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BRIEF COMMUNICATIONS

Dormant Stages of the Green Flagellate *Volvox* in a Natural Habitat

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Abstract—Reproduction and formation of resistant dormant spores in *Volvox aureus* (Ehr.) and *V. tertius* (Meyer) were studied in a biocoenosis of a transient pool in July–August 1996 and 1997. Under these conditions, the populations of two *Volvox* species had species-specific reproductive features. In the *V. tertius* population, a relatively small amount of male individuals and dormant spores (zygospores) appeared sporadically. On the contrary, in the *V. aureus* population, dormant parthenospores were formed, but male individuals were never observed.

Key words: natural populations, development, reproduction, *Volvox*.

The green flagellate *Volvox* is a valuable model object for developmental biology studies (for review see Starr, 1970; Kochert, 1975; Desnitski, 1980). In addition, the experimental data on *Volvox* development are used for analysis of the development of multicellularity and of the evolution of ontogenesis (Desnitski, 1995; Kirk, 1998). During the recent decades, almost all studies on *Volvox* were carried out under laboratory conditions of clonal cultivation. In this respect, a question arises as to what extent the phenomena observed in the laboratory correspond to the natural processes. We have already shown that the diurnal rhythm of divisions of the asexual reproductive cells (gonidia) of *V. tertius* in culture (Desnitski, 1985a, 1995) is similar to that in the natural population of the same species (Desnitski, 1985b).

Analysis of sex differentiation is another very interesting aspect of studying *Volvox* development. In cultures of some species, such as *V. aureus* or *V. carteri*, the development of sexual individuals is induced by highly specific glycoprotein pheromones, while in other species (*V. globator*, *V. spermatosphaera*, and *V. tertius*), no sex pheromones were found under the same conditions (Starr, 1970; Kochert, 1975; Desnitski, 1980). As concerns the development of *V. aureus* and *V. tertius* studied by us in the nature in this work, it has some features already described in literature. First, no special female colonies are formed in the cultures of these two species, and, in the presence of male individuals with spermatozoa, asexual colonies with nondivided gonidia act as facultative female individuals with eggs (Darden, 1966; Starr, 1970; Kochert, 1975). Second, two lines of *V. aureus* with different reproductive strategies are known. In the first of them, male individuals were never observed (Starr, 1968), and, in the second, male colonies formed very rarely (Darden, 1968). With the "aging" of the colonies of both lines, gonidia were enlarged and transformed, without fertilization, into orange haploid parthenospores, indiscernible from the mature zygotes (zygospores) by their external appearance.

Here, the development and reproduction of *V. aureus* and *V. tertius* were analyzed in nature on the example of their population that coexisted in the biocoenosis of a transient rain pool.

MATERIALS AND METHODS

The transient rain pool we studied was located at the forest edge about 60 km to the south of St. Petersburg. In the beginning of observations, the maximum pool diameter was ca. 2 m, and the maximum depth, 10–12 cm. In 1996, six samples were taken with two to nine day intervals from July 12 until August 12 and soon after the pool completely dried. In 1997, samples were taken on July 14 and 20 and on August 5. Note that, by the morning of July 25, the pool dried, but, from July 26–28, it was raining and the pool was again filled with water. Samples were taken during the light hours, between 2 and 9 p.m. The sample volume varied from 50 to 300 ml, with respect to the number of *Volvox* colonies. When samples were taken, water temperature was measured. The samples were fixed by 4–5% formaldehyde and analyzed under a microscope in the laboratory. The *Volvox* species were identified after Smith (1944). In each sample, the number of conspecific colonies were counted, its population density (number of colonies per 1 liter) was determined, and the ratios of life cycle phases and of the number of colonies of *V. aureus* to *V. tertius* were calculated. In 1996, 459 individuals of *V. aureus* and 91 individuals of *V. tertius* were examined, and in 1997, 140 and 792 individuals, respectively. For studying the popula-

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Development of *Volvox* (summer 1996 and 1997)

Date and water temperature, °C	Number of colonies of different species in a water sample	Population density, number of colonies per 1 liter	Colonies at different developmental stages, % of the total number of colonies in a sample			
			1	2	3	4
July 12, 1996	*V. aureus* – 168	1680	10.7	3.0	5.3	81.0
22.3	*V. tertius* – 3	30	0	0	100	0
July 20, 1996	*V. aureus* – 58	193	48.3	31.0	13.8	6.9
13.2	*V. tertius* – 6	20	83.3	0	16.7	0
July 29, 1996	*V. aureus* – 21	105	4.8	47.6	19.0	28.6
18.5	*V. tertius* – 2	10	50.0	0	50.0	0
Aug. 6, 1996	*V. aureus* – 56	280	26.8	51.8	21.4	0
18.0	*V. tertius* – 18	90	38.9	22.2	38.9	0
Aug. 10, 1996	*V. aureus* – 81	405	44.4	39.5	14.8	1.2
21.6	*V. tertius* – 31	155	32.3	41.9	19.4	6.5
Aug. 12, 1996	*V. aureus* – 75	375	38.7	57.3	4.0	0
21.9	*V. tertius* – 31	155	38.7	22.6	35.5	3.2
July 14, 1997	*V. aureus* – 16	160	31.3	43.7	25.0	0
19.0	*V. tertius* – 340	3400	51.5	26.5	11.2	10.8
July 20, 1997	*V. aureus* – 33	660	33.3	51.5	12.1	3.0
21.3	*V. tertius* – 426	8520	38.0	13.9	14.8	33.3
Aug. 5, 1997	*V. aureus* – 91	455	12.1	69.2	18.7	0
21.4	*V. tertius* – 26	130	23.1	15.4	57.7	3.8

tion structure, the life cycle was conditionally divided into four stages: stage 1—young asexual colonies with unicellular (nondividing) gonidia, stage 2—asexual colonies with gonidia at the phase of divisions or stage of inversion (excurvation), stage 3—old asexual colonies with young individuals of the next generation between inversion and release from the parental organism, and stage 4—old colonies with resistant dormant spores (zygospores or parthenospores). These spores were enlarged orange (dark-green at the early stages) reproductive cells with smooth walls consisting of three concentric layers.

In summer 1996, clonal laboratory cultivation of *V. aureus* taken from the same pool began. The flagellates were cultivated on soil water under the conditions described elsewhere (Desnitski, 1985a) for three years: the cultures were regularly examined under a light microscope and, in some cases the colonies with dormant spores were counted.

RESULTS AND DISCUSSION

The quantitative data for four developmental stages of two *Volvox* species are presented in the table. Note, above all, that from July 12 to August 12, 1996, the ratio of *V. aureus* to *V. tertius* colonies underwent changes: 98.2 : 1.8% versus 70.8 : 29.2%. Note also that the samples taken on July 12, 20, and 29 contained a very small amount of *V. tertius* colonies. Hence, we cannot say that no dormant spores were formed in the *V. tertius* population in July 1996.

In 1997, unlike 1996, no mass formation of resistant dormant spores was observed in *V. aureus*. In addition, in July 1997, unlike 1996, *V. tertius* was the predominant species in the pool, when the ratio of *V. aureus* to *V. tertius* colonies was 4.5 : 95.3% (July 14) and 7.2 : 92.8% (July 20). On the contrary, in the last sample taken on August 5, when the pool dried for a short period of time and was then again filled with water, the ratio was 77.8 : 22.2%. Thus, the ratio of these two *Volvox* species was different in different years and underwent changes during the same summer.

Note that during the two years of observations, free swimming male *Volvox* colonies were never observed. However, in the samples fixed on August 6, 10, and 12, 1996 and in all 1997 samples, not only young asexual individuals of the next generation were found in 6–79% old asexual *V. tertius* colonies (stage 3), but also one or two or, sometimes, three to five young male colonies with characteristic ratio of androgonidia and somatic cells (1 : 5). The absence of free swimming male colonies of *V. tertius* may be explained by their degradation immediately after release from parental individuals.

Thus, it can be proposed that the resistant dormant spores of *V. tertius* are zygospores, while the dormant stages of *V. aureus* are parthenospores. The latter suggestion is based on the absence of male individuals in a natural population of *V. aureus* and has been confirmed by analysis of clonal cultures. During three years of cultivation of *V. aureus* taken from the same pool, no male colonies were ever seen. In "old" cultures (1.5–3 months) after replating, 10 to 80% of colonies contained parthenospores. The formation of parthenospores was previously reported for two lines of *V. aureus* from North America (Darden, 1968; Starr, 1968) but was not so far described for the European *V. aureus*.

Note that the observations of Müller (1989) on reproduction involving male individuals in natural populations of *V. aureus* from two ponds in Germany agree with the results obtained by Darden (1966) on line M5 of *V. aureus* originating from North America. In the culture of M5 line, single male colonies spontaneously appear from time to time, which produce a sex pheromone. The latter, in turn, induces mass formation of male individuals in the next generation. However, as was already noted, no sex pheromones were found in the cultures of *V. tertius* and some other *Volvox* species (Starr, 1970; Kochert, 1975).

The results described in the present study do not imply that sexual reproduction of *V. tertius* in a natural habitat is controlled by inducing substances. The appearance of few male colonies in the natural population was not followed by mass development of male individuals in the next generations. The major part of the population was represented by asexual individuals. Thus, the results of the present study and the published data (Müller, 1989) suggest that *V. aureus* and *V. tertius* have different reproductive features not only in culture, as was already shown, but also in natural biocoenoses.

On the whole, it can be believed that the processes of development and reproduction of different *Volvox* species in natural habitats are similar to those during clonal cultivation in the laboratory.

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