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RESEARCH ARTICLE

Changes in valve morphology of two pennate diatom species during long-term culture

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Abstract

The morphology of diatom siliceous is a primary basis for their species identification. This study aims to measure the range of morphological changes induced in the monoclonal cultures of *Fragilaria radians* strains 280 and A6 and *Ulnaria danica* strain BK17 by cultivation in the lab for a year or more. The scanning electron microscopy revealed that the number of abnormal valves increases during the first year of culture maintenance. Specific abnormalities observed include curved valves and apices, axial areas and rimoportulae shifted from their normal positions, disordered or otherwise abnormal striae, and various growths on the valves. Similar morphological abnormalities are known to occur in diatoms exposed to microtubule inhibitors. These results show the limits of morphological variance in studied species and could be used to estimate the effect of toxic agents in natural and experimental conditions.

Keywords

Diatom cultivation, monoclonal culture, pennate diatoms, valve abnormality

Introduction

Diatom algae (Bacillariophyta) are a group of free-living or colonial eukaryotic microalgae. Their most distinctive feature is a siliceous cell; its overall layout and various micro- and nanoscale elements are species-specific. According to the recent works, there is somewhere between 12,000 and 30,000 extant diatom species (Guiry 2012; Mann and Vanormalingen 2013; Malviya et al. 2016; Guiry and Guiry 2020).

Before the advent of molecular genetics, diatom taxonomy was based solely on the valve morphology observed with light or electron microscopy (Schütt 1896; Round and Crawford 1981; Round et al. 1990; Kaczmarska et al. 2013; Mann et al. 2017). The difficulties in species identification are often caused by the phenotypic variance which lets a single species have multiple distinct morphotypes (Cerino et al. 2005; Glemser et al. 2019). On the other hand, there are groups of cryptic species that are morphologically very similar and cannot be reliably distinguished without genetic data (Sorhannus 2007; Alverson 2008; Cox 2014).

Fragilaria radians (Kützing) D.M. Williams & Round (*Synedra acus* subsp. *radians* (Kützing) Scabitchevsky) and *Ulnaria danica* (Kützing) Compère & Bukhtiyarova (*S. ulna* var. *danica* (Kützing) Grunow) are widespread in the freshwater basins of the world (Guiry and Guiry 2020). Monoclonal cultures of these two species isolated from Lake Baikal are relatively easy to culture, which has made them an object of various studies in genomics (Galachyants et al. 2019), molecular biology (Petrova et al. 2007; Marchenkov et al. 2018), and cytology (Annenkov et al. 2013; Shishlyannikov et al. 2014; Kharitonenko et al. 2015). The existence of an axenic culture protocol (Shishlyannikov et al. 2011) and high-volume biomass production technology (Vereshchagin et al. 2008) have also broadened the range of possible studies on these cultures. Our aim was to study the effects of long-term culture on F. radians and U. danica valve morphology and to estimate the abnormal accumulation rates.

Material and methods

This work was performed on *Fragilaria radians* strains A6 and A280, and *Ulnaria danica* strain BK17. All strains were isolated from Lake Baikal according to the previously published protocol (Shishlyannikov et al. 2011), and have been maintained in the lab for more than a year. Cells were grown on the DM medium (Thomson et al 1988) in flasks at 10°C and 16 μmol/m2*sec of light intensity using 12/12 h artificial light/dark cycle. For the present study, clonal cultures grown from one cell were used after several months of cultivation, as soon as their number in a clonal culture became sufficient to clear the valves.

To study the cell morphology, cultures were sedimented on the Mini Spin centrifuge (Eppendorf) for 6 minutes at 3500 g. The cell pellet was washed for 30 minutes at 95°C in 6% sodium dodecyl sulfate solution. After that, the cells were again centrifuged and

the supernatant was removed; this stage was repeated three times. The sediment was washed in distilled water five times and treated with concentrated nitric acid for 1 hour at 95°C. After that, it was thrice washed in ethanol and treated with 36% hydrochloric acid for 24 hours at room temperature. After the final treatment, valves were washed in distilled water at least five times, resuspended in 100 µl 70% ethanol, and placed on SEM stubs. These plates were dried at room temperature and coated with gold.

Microscopy was performed using Quanta 200 scanning electron microscope. All counts were taken on 200 randomly chosen valves.

Results and discussion

Abnormalities were classified based on the data of Kharitonenko et al. (2015) and our results (Fig. 1, Table 1). We registered that after producing the monoclonal culture, 3% of F. radians cells and 15% of U. danica cells had some abnormalities; in both species, the proportion of malformed cells increased during culture maintenance (Figs 2, 3).

During the first year of culture, most valves conformed to the curved shapes (Fig. 2). This anomaly becomes common after a year of culture maintenance. For *F. radians* strain 280, this change was often accompanied by displacement rimoportulae and axial areas, as well as the altered shape of apices (Fig. 1F-I). Unlike *U. danica* strain KB17, misalignment of the rows of areolae (Fig. 1J) was uncommon.

Morphological observations on F. radians strain A6 (which was cultured for more than 7 years) suggest that all cells will eventually become curved after more than a year of culture in a sufficiently old culture (Fig. 3).

Phenotypic anomalies in diatoms are known to be caused by various environmental stresses (Falasco et al. 2009), including anthropogenic pressure (Roubeix et al. 2011; Pandey et al. 2014). It was shown that pennate diatoms are prone to deformation (Cantonati et al. 2014; Pandey et al. 2016). Based on the literature data (despite its scarcity), some species of diatoms seem to be more prone to shell deformations than others. Thus, the single-sutured species *Achnanthidium minutissimum* (Can-

Table 1. Classification of valve abnormalities.

Abnormality type	Description	Kharitonenko et al. 2015	Figure, this study
I	Valve curvature	4b	1F
II	Local displacement of the axial area	4c	1G
III	Altered shape of the valve apex	4e	1H
IV	Displacement of the rimoportula	4f	1I
V	Misalignment of the rows of areolae	4h	1J
VI	Oversized areolae/striae not composed from individual areolae	–	1K
VII	Areolae occlusion	1i	1H

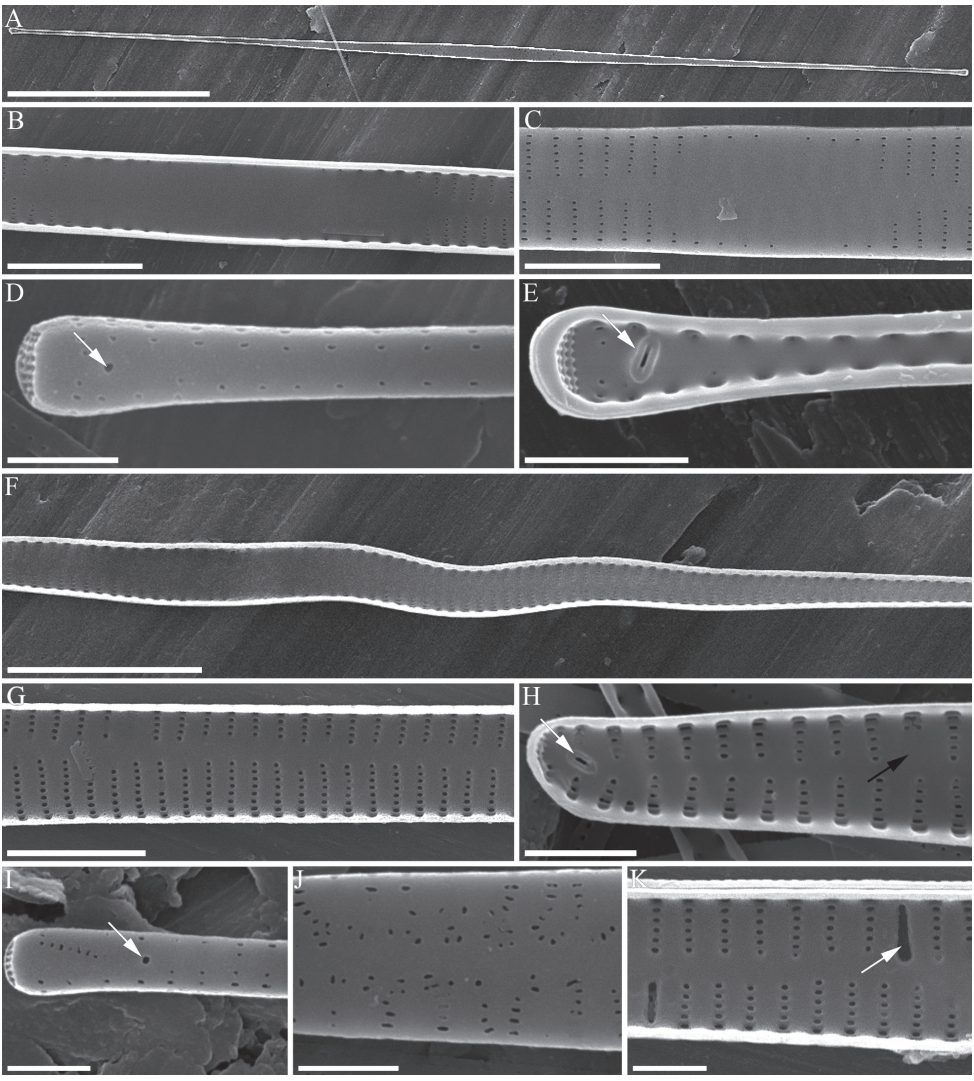

Figure 1. The structure of normal (**A–E**) and abnormal (**D–K**) F. radians valves strain A280 (SEM). **A** – an overall view; **B** – an axial area, inside view; **C** – an axial area, outside view; **D** – valve apex with a rimoportula (arrow), outside view; **E** – valve apex with a rimoportula (arrow), inside view; **F** – a curved valve; **G** – local displacement of the axial area; **H** – an altered shape of the valve apex, rimoportula is marked with a white arrow, non-formed or overgrown stria is marked with a black arrow; **I** – a displacement of the rimoportula; **J** – misalignment of the rows of areolae; **K** – merged areolae within a stria. Scale bars: **A** – 50 μm; **B, C, G** – 5 μm; **D, E, H, I, J** – 2 μm; **F** – 10 μm; **K** – 1 μm.

tonati et al. 2014), as it turned out, is less sensitive to stress conditions, at least this does not affect the structure of valves, unlike other species of pennate diatoms (eg, *Cymbella tumida*, *Gomphonema rosenstockianum*, and *Nitzschia linearis* - Falasco et

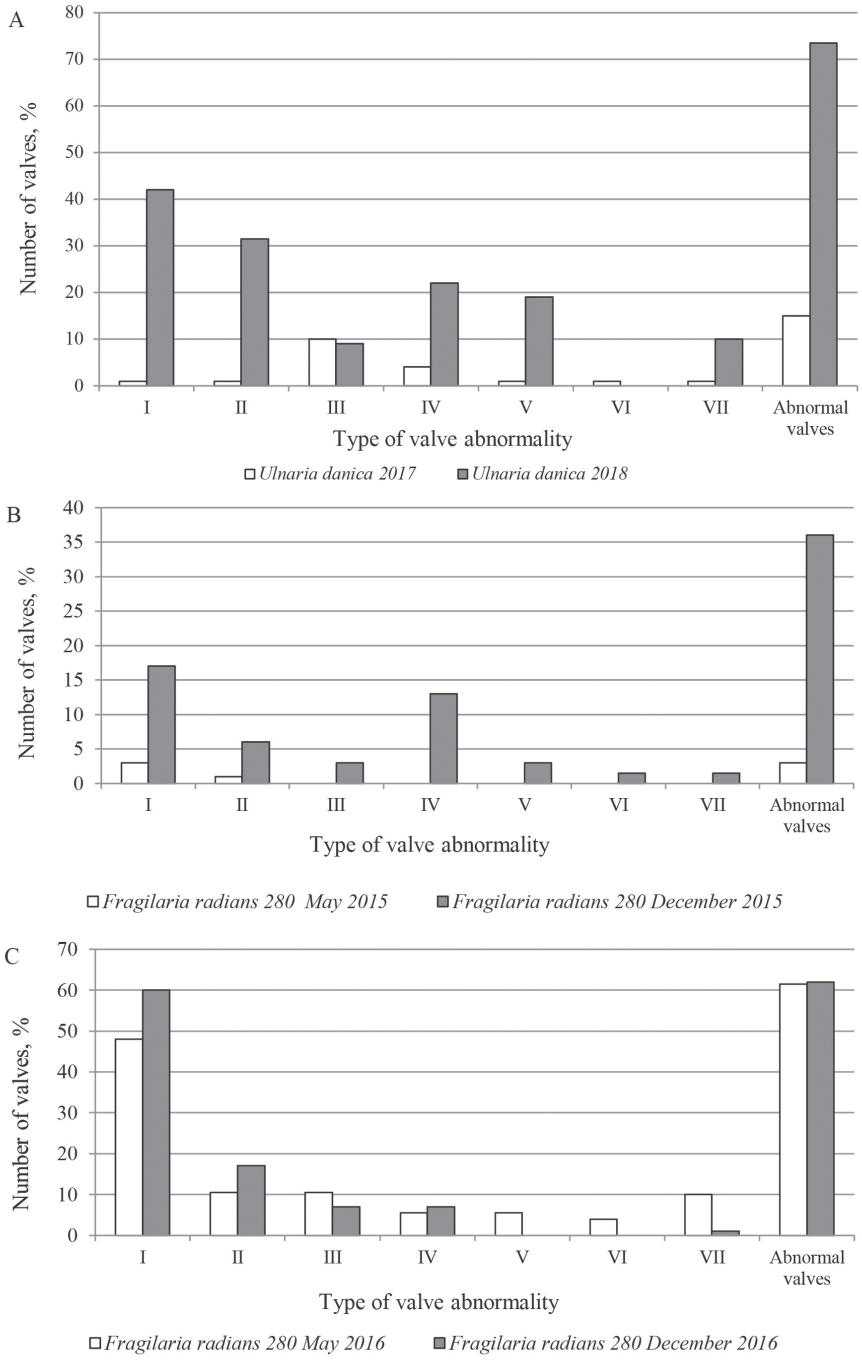

Figure 2. The prevalence of valve abnormalities in *U. danica* KB17 from 2017 to 2018 (A) and in *F. radians* 280 from 2015 to 2016 (B, C). Abnormalities are numbered according to Table 1.

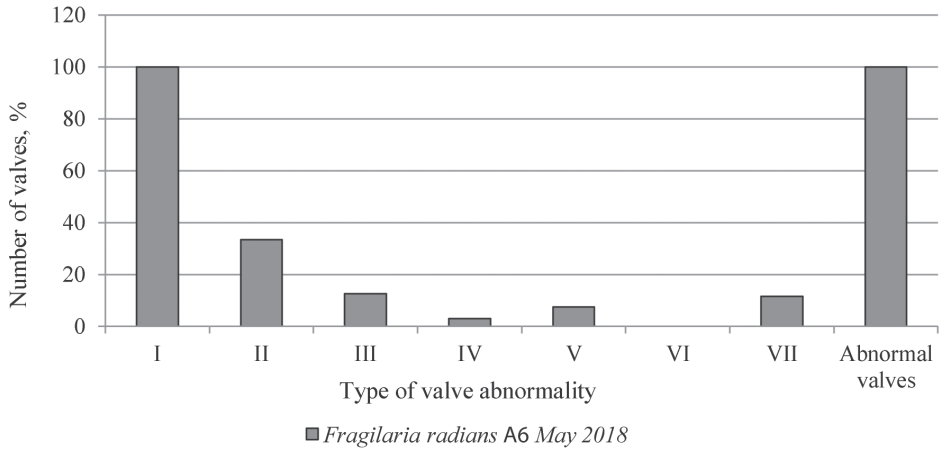

Figure 3. The morphology of *F. radians* A6 valve cells in 2018 (after seven years of culture isolation). Abnormalities are numbered according to Table 1.

al., 2009b). Interestingly, the last three sensitive species turned out to be biraphid. For araphid species *Fragilaria* and *Ulnaria*, the most characteristic deformation of the shell is a change in its shape, according to a 2014 study (Pandey et al. 2014). The present study shows that the last statement is true not only for the cultivation of species of these genera in unfavorable conditions but also for long-term cultivation of monoclonal cultures in laboratory conditions. Although diatom morphogenesis has been studied for several decades, only a few works have documented morphological abnormalities observed in diatom cultures (Estes and Dute 1994), including ones induced artificially by the exposure to microtubule inhibitors (Pickett-Heaps et al. 1979; Blank and Sullivan 1983; Van de Meene and Pickett-Heaps 2002; Tesson and Hildebrand 2010; Bedoshvili et al. 2018). According to the previously published data, structural abnormalities similar to the ones described in this work could be induced in *F. radians* and *U. danica* by microtubule inhibitors (Kharitonenko et al. 2015; Bedoshvili et al. 2017).

This raises a question of the mechanism(s) behind the increase in abnormality prevalence during long-term culture. First of all, culture conditions are not identical to the natural water requirements. A limited medium volume and a lack of mixing could cause most cells to attach themselves to flask walls and bottom with the mucus normally secreted on the outer surface of the valve (Higgins et al. 2003; Edgar 1983). This, in turn, can make normal valve disjunction during the doubling of cell volume before division more difficult, and thus affect the vegetative division process

Second, new valves are formed within the cell and are therefore limited by the shape of mother cell. It means that any deviations in valve shape are propagated to all descendants of the originally malformed cell. It is known that a curved sternum (the

first stage of valve formation) could cause abnormalities in the structures that form later, such as striae, axial and apical fields, and others (Kharitonenko et al. 2015).

Third, it is known that cell cultures generally accumulate somatic mutations (for example, Kim et al. 2017). Since, as we have noted before, the abnormalities observed in old cultures are similar to the ones induced by cytoskeleton inhibition, diatom cells may accumulate deleterious mutations in cytoskeleton-related genes. Unlike mesenchyme cells, which were shown to accumulate somatic mutations without changes in the phenotype (Kim et al. 2017), cytoskeleton mutations could immediately lead to valve abnormalities in diatoms, since valve formation is highly dependent on the cytoskeleton (Van de Meene and Pickett-Heaps 2002).

Fourth, valve abnormalities are not selected against in culture conditions, since they do not directly kill the cells or prevent asexual reproduction. In natural populations, on the other hand, they are quickly eliminated perhaps through sexual reproduction.

These data on the accumulation of morphological changes in monoclonal cultures *F. radians* and *U. danica* extend the range of known variations in diatom valve structure of these genera and can be used for estimating the toxic effects of various agents in the laboratory and environmental conditions.

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