



Differentiation of reproductive structures and experimental sex change in *Volvox* (Chlorophyta, Volvocaceae)

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ABSTRACT

This paper represents a brief review on current topics in both asexual and sexual reproduction of *Volvox* L., a green colonial freshwater alga, which is a valuable model of contemporary developmental biology of plants. In the case of asexual life cycle, the main attention is paid to published data on the mechanisms of differentiation into reproductive and somatic cells of *Volvox carteri* f. *nagariensis* Iyengar (the most thoroughly studied species and forma of *Volvox*) as well as to author's data showing considerable ontogenetic diversity and different types (programs) of asexual development and reproduction processes within the genus *Volvox*. Published data on sexual reproduction and experimental sex change in *Volvox* are considered with respect to the abovementioned problem of ontogenetic diversity.

Keywords : cell differentiation, gene *MID*, green algae, reproduction, sex transformation, *Volvox*.

Colonial volvocine algae, including more than 60 species, have been recently studied with respect to taxonomy (Coleman 2012, Nozaki *et al.* 2014, 2015), evolution (Herron *et al.* 2009, 2010, Umen 2011, Herron and Nedelcu 2015, Desnitskiy 2016, Olson and Nedelcu 2016, Yamashita *et al.* 2016), cell differentiation (Shelton *et al.* 2012, Hanschen *et al.* 2014, Nozaki 2014, Matt and Umen 2016), morphogenesis (Höhn and Hallmann 2011, 2016) and genomics (Ferris *et al.* 2010, Prochnik *et al.* 2010, Umen and Olson 2012). The main attention is paid to the series *Gonium* – *Pandorina* – *Eudorina* – *Pleodorina* – *Volvox*, showing the complication of colonial organization and development including the processes of sexual reproduction (Table 1), and to the genus *Volvox* L., comprising 22 species, which are characterized by differentiation into several hundred or thousand small somatic cells and a few large reproductive cells. Despite a relatively simple organization,

during last 50 years the eukaryotic microorganism *Volvox* was a very popular model for studying a number of interesting phenomena and processes: growth and subsequent series of divisions in the asexual reproductive cells (gonidia), which proceeds differently in various species, formation of cell lineages, asymmetric divisions (in several, but not all *Volvox* species), morphogenesis, control of cell differentiation by sex pheromones, etc. (for reviews see Starr 1970, Kirk 2001, Desnitskiy 2008). The studies were mostly carried out on *Volvox carteri* Stein and, in rare cases, on other species.

The data on sequencing the nuclear genomes of two volvocine species, unicell *Chlamydomonas reinhardtii* Dangeard (Merchant *et al.* 2007) (one of the most intensely studied algae) and colonial *Volvox carteri* f. *nagariensis* Iyengar (Prochnik *et al.* 2010), showed that they have similar number of genes (about 14500). Evolutionary transition to the *Volvox* level

Table 1 – Characteristics of selected representatives of colonial volvocine algae

Species	Colony morphology and cell number	Cell types in asexual colony	Sexual reproduction	References
<i>Gonium pectorale</i> Müller	Flat or slightly curved plate, 8 or 16 cells	All cells reproductive	Isogamous	Iida <i>et al.</i> 2013
<i>Pandorina morum</i> (Müller) Bory	Spheroid, 8 or 16 cells	All cells reproductive	Isogamous	Ettl 1983
<i>Eudorina elegans</i> Ehrenberg	Spheroid, 16 or 32 cells	All cells reproductive	Anisogamous, with antheridial (sperm) packets	Ettl 1983
<i>Pleodorina starrii</i> Nozaki, Ott et Coleman	Spheroid, 32 or 64 cells	Reproductive cells: 62.5-75%; somatic cells: 25-37.5%	Anisogamous, with antheridial packets	Nozaki <i>et al.</i> 2006b
<i>Volvox carteri</i> f. <i>nagariensis</i> Iyengar	Spheroid, 2000-4000 cells	Reproductive cells: <1%; somatic cells: •99%	Oogamous, with antheridial packets	Kirk 1998

of organization was produced by relatively small genetic changes (Pennisi 2010). This fact, however, cannot explain the well known data on the ontogenetic diversity (Smith 1944, Starr 1970, Desnitskiy 2009, 2014, Herron *et al.* 2010) within the genus *Volvox*, which is polyphyletic (Kirk 1998, Herron *et al.* 2009, 2010, Nozaki *et al.* 2014). Firstly, this short review briefly considers recent data on the features and mechanisms of asexual reproduction of *Volvox*. Secondly, recent exiting data on experimental sex change in *Volvox* (mainly in *V. carteri* f. *nagariensis*) (Geng *et al.* 2014) are discussed with respect to the abovementioned problem of ontogenetic diversity.

BASIC DATA ON ASEQUAL REPRODUCTION IN VOLVOX—Asexual cycle of development in *Volvox* includes growth of gonidia, period of their cleavage (a series of 9-15 synchronous divisions in various species), inversion (turning inside out) of young colonies, their growth inside the parental organism, liberation from the parent, etc. Two main types of *Volvox* asexual ontogeny and reproduction were traditionally distinguished (Starr 1970, Desnitskiy 2009). In the one (e.g., *V. africanus* West, *V. carteri*, *V. gigas* Pocock), gonidia reach very large size as result of long growth before the beginning of divisions and exceed somatic cells in diameter at least in six or eight times; cellular growth is absent during the period of consecutive divisions of cleavage. In the other type, (e.g., *V. aureus* Ehrenberg, *V. globator* L., *V. rousseletii* West), mature gonidia are relatively small and exceed somatic cells in diameter no more than in three or four times, since the period of gonidial growth is rather short and embryonic cells grow during intervals between cleavage divisions. It should be remarked, though, that in this case the cell growth between consecutive divisions does not compensate fully a two fold reduction of cell volume during each division.

Let us also remember (Karn *et al.* 1974, Kirk 1998, Desnitskiy 2016) that *V. africanus*, *V. carteri*, *V. obversus* (Shaw) Printz, and *V. reticuliferus* Nozaki are characterized by asymmetric divisions and differentiation of the lineages of presumptive reproductive and somatic cells at relatively early developmental stages, during transition from 16-32-celled stages to 32-64-celled stages or at a bit later stages of cleavage. In other *Volvox* species, no asymmetric divisions occur and gonidia become morphologically distinct from somatic cells only after the end of cleavage or even after inversion of the young colony.

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* can be distinguished (Desnitski 1995, Desnitskiy 2006, 2016, Herron *et al.* 2010):

- The first developmental program is characteristic of *V. gigas*, *V. pocockiae* Starr, *V. powersii* (Shaw) Printz, and

V. spermatosphaera Powers. These species have large gonidia, the rate of their division is high, and there is no unequal (asymmetric) division into two cell types.

- The second program is characteristic of *V. africanus*, *V. carteri*, *V. obversus*, and *V. reticuliferus* 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 of *V. tertius* Meyer, in which gonidia are large, but the rate of division is low, and unequal division is absent.
- The fourth program is characteristic of *V. aureus*, *V. globator*, *V. rousseletii*, *V. ferrisii* Isaka, Matzuzaki et Nozaki and several other species, in which small gonidia divide slowly and without differentiation of cell lineages.

I will now proceed to molecular-genetic mechanisms of the differentiation of gonidial and somatic cells in *V. carteri* f. *nagariensis*, the only thoroughly studied representative of the genus in this respect (Kirk 2001, Pappas and Miller 2009, Hanschen *et al.* 2014, Matt and Umen 2016). When an embryo of this species and forma of *Volvox* reaches the 32-celled stage of cleavage (after five symmetric divisions), 16 anterior cells divide asymmetrically into large and small cells, presumptive gonidial and somatic cell lineages respectively. By contrast, 16 cells in posterior part of the embryo undergo symmetric divisions and contribute only to the lineage of small somatic cells of the next generation. The asymmetric divisions in anterior cells occur under the control of *glsA* gene, the products of which are associated with the mitotic spindle and shift of cell-division planes (Pappas and Miller 2009). Interestingly, there are a few references revealing that the gene *glsA* may be also involved in male gametogenesis (e.g., Mori *et al.* 2003, Igawa *et al.* 2009) or other morphogenetic processes (Guzman-Lopez *et al.* 2016) in higher plants.

During development of *V. carteri* f. *nagariensis* embryo after the asymmetric cleavage division, *lag* gene products act in the large cells to repress somatic genes, while in the small cells *regA* gene product acts to repress gonidial genes and prevent chloroplast biogenesis (Kirk 2001). Therefore, the large and small cells differentiate into large asexual reproductive cells (gonidia) and small somatic cells respectively. The *regA* gene, which is crucial for somatic differentiation in *V. carteri* f. *nagariensis* (the second program of *Volvox* asexual development), has recently been identified in several other members of the genus, including even *V. ferrisii* (Hanschen *et al.* 2014), a species with relatively small size of mature gonidia and without the asymmetric division (the fourth program of development). On the other hand, the *lag* genes (unlike the *glsA* and *regA* genes) have not been

cloned so far and “their function in germ-soma differentiation awaits further study” (Matt and Umen 2016, p. 110).

A review on the recent data concerning the process of inversion of young *Volvox* colony (e.g., Höhn and Hallmann 2011, Haas and Goldstein 2015), which occurs immediately after completing of the embryonic cleavage period, is not within the scope of this paper.

SEXUAL REPRODUCTION AND EXPERIMENTAL SEX CHANGE IN VOLVOX—Gametogenesis is induced under conditions of nitrogen deficiency and this process is associated with gene *MID* expression in *C. reinhardtii* (Ferris & Goodenough 1997, Goodenough *et al.* 2007), *Gonium pectorale* Müller (Hamaji *et al.* 2008, 2013, 2016), and *Pleodorina starrii* Nozaki, Ott et Coleman (Nozaki *et al.* 2006a, Nozaki 2008). However, in *V. carteri* f. *nagariensis* and several other members of the genus *Volvox*, species-specific glycoprotein pheromones have been shown to induce sexual reproduction (Kirk 1998, Hallmann 2011, Coleman 2012). Under the influence of the inducer (produced by sexual males or by somatic cells of asexual colony of *V. carteri* f. *nagariensis* after such stress factors as heat shock or mechanical wounding) the gonidia undergo modified patterns of cleavage and form egg-bearing colonies in the female strain or colonies with androgonidia (which, in turn, form sperm packets) in the male strain (see Table 2 for details of development and specific features of different types of colonies).

The regulatory gene *MID* has been recently identified in heterothallic *V. carteri* f. *nagariensis* (Ferris *et al.* 2010, Geng *et al.* 2014). This *VcMID* gene acquired new functions (associated with the evolution of spermatogenesis and oogenesis) compared to a related gene in *C. reinhardtii*. The *VcMID* gene is present only in the genome of male clone and the knockdown of *VcMID* leads to the formation of *Volvox* colonies with fertilizable eggs in the next generation (Geng *et*

al. 2014). Interestingly, partial *VcMID* knockdown generates self-fertile hermaphrodites. By contrast, expression of this gene after its introduction into the female *V. carteri* f. *nagariensis* clone results in the formation of colonies with sperm packets. However, after such transformations of germ cells (eggs or androgonidia) their number and distribution pattern in colonies remain unchanged. A pseudo-female colony is characterized by the 1:1 ratio of evenly scattered eggs and somatic cells. A pseudo-male colony contains approximately 35 androgonidia, which are located in posterior and equatorial regions of the colony. Therefore, it is clear that sexual reproductive cell patterning (i.e., the number and distribution of sexual reproductive cells and ratio of sexual reproductive cells to somatic cells) is separable from the events of differentiation in two kinds of sexual reproductive cells and that the patterning (unlike the differentiation of sexual cells) is not controlled by the *VcMid* pathway. From my point of view, these results of Geng *et al.* (2014) show that the well known concept of “positional information” in the sense of Wolpert (1969, 2011) may be used to comment the morphogenesis of reproductive structures in this species and forma of *Volvox*. It is reasonable to think that two sex-specific cleavage patterns of normal development were not the subjects of change during these experiments on sex transformation. Nonetheless, it would be of interest to carry out a special analysis of this point.

In the light of heterothallic *V. carteri* f. *nagariensis* data, Geng *et al.* (2014) suggested that in homothallic *Volvox* species, the *MID* expression is insufficient for the development of male reproductive structures in all colonies. Then relatively early or late expression of this gene during development would produce dioecious or monoecious colonies respectively. It should be also noted that expression of the isogamous unicell *C. reinhardtii* *MID* gene introduced

Table 2— Specific features of asexual, female, and male colonies of *Volvox carteri* f. *nagariensis*. After Starr (1970) and Kirk (1998)

Types of colonies	Details of development of the colonies of a given type	Morphology of the adult colonies
Asexual colonies, which are identical in female and male strains	A series of 11-12 synchronous embryonic divisions; asymmetric (differentiating) divisions occur in 16 anterior cells when 32-celled stage divides (the sixth cleavage).	2000-4000 somatic cells and (under optimal conditions) 16 gonidia, which are located in the posterior and equatorial regions of the colony.
Female colonies	A series of 11-12 synchronous embryonic divisions; asymmetric divisions occur in 35-45 cells when 64-celled stage divides (the seventh cleavage).	2000-4000 somatic cells and 35-45 eggs, which are located in the posterior and equatorial regions of the colony.
Male colonies (“dwarf males”)	A series of nine synchronous embryonic divisions; the final division is asymmetric in all cells of the embryo.	A dwarfish colony with 1:1 ratio of two cell types: 256 somatic cells and 256 androgonidia under optimal conditions; the androgonidia are evenly scattered throughout the colony.

into the female clone of *V. carteri* f. *nagariensis* fails to transform gender, while the *MID* gene of colonial, but isogamous *G. pectorale* is nevertheless capable of inducing *Volvox* spermatogenesis (see Geng *et al.* 2014, Herron 2016).

In concluding, it is appropriate to remark that homothallic and dioecious *V. aureus*, which is the only almost cosmopolitan representative of the genus (Desnitskiy 2016), does not form special females with eggs; the asexual gonidial cells act as facultative eggs in the presence of male colonies with sperm packets (Starr 1970). Moreover, the induction of male colony differentiation in *V. aureus* is not associated with the modification of embryonic cleavage pattern (unlike *V. carteri* f. *nagariensis*). Finally, let me also remind about the discovery of several parthenosporic strains of *V. aureus*, in which male colonies appear extremely rarely or even completely absent (Starr 1970, Starr and Zeikus 1993, Desnitskiy 2000). It would be interesting to carry out an attempt to analyze the ectopic *VcMID* gene expression in various strains of this species.

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