Development and reproduction of two species of the genus *Volvox* in a shallow temporary pool

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Summary

The reproduction and the formation of resistant dormant spores in *Volvox aureus* Ehr. and *V. tertius* Meyer were studied in a temporary pool during July–August 1996 and 1997. It has been shown that the populations of two species of *Volvox* (L.) Ehr. from this pool were characterized by different reproductive traits. Comparatively small quantity of male individuals and dormant zygotes (zygospores) episodically formed in the population of *V. tertius*. By contrast, in the population of *V. aureus* the formation of dormant parthenospores occurred and the male individuals were never observed. The ratio *V. aureus* : *V. tertius* was different in different years and underwent changes during one and the same summer.

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

Introduction

During the first half of XX century, all studies of both sexual and asexual reproduction of *Volvox* were carried out on the material taken from natural populations (Powers, 1908; Iyengar, 1933; Pocock, 1933; Smith, 1944, and others). In contrast, during the second half of the century, almost all investigations of *Volvox* ontogeny were fulfilled under laboratory culture conditions (Darden, 1966, 1968; Starr, 1968, 1970; Kochert, 1975; Desnitski, 1995; Kirk, 1998, and others).

During recent 30 years, *Volvox* was also a subject (sometimes along with other algae or protists) of some ecological research in its natural habitat: lakes, reservoirs and ponds (Moss, 1973; Ganf et al., 1983; Pentecost, 1983; Reynolds, 1983; Sommer and Gliwicz, 1986; Mueller, 1989, and others). It should be noted that, as a rule, the ecological works (with the exception of Mueller's (1989) study) did not analyse and record separate phases of *Volvox* life cycle.

Many species of *Volvox* may be also found, sometimes in great abundance, in temporary pools of rain water. It is appropriate to emphasize that the transition from asexual to sexual reproduction seems to be more frequent and important event in the small temporary pools than in big lakes and reservoirs, in which *Volvox* is able to reproduce asexually for comparatively long time (sometimes even during several months – Ganf et al., 1983; Sommer, Gliwicz, 1986). Life in the small pools is more connected with variations of temperature and more dependent on occurrence of rains. Besides, in shallow pools *Volvox* and other phytoflagellates are unable to accomplish long range vertical migrations as they do in deep lakes and reservoirs (Sommer and Gliwicz, 1986; Sommer, 1988).

More than 90 years ago, Powers (1908, p. 141) wrote that "in the full blaze of Nebraska sunlight, *Volvox* is able to appear, multiply and riot in sexual reproduction in pools of rainwater of scarcely a fortnight's duration". To our knowledge, however, during several recent decades nobody studied *Volvox* development and reproduction in this kind of natural habitat.

It is well-known that in a temporary pool the populations of 2 or even 3 species of *Volvox* may coexist (Powers, 1908; Iyengar, 1933). We have chosen for our analysis a small pool containing simultaneously *V. aureus* and *V. tertius*. Previously we had carried out a detailed study of the control of asexual reproduction in these 2 species under laboratory culture conditions (Desnitski, 1995). Data on sexual reproduction in the cultures of *V. aureus* and *V. tertius* are known from the literature and it is appropriate to recall some important information. First, *V. aureus* and *V. tertius* are homothallic species and both do not form special female coenobia; in the presence of sperm-bearing males, the asexual coenobia with undivided gonidia act as facultative females with eggs (Darden, 1966; Starr, 1970; Kochert, 1975). Secondly, two American strains of *V. aureus* with quite different reproductive strategy are also known. Males were not observed in 65–98 strain (Starr, 1968), while DS strain (Darden, 1968) formed males extremely rarely. As cultures of both strains aged, gonidia

enlarged and, without dividing or fertilization, transformed into orange-coloured parthenospores. The parthenospores had the same appearance as mature zygotes (zygospores) and it was assumed that they were haploid (Starr, 1970).

The present work deals with some aspects of development and reproduction in natural populations of *V. aureus* and *V. tertius* in 1996 and 1997. It was of interest to compare the life of these two species under the same natural conditions.

Material and Methods

The temporary pool located in the path at the edge of the wood (approximately 60 km south of St. Petersburg, Russia) was chosen for investigation. The maximal diameter of the pool in the beginning of the study was approximately 2 m and the maximal depth was approximately 10–12 cm. Water samples (50–300 ml each, depending on the quantity of *Volvox* coenobia) were collected between 14 and 21 h, in most cases at the intervals of 2–9 days in July and August 1996 and 1997. Choosing the periodicity of sample collections, we have kept in mind the studies on *Volvox* ecology in lakes and reservoirs in which the samples had been collected at the intervals of 6–10 days (Moss, 1973; Ganf et al., 1983). It is also important that the asexual cycle of *Volvox* development lasts for 4–5 days in actively growing cultures (Desnitski, 1995), but is usually longer under natural conditions in Europe (Reynolds, 1983).

The species of *Volvox* were identified using Smith (1944). The measurements of water temperature in the pool at the moments of sample collections were also made. The samples were fixed with formalin (final concentration 4–5%) and then transported to the laboratory for the microscopic analysis of *Volvox* populations.

To study the population structures of *V. aureus* and *V. tertius* in the pool, we have subdivided the life cycle into four stages (phases). Stage 1 – young asexual coenobia with unicellular (undivided) gonidia. Stage 2 – asexual coenobia with dividing or inverting gonidia.

Stage 3 – old asexual coenobia with young individuals of the next generation between inversion and liberation from the parent. Young males together with more abundant young asexual individuals were episodically observed inside some old parental coenobia of *V. tertius* of the stage 3. Therefore, these young males were scored separately (but not as an additional stage for male differentiation). Stage 4 – old coenobia with resistant dormant spores (zygospores or parthenospores). They are enlarged orange-coloured (at first enlarged dark-green) reproductive cells with a smooth wall consisting of 3 concentric layers.

For each sample we have determined the total number of coenobia of a given species, the density of population (coenobial number per 1 liter), the percentage of each of the four stages and the ratio *V. aureus* : *V. tertius*.

In the investigation of 1996, 459 coenobia of *V. aureus* and 91 coenobia of *V. tertius* were used. In 1997, 140 coenobia of *V. aureus* and 792 coenobia of *V. tertius* were studied.

In August 1996, a clonal culture of *V. aureus* from the same pool was started and maintained on soilwater medium containing $CaCO_3$ (Starr and Zeikus, 1993) at 18–24 °C. The illumination was provided by cool white fluorescent tubes (approximately 2000 lux, a light/dark regime of 16 h – 8 h) or by the natural light (the north-west window). The culture was routinely transferred to 10–50 ml of fresh medium (in test-tubes or small flasks) at 2–6 weeks intervals. During 3 years, we repeatedly checked under the light microscope both actively growing culture (1–3 weeks after inoculation) and old culture (up to 2–3 months after inoculation). In some cases, the percentage of *V. aureus* coenobia with resistant dormant spores was calculated.

Results and Discussion

The quantitative data on the four developmental stages of 2 species of *Volvox* during two consecutive years are given in Table 1. First of all, it should be noted that during the period from 12 July to 12 August 1996, the ratio *V. aureus* : *V. tertius* changed from 98.2% : 1.8% to 70.8% : 29.2%. It may be supposed that the drastic decline of the population density of *V. aureus* by 20 July was due to the fact that on 12 July the majority of the population (81%) was represented by coenobia with resistant dormant spores, unable to produce new coenobia at once. Further decline of the population density in both species (after 20 July) may be explained by a period of low temperature. It is appropriate to note that scarce numbers of *V. tertius* coenobia were found in the samples collected on 12, 20 and 29 July (only 3, 6 and 2 individuals respectively). For this reason it is probably wise to refrain from saying that the formation of dormant spores did not occur in the population of *V. tertius* during July 1996.

The increase of the population density in both species of *Volvox* after 29 July 1996 was partly due to intensive asexual reproduction, partly due to the progressive evaporation of water from the pool (there were no rains during this period). By 16 August the pool had dried up completely and it was impossible to take new water samples.

By contrast with the data of the preceding year, in 1997 a mass formation of resistant dormant stages of *V. aureus* was not observed. Besides, in contrast with the summer of 1996, in July 1997 *V. tertius* was predominant species in the pool. The ratio *V. aureus* : *V. tertius* was 4.5% : 95.5% on 14 July and 7.2% : 92.8% on 20 July. It is difficult to explain such great differences between the data obtained for two consecutive years.

Table 1. Changes in the populations of *Volvox* during July-August 1996 and 1997

Date, temperature °C	Volvox species (in parentheses: coenobial number in a fixed water sample)	Population density (coenobia per 1 liter)	Coenobia at various developmental stages (% from the total coenobial number of a given species in a sample)			
			1	2	3	4
12.07.96 22.3	*V. aureus* (168)	1680	10.7	3.0	5.3	81.0
	V. tertius (3)	30	0	0	100.0	0
20.07.96 13.2	*V. aureus* (58)	193	48.3	31.0	13.8	6.9
	V. tertius (6)	20	83.3	0	16.7	0
29.07.96 18.5	*V. aureus* (21)	105	4.8	47.6	19.0	28.6
	V. tertius (2)	10	50.0	0	50.0	0
06.08.96 18.0	*V. aureus* (56)	280	26.8	51.8	21.4	0
	V. tertius (18)	90	38.9	22.2	38.9	0
10.08.96 21.6	*V. aureus* (81)	405	44.4	39.5	14.8	1.2
	V. tertius (31)	155	32.3	41.9	19.4	6.5
12.08.96 21.9	*V. aureus* (75)	375	38.7	57.3	4.0	0
	V. tertius (31)	155	38.7	22.6	35.5	3.2
14.07.97 19.0	*V. aureus* (16)	160	31.3	43.7	25.0	0
	V. tertius (340)	3400	51.5	26.5	11.2	10.8
20.07.97 21.3	*V. aureus* (33)	660	33.3	51.5	12.1	3.0
	V. tertius (426)	8520	38.0	13.9	14.8	33.3
05.08.97 21.4	*V. aureus* (91)	455	12.1	69.2	18.7	0
	V. tertius (26)	130	23.1	15.4	57.7	3.8

By noon on 26 July 1997 the pool had dried up and it was impossible to take a sample. However, there were rains on 26, 27 and 28 July. The next sample was taken on 5 August and significant changes in *Volvox* populations had been revealed. (This sample was the last, because during next few days the pool had dried up again.) The ratio *V. aureus* : *V. tertius* happened to be 77.8% : 22.2%. It seems reasonable to suppose that such changes in the ratio between two *Volvox* species were at least partly due to different ability of asexual coenobia to survive for comparatively short periods of time in the damp mud. In this respect *V. aureus* is probably more resistant than *V. tertius*.

It should be noted that for 2 year observations on the natural populations we never saw free-swimming male coenobia of any species of *Volvox*. However, in the samples fixed on 6, 10 and 12 August 1996 as well as in all samples fixed in the summer of 1997, inside 6–79% of old asexual coenobia (stage 3) of *V. tertius*, there were not only several young asexual individuals of the next generation, but also 1–2 (sometimes 3–5) young male coenobia with characteristic ratio androgonidia : somatic cells approximately 1 : 5.

Thus, the resistant dormant stages of *V. tertius* may be considered zygotes, whereas resistant dormant stages of *V. aureus* seem to be the parthenospores. This assumption was based on the fact that we had never observed male coenobia of *V. aureus*. The idea about the peculiarities of *V. aureus* reproduction in this pool has gained strong support in the study of clonal culture. During 3 years of cultivation of *V. aureus* originating from the same pool, we never observed any male individuals. The actively growing cultures (1–3 weeks after inoculation) usually contained only asexual coenobia with growing or dividing gonidia rather than coenobia with resistant dormant spores. However, in the old cultures, 1.5 and 3 months after inoculation, approximately 10 and 80% of coenobia respectively contained parthenospores (in the absence of male coenobia). The parthenosporic type of reproduction was earlier described for several strains of *V. aureus* originating from North America (Darden, 1968; Starr, 1968; Starr and Zeikus, 1993), but it was not reported for *V. aureus* from Europe.

It is appropriate to note that Mueller's (1989) observations on the reproduction with the participation of male coenobia in natural populations of *V. aureus* from two

ponds in Germany seem to be in accord with Darden's (1966) study of the American M5 strain of *V. aureus*. The male coenobia episodically appeared in this strain and produced sexual pheromone which induced mass formation of further male individuals in the culture. Sexual pheromones have not been found, however, in cultures of *V. tertius* and several other species of *Volvox* (see reviews: Starr, 1970; Kochert, 1975). Present work does not give any reason to suppose that sexual reproduction of *V. tertius* in its natural habitat is under the control of inducing substance. The appearance of first male coenobia in the natural population has not been followed by mass development of male individuals in the next generation. The majority of the population has been represented by asexual coenobia. Thus, our study, along with the published data (Mueller, 1989), shows that *V. aureus* and *V. tertius* have different reproductive traits not only under laboratory culture conditions, as was previously demonstrated, but probably also under natural conditions.

Our study is in accord with some aspects of Pocock's (1933) work who investigated the development of two South African species, *V. capensis* Rich et Pocock and *V. rousseletii* West, in the natural habitat (pools and small ponds) and came to the conclusion about important role of temperature changes and rains. In contrast with Pocock's study, however, we did not observe sudden disappearance of *Volvox* populations from the pools which still contained water.

In concluding, it should be emphasized again that *Volvox* reproduction under natural conditions is still very poorly investigated field. To understand the mechanisms of *Volvox* development and evolution thoroughly, further studies on natural populations of *Volvox* and its closest relatives (certainly, along with using laboratory cultures) are required.

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