and diatom algae *Thalassiosira pseudonana* (Heath and Spencer, 1985). In both species, the changes of temperature in rather wide limits (20 to 30 and 7.5 to 17.5°C, respectively) do not practically affect the rate of progression through the light-dependent part of cell cycle.

Thus, among representatives of the family Volvocaceae with obligatory differentiated somatic cells (22 *Volvox* and *Pleodorina* species) only three *Volvox* species occur in high latitudes (to the north of 50–57 degrees), in which the formation of new coenobia (series of consecutive gonidial divisions) is initiated in the beginning of light period (in the morning), the rate of divisions is low, and divisions are temporarily blocked in darkness (during the night). These features have adaptive significance under the conditions of long summer day and might have been important for the for-

mation of the contemporary flora of freshwater green algae in high latitudes of the Northern hemisphere.

EFFECTS OF INHIBITORS ON CELL DIVISION IN THREE *VOLVOX* SPECIES

In our experiments on *V. aureus*, *V. carteri* and *V. tertius*, aminopterin (an inhibitor of DNA precursor synthesis), actinomycin D (an inhibitor of RNA synthesis), cycloheximide, and streptomycin (inhibitors of protein synthesis on ribosomes 80S and 70S, respectively) have been used (Desnitskiy, 1985c, 1986, 1987, 1990, 1992) (Table 2). The divisions of *V. aureus* gonidia are very sensitive to all inhibitors, while divisions of *V. carteri* f. *nagariensis* are much more resistant. In *V. tertius*, the divisions are also resistant to actinomycin, streptomycin, and cycloheximide, but they are not so resistant against aminopterin, as in *V. carteri* f. *nagariensis*. Note that our data on high resistance of *V. carteri* divisions to aminopterin agree with the data of Weinheimer (1983), who used 5-fluorouracil

Table 2. Effects of inhibitors on gonidial division in three *Volvox* species (mean number of cell divisions in the presence of inhibitor)

Inhibitor concentration, µg/ml	<i>V. aureus</i>		<i>V. carteri</i> f. <i>nagariensis</i>		<i>V. tertius</i>	
	Aminopterin					
0*	≥4.90	≥4.92	–		–	
5	1.14	1.20	No effect		No effect	
10	0.45	0.92	No effect		2.43	2.77
25	0.24	0.42	No effect		2.07	2.66
	Actinomycin D					
0*	≥5.72	≥5.29	–		–	
1	4.44	≥4.75	No effect		No effect	
5	0.67	0.66	≥4.79	≥5.35	≥5.00	≥5.16
20	0.60	0.44	0.32	0.55	0.71	0.74
	Cycloheximide					
0*	≥5.16	≥5.15	–		–	
0.1	0.35	0.53	≥4.99	≥5.12	4.08	≥4.54
0.4	0.28	0.23	1.26	0.79	0.75	0.60
1.0	0.20	0.20	0.47	0.12	0.42	0.28
	Streptomycin					
10	3.97	3.40	Not tested		No effect	
25	2.24	2.05	Not tested		No effect	
100	1.06	0.67	≥6.15	≥5.86	≥5.97	≥5.63
200	Not tested		4.94	4.70	3.78	4.23
500	Not tested		0.70	0.92	1.44	1.06

Note: Experiments were repeated twice. 0*, control (material not treated with inhibitors). Control gonidia of *V. aureus*, *V. carteri* f. *nagariensis*, and *V. tertius* underwent 5–6, 11–12, and 7–8 divisions, respectively. In most cases, it was impossible to calculate the number of division in the control, since by the end of the period of observations, the gonidia were at too advanced developmental stages, when the number of cells could not be estimated precisely.

(another inhibitor of DNA precursor synthesis) on gonidia of the same species.

Thus, it can be proposed that the *V. carteri* gonidia have a vast reserve of intracellular DNA precursors already in the beginning of the period of divisions, which provides for a high division rate. Seemingly, these data on the cell cycle control in *V. carteri* should have been similar to those for early sea urchin embryos (Niikura et al., 1984) and amphibian embryos (Grant, 1960; Landström et al., 1975). On the contrary, the results of experiments on *V. aureus* and *V. tertius* suggest that in both species, especially in *V. aureus*, endogenous pools of DNA precursors are rather small in the beginning of the period of divisions. Apparently, formation of DNA precursors directly during the period of divisions plays an essential role in the control of gonidial divisions in these *Volvox* species.

The results of our studies suggesting that in *V. aureus* cell divisions are much more sensitive to the inhibitor of transcription actinomycin D than in *V. carteri* agree quite well with the published data on differences in the dynamics of RNA synthesis in the course of asexual development in the same *Volvox* species (Tucker and Darden, 1972; Kochert, 1975). Similar results have been obtained in our experiments with cycloheximide and streptomycin. A high sensitivity of *V. aureus* gonidia to diverse inhibitors of RNA and protein synthesis appears to be explained by the growth of cells during long-term intervals between consecutive divisions in this species, unlike the two others. The divisions of *V. carteri* and *V. tertius* gonidia are approximately equally resistant to the inhibitors of transcription and translation irrespective of the differences in the rate of division. This circumstance excludes the possibility of trivial explanation of significant resistance of *V. carteri* gonidia to any inhibitors as a simple consequence of the high rate of cell division. On the other hand, the high resistance of gonidial divisions in the same *Volvox* species to the effect of aminopterin appears to correlate above all with a high division rate.

ANALYSIS OF EVOOLUTIONARY REORGANIZATIONS OF ONTOGENESIS IN COENOBIAL VOLVOCINE ALGAE

An attempt was undertaken to create a formal model of the establishment of multicellularity on the basis of genetic data on the control of development in *V. carteri* f. *nagariensis* (Kirk, 1988, 1998, 2001). It was proposed that successive appearance of three regulatory loci, *regA*, *gls*, and *lag*, in the genome of volvocine algae became a prerequisite for evolution from the unicellular (a *Chlamydomonas*-like organism) or colonial state (a *Pandorina*-like organism with pluripotent cells) to the multicellular one with irreversible "division of labor" between two specialized cell types. An "evolutionary program" has recently been proposed (Kirk, 2005), comprising already 12 successive stages, of which the initial and final steps are a unicellular organ-

ism similar to *Chlamydomonas reinhardtii* and a multicellular *Volvox carteri*-like organism, respectively. For this model to be constructed, the data on *Pandorina*, *Eudorina*, *Pleodorina* and some other coenobial Volvocales, for example, genus *Gonium* from the family Goniaceae, have also been used, rather than the data on other *Volvox* species, although the availability of such an information is evident.

What are evolutionary interrelations between the two main types of asexual development, palintomic and nonpalintomic, which can be conditionally called *V. carteri* and *V. aureus* developmental types, respectively? In order to answer this question, some facts should be taken into consideration. First, *V. powersii* and *V. gigas*, two relatively primitive species of this genus possessing some morphological (Vande Berg and Starr, 1971) and molecular features (Coleman, 2002) common with the genus *Pleodorina*, are characterized by palintomy. Second, the data on patterns of asexual development in other genera of coenobial Volvocales, as well as in the unicellular alga *Ch. reinhardtii*, suggest that all these organisms are palintomic (for review see Desnitskiy, 1981b). Hence, there are grounds to believe that in *V. aureus*, palintomy is reduced. Thus, the type of *V. aureus* asexual development is evolutionarily more advanced than the palintomic type of asexual development, characteristic, for instance, of *V. carteri*.

The above described results of our studies shed light on cellular mechanisms underlying the evolution of asexual development in *Volvox* and Table 3 provides some comparative characteristics of three *Volvox* species. Comparison of development in *V. carteri* and *V. aureus* has shown that the reduction of palintomy in the latter species is conditioned by a shortened period of gonidial growth and delayed rate of subsequent divisions. The cells of young coenobia grow during long-term intervals between divisions. The light/dark control of cell divisions undergoes changes: it becomes light-dependent and the moment of initiation of divisions is displaced from the second half of the light period to the first one. RNA and protein syntheses proceed in *V. aureus* at the highest rates not during the growth of gonidia, like in *V. carteri*, but directly during the period of divisions. The same pattern is also characteristic of the dynamics of formation of the intracellular pools (reserves) of DNA precursors during the asexual developmental cycle.

On the other hand, the results of our studies of asexual development in *V. tertius* suggest that the reduction of palintomy is also found within section *Meriliosphaera*, irrespective of evolutionary reorganizations in section *Janetosphaera*. However, only certain palintomic traits were lost in *V. tertius*: some features of its development are similar to those of *V. aureus*, while others resemble the development of *V. carteri* (Table 3). Thus, the initial step of palintomic reduction observed in *V. tertius* is connected with alteration of the light/dark control of cell divisions (photo-

Table 3. Comparative characteristics of asexual development in three *Volvox* species

Comparative characteristics	<i>V. carteri</i> f. <i>nagariensis</i>	<i>V. aureus</i>	<i>V. tertius</i>
Size of gonidia before the beginning of a series of divisions, μm	50–60	20–25	42–45
Presence or absence of cell growth during intervals between consecutive divisions	–	+	–
Duration of cell cycle, h	1	4	3
Is light essential for cell divisions?	No	Yes	Yes
Initiation of a series of divisions	Second half of light period	Beginning of light period	Beginning of light period
Relative resistance of divisions against inhibitors of RNA and protein synthesis	High	Low	High
Relative resistance of divisions against inhibitor of synthesis of DNA precursors	High	Low	Low

periodic reaction), slowing down of the rate of cell division, and, at the molecular level, changes in metabolism of DNA precursors (Desnitskiy, 1991, 1992). However, there appear to be no marked changes (as compared to the palintomic species *V. carteri*) in the dynamics of RNA and protein syntheses in the course of asexual cycle due to the absence of cellular growth in young coenobia during the period of divisions in all representatives of section *Merrillosphaera*.

Nevertheless, the presence of large gonidia dividing without cell growth does not mean *per se* a high rate of divisions. It can be proposed that the program of asexual development in *V. tertius* is a modification of the palintomic program characteristic for most species of section *Merrillosphaera*, in which, however, the supply of gonidia with an endogenous reserve of DNA precursors in the beginning of the series of divisions is not provided.

Finally, let us remember (see Karn et al., 1974; Desnitskiy, 1996a; Kirk and Kirk, 2004) that the palintomic *V. carteri* and *V. obversus*, two evolutionarily advanced species from section *Merrillosphaera*, are characterized by differentiation of the lineages of presumptive reproductive and somatic cells at relatively early developmental stages, during transition from 16–32-cell stages to 32–64-cell stages. In other *Volvox* species, gonidia become morphologically distinct from somatic cells after termination of the series of divisions.

Thus, although the concept of two main types of asexual development in *Volvox* (Starr, 1970a; Kochert, 1975; Desnitskiy, 1980) and above considerations about evolutionary relations between them (Desnitskiy, 1991, 1992) stand good, a perspective is opened of creating a more detailed concept of the evolution of asexual development in *Volvox* (Desnitskiy, 1995b). Taking into account the size of mature gonidia, rate of their division, and specific features of the formation of cell lineages, four programs (types) of asexual development of *Volvox* and *Pleodorina* can be distinguished.

The first developmental program is characteristic of *V. powersii*, *V. spermato-sphaera*, *V. gigas*, *V. pocockiae*, and *Pleodorina californica*. These species have large gonidia, the rate of their division is high, and there is no unequal (asymmetrical) division into two cell types.

The second program is characteristic of *V. carteri* and *V. obversus* and differs from the first program in having an asymmetric division, which forms presumptive reproductive and somatic cells of the next generation.

The third program is characteristic for *V. tertius*, in which gonidia are large, but the rate of development is low, and unequal division is absent.

The fourth program is characteristic of *V. aureus* and *V. globator*, but is also probable for *V. dissipatrix* and most species of section *Euvolvox*, in which small gonidia divide at a slow rate and without differentiation of cell lineages.

The first program is the most primitive and has ancestral features: large gonidia, fast division, and differentiation into two cell types without unequal division. The second, third, and fourth programs are evolutionary advanced, each of them having certain derived features: asymmetric division, slow divisions, and small gonidia and slow divisions, respectively. From the viewpoint of contemporary cladistics, the first developmental program represents symplesiomorphy, second—synapomorphy, third—autapomorphy, and fourth, observed in three *Volvox* sections,—homoplasy.

Series of consecutive cell divisions in all *Volvox* species and other coenobial Volvocales, such as *Pandorina*, *Eudorina*, *Pleodorina*, etc., differ radically from division into two daughter cells in many unicellular planktonic algae (euglenoids, diatoms, dinoflagellates, coccolithophorids), which have a typical monotomic (binary) cell cycle consisting of phases G_1 , S , G_2 , and M (for reviews see Chisholm et al., 1984; Desnitskiy, 1984b, 1989).

Asexual (vegetative) cycle of the unicellular alga *Chlamydomonas reinhardtii* (a species evolutionarily

very closely linked with coenobial Volvocales) can be monotomic under unfavorable conditions, such as weak illumination, but is usually palintomic under favorable conditions and includes the phases G_1 - S - M - S - M - S - M , i.e., a long period of cell growth (phase G_1) provides for a series of rapidly alternating nuclear DNA replications and mitoses (Coleman, 1982; Harper and John, 1986). Thus, it can easily be imagined how asexual developmental cycles of coenobial Volvocales originated from this basic cell cycle, although, according to the current concepts (Larson et al., 1992; Hoops et al., 1994; Kirk, 1998; Coleman, 1999; Nozaki et al., 2000), the genera *Pandorina*, *Eudorina*, *Pleodorina*, and *Volvox* do not represent a successive evolutionary series. In the course of evolution, phase G_1 was transformed into ever longer period of hypertrophied light-dependent cell growth, which provided for more and more extensive series of rapidly alternating DNA replications and mitoses: up to 10–13 rounds of replications and mitoses in palintomic *Volvox* species. Finally, different stages of palintomic reduction in several representatives of the genus *Volvox* appear to be evolutionary changes of organisms that are already at the multicellular level of organization.

It is well known (Bold and Wynne, 1985) that the unicellular *Chlamydomonas* and coenobial *Pandorina* and *Eudorina* become transiently immobile during the period of palintomic divisions. Note that in *Volvox*, the differentiation into a small number of large reproductive cells and many small biflagellate somatic cells provides for motility throughout the cycle of asexual development. This is, beyond doubt, an important advantage over other Volvocaceae (Desnitskiy, 1983; Bell, 1985; Koufopanou, 1994). It is evident that such a differentiation into two cell types might have arisen only after a certain (more or less considerable) number of cells had been attained in a coenobium.

Within the family Volvocaceae, nonpalintomic type of asexual reproduction occurs only in a few *Volvox* species. For coenobia of *Volvulina*, *Pandorina*, *Eudorina*, and other related Volvocaceae, which are not yet at the multicellular level of organization and have no irreversibly differentiated cell types, the palintomic reduction would be, in our opinion, a “forbidden way of evolution” because of considerable increase in the duration of the immobile developmental phase, which, in addition, is light-dependent. For the same reason, palintomy could not have been reduced in any *Volvox* species during germination of a unicellular zygote proceeding in the absence of somatic cells (Pocock, 1933; Smith, 1944; Darden, 1966).

A shift of morphological segregation of the reproductive cells to earlier stages of asexual development under the conditions of palintomy (*V. carteri* and *V. obversus* in section *Merillosphaera*) is an essential evolutionary trend within the genus *Volvox*. Reduction of certain palintomic features, starting from the changes in diurnal rhythm, light/dark control, and rate of gonidial divisions (*V. tertius* in section *Meril-*

losphaera) is another important evolutionary trend observed in all four sections of the genus *Volvox*. In *V. aureus* from section *Janetosphaera* and in all species of sections *Copelandosphaera* and *Euvolvox*, palintomy is reduced to a greater extent: the period of growth in unicellular gonidia is sharply reduced, while the basic cell growth proceeds during a series of divisions. However, the growth does not fully compensate a two-fold decrease of cell volume occurring during each division and, therefore, reduction of palintomy during asexual development in these *Volvox* species cannot be considered a return to monotomy.

Independently of us, Kirk (1998, 2005) also put forward an idea of programmed (directional) evolution in coenobial volvocine algae but he compared representatives of different Volvocales genera, rather than different *Volvox* species. On the other hand, some researchers sometimes underestimated a possibility of parallel evolution in coenobial Volvocales and considered it unlikely (Nozaki, 1996).

The idea that reorganization of ontogenesis can be a factor of evolution of the related species was put forward in studies on comparative and evolutionary embryology of insects (Tikhomirova, 1991), starfishes (Byrne and Cerra, 1996), and gastropods (Duda and Palumbi, 1999). We believe that this should also be true for *Volvox*. Alteration of the developmental type enhances ecological diversification and stable coexistence of related species in the same territory. As a matter of fact, populations of two or even three species of coenobial Volvocales sometimes coexist in a small temporary pool (Powers, 1908; Iyengar, 1933; Desnitskiy, 2000, 2002). It can be proposed that this mode of life enhanced the appearance of a significant number of developmental types, i.e., ontogenetic diversity, in this small and evolutionarily young taxonomic group.

Changes of the developmental type, in a given case: reduction of palintomy and change of the light/dark control of gonidial divisions, may assist in colonization of new territories, for example, penetration of several evolutionarily advanced *Volvox* species into high latitudes of the Northern hemisphere (Desnitskiy, 2003b). Let us remember also the studies on sea urchins (Emlet, 1995) and gastropods (Collin, 2001, 2003) suggesting a certain relationship between the mode of development of the related species and their geographical distribution. Apparently, very significant changes of development may proceed during evolution of diverse groups of multicellular organisms under the influence of ecological factors. It would be promising to use the concepts of contemporary evolutionary ecology (Gause, 1984; Khlebovich, 2002) in such an analysis.

The presence of parallel (directional) evolutionary reorganizations in different representatives of the same group and variation in the degree of ontogenetic reorganization within this group, i.e., existence of intermediate, transitional developmental types, can be given as an example of general patterns in the evolution of

development in coenobial Volvocales (Desnitskiy, 1995), sea urchins (Raff, 1987; Desnitskiy, 2005), ascidians (Jeffery, 1997; Jeffery et al., 1999), and anuran amphibians (Callery et al., 2001; Desnitskiy, 2004). In the case of genus *Volvox*, the type of *V. tertius* development is intermediate, when only some palintomic features are reduced. In many orders of the class Echinoidea and many families of the order Anura, the sizes of eggs and reserves of nutrients in them increased in the course of evolution and lecithotrophic larvae appeared or the larval stage was even eliminated. For example, in sea urchins, the type of *Peronella japonica* development is intermediate.

However, in *Volvox*, unlike some sea urchins and anuran amphibians (Desnitskiy, 2004, 2005), the development acceleration and autonomization have not been found. In the course of evolution, on the contrary, the series of gonidial divisions forming a new individual was slowed down and became light-dependent and a trend was observed to a decreased size of mature gonidia. This appears to be explained by the algal status of *Volvox* and hence, a very primitive level of multicellular organization. Note that heterochronies played a certain role in the evolution of *Volvox* but they were realized in a different way from Metazoa: in *Volvox*, evolutionary reorganizations affect mostly asexual developmental cycles, which, however, play a leading role in the life of natural populations.

As concerns the green algae of the genus *Volvox* (Liss et al., 1997), sea urchins of the genus *Heliocidaris* (McMillan et al., 1992, and ascidians of the genus *Molgula* (Jeffery, 1997), the molecular phylogenetic data suggest clearly that the reorganizations of ontogenesis in these three genera are relatively recent: within several last million of years. Recent publications on the evolution of development in gastropods of the family Calyptraeidae (Collin, 2003, 2004) suggest that the concept of "punctuated evolution" of embryos cannot be applied to them, unlike the situation with sea urchins (Raff et al., 2003). By the way, Kirk (2005) also tends to interpret the phylogeny of the algal family Volvocaceae as a gradual process.

CONCLUSION

The rate, diurnal rhythms, and light/dark control of cell divisions during the asexual reproduction can serve as important ecophysiological and ontogenetic features that characterize the Volvocaceae species. The palintomic cycle of asexual reproduction is more primitive (ancestral): hypertrophied growth of gonidia is accompanied by a series of fast divisions without cell growth. This type of development occurs in several *Volvox* species, such as *V. carteri*, *V. poccockiae*, *V. powersii*, and *V. spermato-sphaera*, and other genera of coenobial Volvocales.

Independent reduction of palintomy in different *Volvox* species is an important feature of the evolution of

asexual developmental cycles. For example, the initial step of palintomic reduction observed in *V. tertius* is connected with changes in the diurnal rhythm and light/dark control of gonidial divisions, slowdown of divisions, and, at the molecular level, changes in the metabolism of DNA precursors. A more advanced step observed, for example, in *V. aureus*, is characterized also by changes in the dynamics of RNA and protein synthesis during asexual development and smaller mature gonidia due to a reduced period of their growth. The palintomic reduction is least expressed in section *Merillosphaera* and most expressed in section *Euvolvox*. However, in the course of sexual development, during zygote germination in the absence of somatic cells, all *Volvox* species retain the ancestral palintomic features.

It cannot be excluded that based on novel molecular-phylogenetic data (Nozaki et al., 2002; Nozaki, 2003), a taxonomic revision of the genus *Volvox* will soon be carried out, which almost certainly is not monophyletic. Nevertheless, according to these data, all *Volvox* species with reduced palintomy are evolutionarily advanced and, hence, there are strong reasons to believe that our concept on patterns and mechanisms of evolutionary reorganizations of ontogenesis will not contradict such a taxonomic revision.

REFERENCES

- Bell, G., The Origin and Early Evolution of Germ Cells as Illustrated by the Volvocales, *The Origin and Evolution of Sex*, Halvorson, H.O. and Monroy, A., Eds., New York: Alan R. Liss, 1985, pp. 221–256.
- Bold, H.C. and Wynne, M.J., *Introduction to the Algae*, 2nd edition, Englewood Cliffs, NJ (New Jersey): Prentice-Hall, 1985.
- Byrne, M. and Cerra, A., Evolution of Intra-gonadal Development in the Diminutive Asterinid Sea Stars *Patiriella vivipara* and *P. parvivipara* with an Overview of Development in the Asterinidae, *Biol. Bull.*, 1996, vol. 191, pp. 17–26.
- Callery, E.M., Fang, H., and Elinson, R.P., Frogs without Polliwogs: Evolution of Anuran Direct Development, *BioEssays*, 2001, vol. 23, pp. 233–241.
- Cerra, A. and Byrne, M., Evolution of Development in the Sea Star Genus *Patiriella*: Clade-Specific Alterations in Cleavage, *Evol. Devel.*, 2004, vol. 6, pp. 105–113.
- Chisholm, S.W., Vaultot, D., and Olson, R.J., Cell Cycle Controls in Phytoplankton. Comparative Physiology and Ecology, *Cell Cycle Clocks*, Edmunds, L.N., Ed., New York: Marcel Dekker, Inc., 1984, pp. 365–394.
- Coleman, A.W., The Nuclear Cell Cycle in *Chlamydomonas* (Chlorophyceae), *J. Phycol.*, 1982, vol. 18, pp. 192–195.
- Coleman, A., W., Phylogenetic Analysis of "Volvocaceae" for Comparative Genetic Studies, *Proc. Natl. Acad. Sci. USA*, 1999, vol. 96, pp. 13892–13897.
- Coleman, A.W., Comparison of *Eudorina/Pleodorina* ITS Sequences of Isolates from Nature with Those from Experimental Hybrids, *Am. J. Bot.*, 2002, vol. 89, pp. 1523–1530.

- Coleman, A.W. and Maguire, M.J., A Microspectrofluorometric Analysis of Nuclear and Chloroplast DNA in *Volvox*, *Devel. Biol.*, 1982, vol. 94, pp. 441–450.
- Collin, R., The Effects of Mode of Development on Phylogeography and Population Structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae), *Mol. Ecol.*, 2001, vol. 10, pp. 2249–2262.
- Collin, R., Worldwide Patterns in Mode of Development in Calyptraeid Gastropods, *Mar. Ecol. Progr. Ser.*, 2003, vol. 247, pp. 103–122.
- Collin, R., Phylogenetic Effects, the Loss of Complex Characters, and the Evolution of Development in Calyptraeid Gastropods, *Evolution*, 2004, vol. 58, pp. 1488–1502.
- Darden, W.H., Sexual Differentiation in *Volvox aureus*, *J. Protozool.*, 1966, vol. 13, pp. 239–255.
- Desnitskiy, A.G., Cell Differentiation and Morphogenesis in *Volvox*, *Ontogenez*, 1980, vol. 11, no. 4, pp. 339–350.
- Desnitskiy, A.G., A Study of Development of *Volvox aureus* Ehrenberg (Petergofskaya liniya P-1), *Vestn. Leningr. Univ.*, 1981a, no. 3, pp. 29–32.
- Desnitskiy, A.G., Cell Cycles in *Volvox* and Some Other Green Flagellates (Volvocales), *Tsitologiya*, 1981b, vol. 23, no. 3, pp. 243–253.
- Desnitskiy, A.G., An Autoradiographic Study of Incorporation of Nucleic Acid Precursors in *Volvox* Cells, *Tsitologiya* 1982a, vol. 24, no. 2, pp. 172–176.
- Desnitskiy, A.G., Specific Features of ³H-Thymidine Incorporation in Embryos of *Volvox aureus*, *Ontogenez*, 1982b, vol. 13, no. 4, pp. 424–426.
- Desnitskiy, A.G., Problem of Establishment and Early Stages of the Evolution of Multicellularity in Volvocales, *Tsitologiya*, 1983, vol. 25, no. 6, pp. 635–642.
- Desnitskiy, A.G., Some Features of Regulation of Cell Divisions in *Volvox*, *Tsitologiya*, 1984a, vol. 26, no. 3, pp. 269–274.
- Desnitskiy, A.G., Regulation of Cell Cycles in Phytoflagellates, *Tsitologiya*, 1984b, vol. 26, no. 6, pp. 635–642.
- Desnitskiy, A.G., Determination of the Time of Beginning of Gonidial Division in *Volvox aureus* and *Volvox tertius*, *Tsitologiya*, 1985a, vol. 27, no. 2, pp. 227–229.
- Desnitskiy, A.G., Diurnal Rhythm of Gonidial Cleavage in Natural *Volvox* Populations, *Tsitologiya*, 1985b, vol. 27, no. 9, pp. 1075–1077.
- Desnitskiy, A.G., Influence of Streptomycin on Cell Divisions and Growth in Three *Volvox* Species, *Tsitologiya*, 1985c, vol. 27, no. 8, pp. 921–927.
- Desnitskiy, A.G., Influence of Aminopterin on Cell Divisions in *Volvox*, *Tsitologiya*, 1986, vol. 28, no. 5, pp. 545–551.
- Desnitskiy, A.G., Influence of Actinomycin D on Cell Divisions in Three *Volvox* Species, *Tsitologiya*, 1987, vol. 29, no. 4, pp. 448–453.
- Desnitskiy, A.G., Regulation of Divisions and DNA Synthesis in Unicellular Algae, *Tsitologiya*, 1989, vol. 31, no. 2, pp. 148–153.
- Desnitskiy, A.G., Influence of Cycloheximide on Cell Divisions in *Volvox*, *Bot. Zh.*, 1990, vol. 75, no. 2, pp. 181–186.
- Desnitskiy, A.G., Mechanisms and Evolutionary Aspects of Ontogenesis in the Genus *Volvox* (Chlorophyta, Volvocales), *Bot. Zh.*, 1991, vol. 76, no. 5, pp. 657–668.
- Desnitskiy, A.G., Cellular Mechanisms of the Evolution of Ontogenesis in *Volvox*, *Arch. Protistenkd.*, 1992, vol. 141, pp. 171–178.
- Desnitskiy, A.G., On the Rate of Cell Divisions during Asexual Reproduction in *Volvox globator* and *V. spermatozphaera* (Chlorophyta, Volvocales), *Bot. Zh.*, 1995a, vol. 80, no. 8, pp. 40–42.
- Desnitskiy, A.G., A Review on the Evolution of Development in *Volvox*—Morphological and Physiological Aspects, *Eur. J. Protistol.*, 1995b, vol. 31, pp. 241–247.
- Desnitskiy, A.G., Comparative Analysis of the Mechanisms Underlying Cell Differentiation in the Genus *Volvox* (Chlorophyta, Volvocales), *Bot. Zh.*, 1996a, vol. 81, no. 6, pp. 1–9.
- Desnitskiy, A.G., On Geographic Distribution of Species of the Genus *Volvox* (Chlorophyta, Volvocales), *Bot. Zh.*, 1996b, vol. 81, no. 3, pp. 28–33.
- Desnitskiy, A.G., A Review of Species of the Genus *Volvox*, *Vestn. St. Petersburg Univ. Ser. 3*, 1997, no. 4(24), pp. 19–29.
- Desnitskiy, A.G., Development and Reproduction of Two Species of the Genus *Volvox* in a Shallow Temporary Pool, *Protistology*, 2000, vol. 1, pp. 195–198.
- Desnitskiy, A.G., Dormant Stages of Green Flagellate *Volvox* in Nature, *Ontogenez*, 2002, vol. 33, no. 2, pp. 136–138.
- Desnitskiy, A.G., A Review of Green Microalgae of the Genus *Pleodorina*, *Vestn. St. Petersburg Univ. Ser. 3*, 2003a, no. 1(3), pp. 98–102.
- Desnitskiy, A.G., Specific Features of Geographical Distribution of Coenobial Volvocaceae (Chlorophyta), *Bot. Zh.*, 2003b, vol. 88, no. 11, pp. 52–61.
- Desnitskiy, A.G., Evolutionary Reorganizations of Ontogenesis in Anuran Amphibians, *Ontogenez*, 2004, vol. 35, no. 3, pp. 165–170.
- Desnitskiy, A.G., Evolutionary Reorganization of Ontogenesis in Sea Urchins, *Ontogenez*, 2005, vol. 36, no. 3, pp. 182–189.
- Donnan, L. and John, P.C.L., Cell Cycle Control by Timer and Sizer in *Chlamydomonas*, *Nature*, 1983, vol. 304, pp. 630–633.
- Duda, T.F. and Palumbi, S.R., Developmental Shifts and Species Selection in Gastropods, *Proc. Natl. Acad. Sci. USA*, 1999, vol. 96, pp. 10272–10277.
- Emler, R.B., Developmental Mode and Species Geographic Range in Regular Sea Urchins (Echinodermata: Echinoidea), *Evolution*, 1995, vol. 49, pp. 476–489.
- Gause, G.F., Ecology and Some Problems of Origin of Species, *Ekologiya i evolyutsionnaya teoriya* (Ecology and Evolutionary Theory), Leningrad: Nauka, 1984, pp. 5–108.
- Grant, P., The Influence of Folic Acid Analogs on Development and Nucleic Acid Metabolism in *Rana pipiens* Embryos, *Devel. Biol.*, 1960, vol. 2, pp. 197–251.
- Grbič, M., Polyembryony in Parasitic Wasps: Evolution of a Novel Mode of Development, *Int. J. Devel. Biol.*, 2003, vol. 47, pp. 633–642.
- Hallmann, A., Extracellular Matrix and Sex-Inducing Pheromone in *Volvox*, *Int. Rev. Cytol.*, 2003, vol. 227, pp. 131–182.
- Harper, J.D.I. and John, P.C.L., Coordination of Division Events in the *Chlamydomonas* Cell Cycle, *Protoplasma*, 1986, vol. 131, pp. 118–130.
- Heath, M.R. and Spencer, C.P., A Model of the Cell Cycle and Cell Division Phasing in a Marine Diatom, *J. Gen. Microbiol.*, 1985, vol. 131, pp. 411–425.

- Hoops, H.J., Long, J.J., and Hile, E.S., Flagellar Apparatus Structure Is Similar but not Identical in *Volvox steinii*, *Eudorina elegans*, and *Pleodorina illinoisensis* (Chlorophyta): Implications for the "Volvocine Evolutionary Lineage", *J. Phycol.*, 1994, vol. 30, pp. 679–689.
- Ivanova-Kazas, O.M., *Sravnitel'naya embriologiya bespozvonochnykh zhivotnykh. Prosteishie i nizshie mnogokletchnye* (Comparative Embryology of Invertebrate Animals. Protoists and Lower Multicellular Animals), Novosibirsk: Nauka, 1975.
- Iyengar, M.O.P., Contributions to Our Knowledge of the Colonial Volvocales of South India, *J. Linn. Soc. London: Bot.*, 1933, vol. 49, pp. 323–373.
- Jeffery, W.R., Evolution of Ascidian Development, *BioScience*, 1997, vol. 47, pp. 417–425.
- Jeffery, W.R., Swalla, B.J., Ewing, N., and Kusakabe, T., Evolution of the Ascidian Anural Larva: Evidence from Embryos and Molecules, *Mol. Biol. Evol.*, 1999, vol. 16, pp. 646–654.
- Karn, R.C., Starr, R.C., and Hudock, G.A., Sexual and Asexual Differentiation in *Volvox obversus* (Shaw) Printz, Strains WD3 and WD7, *Arch. Protistenkd.*, 1974, vol. 116, pp. 142–148.
- Khlebovich, V.V., Adaptations of an Individual and Clone: Mechanisms and Role in Evolution, *Usp. Sovrem. Biol.*, 2002, vol. 122, no. 1, pp. 16–25.
- Kirk, D.L., The Ontogeny and Phylogeny of Cellular Differentiation in *Volvox*, *Trends Genet.*, 1988, vol. 4, pp. 32–36.
- Kirk, D.L., *Volvox: Molecular-Genetic Origins of Multicellularity and Cellular Differentiation*, New York: Cambridge Univ., 1998.
- Kirk, D.L., Germ-Soma Differentiation in *Volvox*, *Devel. Biol.*, 2001, vol. 238, pp. 213–223.
- Kirk, D.L., A Twelve-Step Program for Evolving Multicellularity and a Division of Labor, *BioEssays*, 2005, vol. 27, pp. 299–310.
- Kirk, D.L. and Harper, J.F., Genetic, Biochemical and Molecular Approaches to *Volvox* Development and Evolution, *Int. Rev. Cytol.*, 1986, vol. 99, pp. 217–293.
- Kirk, M.M. and Kirk, D.L., Exploring Germ-Soma Differentiation in *Volvox*, *J. Biosci.*, 2004, vol. 29, pp. 143–152.
- Kobl, I., Kirk, D.L., and Schmitt, R., Quantitative PCR Data Falsify the Chromosomal Endoreduplication Hypothesis for *Volvox carteri* (Volvocales, Chlorophyta), *J. Phycol.*, 1998, vol. 34, pp. 981–988.
- Kochert, G., Developmental Mechanisms in *Volvox* Reproduction, *The Developmental Biology of Reproduction*, Markert, C.L. and Papaconstantinou, J., Eds., New York: Academic, 1975, pp. 55–90.
- Koufopanou, V., The Evolution of Soma in the Volvocales, *Am. Nat.*, 1994, vol. 143, pp. 907–931.
- Landström, U., Løvtrup-Rein, U., and Løvtrup, S., Control of Cell Division and Cell Differentiation by Deoxynucleotides in the Early Embryo of *Xenopus laevis*, *Cell Differ.*, 1975, vol. 4, pp. 313–325.
- Larson, A., Kirk, M.M., and Kirk, D.L., Molecular Phylogeny of the Volvocine Flagellates, *Mol. Biol. Evol.*, 1992, vol. 9, pp. 85–105.
- Liss, M., Kirk, D.L., Beyser, K., and Fabry, S., Intron Sequences Provide a Tool for High-Resolution Phylogenetic Analysis of Volvocine Algae, *Curr. Genet.*, 1997, vol. 31, pp. 214–227.
- Luntz, A.M., On Periodicity of Divisions in *Eudorina elegans* Ehrbg, *Zh. Obshch. Biol.*, 1968, vol. 29, no. 2, pp. 250–251.
- Mayr, E., *Populations, Species, and Evolution*, Cambridge (Massachusetts): Harvard Univ., 1970.
- McCracken, M.D. and Starr, R.C., Induction and Development of Reproductive Cells in the K-32 Strains of *Volvox rousseletii*, *Arch. Protistenkd.*, 1970, vol. 112, pp. 262–282.
- McMillan, W.O., Raff, R.A., and Palumbi, S.R., Population Genetic Consequences of Developmental Evolution in Sea Urchins (Genus *Heliocidaris*), *Evolution*, 1992, vol. 46, pp. 1299–1312.
- Nedelcu, A.M. and Michod, R.E., Evolvability, Modularity, and Individuality during the Transition to Multicellularity in Volvocalean Green Alga, *Modularity in Development and Evolution*, Schlosser, G. and Wagner, G., Eds., Chicago: Chicago Univ., 2004, pp. 466–489.
- Niikura, K., Fujiwara, A., and Yasumasu, I., Effect of Aminopterin and Deoxyribonucleosides on the Cleavage and Embryogenesis of the Sea Urchin, *Hemicentrotus pulcherrimus*, *Devel. Growth Differ.*, 1984, vol. 26, pp. 451–463.
- Nozaki, H., Morphology and Evolution of Sexual Reproduction in the Volvocaceae (Chlorophyta), *J. Plant Res.*, 1996, vol. 109, pp. 353–361.
- Nozaki, H., Origin and Evolution of the Genera *Pleodorina* and *Volvox* (Volvocales), *Biologia (Bratislava)*, 2003, vol. 58, pp. 425–431.
- Nozaki, H. and Itoh, M., Phylogenetic Relationships within the Colonial Volvocales (Chlorophyta) Inferred from Cladistic Analysis Based on Morphological Data, *J. Phycol.*, 1994, vol. 30, pp. 353–365.
- Nozaki, H. and Kuroiwa, T., Ultrastructure of the Extracellular Matrix and Taxonomy of *Eudorina*, *Pleodorina* and *Yamagishiella* Gen. Nov. (Volvocaceae, Chlorophyta), *Phycologia*, 1992, vol. 31, pp. 529–541.
- Nozaki, H., Misawa, K., Kajita, T., et al., Origin and Evolution of the Colonial Volvocales (Chlorophyceae) as Inferred from Multiple, Chloroplast Gene Sequences, *Mol. Phylogenet. Evol.*, 2000, vol. 17, pp. 256–268.
- Nozaki, H., Takahara, M., Nakazawa, A., et al., Evolution of rbcL Group IA Introns and Intron Open Reading Frames within the Colonial Volvocales (Chlorophyceae), *Mol. Phylogenet. Evol.*, 2002, vol. 23, pp. 326–338.
- del Pino, E.M. and Elinson, R.P., The Organizer in Amphibians with Large Eggs: Problems and Perspectives, *The Vertebrate Organizer*, Grunz, H., Ed., Berlin: Springer, 2003, pp. 359–374.
- Pocock, M.A., *Volvox* in South Africa, *Ann. South African Mus.*, 1933, vol. 16, pp. 523–646.
- Powers, J.H., Further Studies in *Volvox*, with Descriptions of Three New Species, *Trans. Amer. Microsc. Soc.*, 1908, vol. 28, pp. 141–175.
- Raff, E.C., Popodi, E.M., Kauffman, J.S., et al., Regulatory Punctuated Equilibrium and Convergence in the Evolution of Developmental Pathways in Direct-Developing Sea Urchins, *Evol. Devel.*, 2003, vol. 5, pp. 478–493.
- Raff, R.A., Constraint, Flexibility, and Phylogenetic History in the Evolution of Direct Development in Sea Urchins, *Devel. Biol.*, 1987, vol. 119, pp. 6–19.

- Raff, R.A., Larval Homologies and Radical Evolutionary Changes in Early Development, *Novartis Found. Symp.*, 1999, vol. 222, pp. 110–124.
- Raff, R. and Kaufman, T., *Embryony, geny i evolyutsiya* (Embryos, Genes, and Evolution), Moscow: Mir, 1986.
- Raff, R.A. and Love, A.C., Kowalevsky, Comparative Evolutionary Embryology, and the Intellectual Lineage of Evo-Devo, *J. Exp. Zool.*, 2004, vol. 302, pp. 19–34.
- Schmitt, R., Fabry, S., and Kirk, D.L., In Search of Molecular Origins of Cellular Differentiation in *Volvox* and Its Relatives, *Int. Rev. Cytol.*, 1992, vol. 139, pp. 189–265.
- Skripchinsky, V.V., *Fotoperiodizm—ego proiskhozhdenie i evolyutsiya* (Photoperiodism: Its Origin and Evolution), Leningrad: Nauka, 1975.
- Sleigh, M.A., *Protozoa and Other Protists*, London: Edward Arnold, 1989.
- Smith, G.M., A Comparative Study of the Species of *Volvox*, *Trans. Amer. Microsc. Soc.*, 1944, vol. 63, pp. 265–310.
- Spudich, J.L. and Sager, R., Regulation of the *Chlamydomonas* Cell Cycle by Light and Dark, *J. Cell Biol.*, 1980, vol. 85, pp. 136–145.
- Starr, R.C., Control of Differentiation in *Volvox*, *Devel. Biol.*, 1970a, Suppl. 4, pp. 59–100.
- Starr, R.C., *Volvox pocockiae*, a New Species with Dwarf Males, *J. Phycol.*, 1970b, vol. 6, pp. 234–239.
- Tikhomirova, A.L., *Perestroika ontogeneza kak mekhanizm evolyutsii nasekomykh* (Reorganization of Ontogenesis as a Mechanism of Evolution in Insects), Moscow: Nauka, 1991.
- Tucker, R.G. and Darden, W.H., Nucleic Acid Synthesis during the Vegetative Life Cycle of *Volvox aureus* M5, *Arch. Microbiol.*, 1972, vol. 84, pp. 87–94.
- Vande Berg, W.J. and Starr, R.C., Structure, Reproduction and Differentiation in *Volvox gigas* and *Volvox powersii*, *Arch. Protistenkd.*, 1971, vol. 113, pp. 195–219.
- Weinheimer, T., Cellular Development in the Green Alga *Volvox carteri*, *Cytobios*, 1983, vol. 36, pp. 161–174.
- Wilkins, A.S., *The Evolution of Developmental Pathways*, Sunderland (Massachusetts): Sinauer, 2001.