FINE STRUCTURE OF THE VELUM AND GIRDLE BANDS IN
AULACOSEIRA BAICALENSIS

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The fine structure of Aulacoseira (Melosira) baicalensis frustules has been studied by means of scanning and transmission electron microscopy. It appears that the form of the suture spines is conservative. Areolae are of two types. Type 1 areolae are loculate and are covered internally with a velum, with an additional finely perforate membrane, which is bell-shaped. Areolae of type 2 are lamellar (non-loculate) with vela of the vela and rota types and do not have fine membranes, or have only minute ones. Intermediate forms are also present. The fine structure of the copulae has been investigated: they are perforated by fine pores (20-40 nm in diameter) in regular rows.

INTRODUCTION

Aulacoseira baicalensis (Meyer) Simonsen (= Melosira baicalensis; see Simonsen 1979), is one of the dominant planktonic diatom algae of Lake Baikal (East Siberia). During blooms of this alga which start in March-April, the lake is covered by ice. The bloom continues during May and June, concentrations of A. baicalensis in the upper 25 m layer can reach 4-6 \times 10^5 cells l^{-1} before the population begins to sink to the lake bottom (Popovskaya & Skabichevsky 1970).

A. baicalensis has cylindrical frustules (Figs 1, 2), which are linked together into filamentous colonies. Growth and division of cells takes place within filaments. The region where cell division takes place is marked by a conspicuous girdle easily seen under the light microscope. The girdle is thin, has no obvious pores (Popovskaya & Skabichevsky 1970), and consists of several elements (Kobayasi & Nozawa 1982).

The areolae are large and round or oval (Likhoshway et al. 1989), and form longitudinal rows (5-11 per 10 \mu m of the frustule perimeter). The number of areolae per row varies between 3 and 8 (Genkal & Popovskaya, in press).

Scanning electron microscopy has shown that the structures linking adjacent cells are paddle-like spines. The form of the spines, and hence of the suture between sibling cells, is one of the most important species-specific features of Aulacoseira (Crawford 1979, Genkal & Popovskaya, in press). Fortunately, the sutures are well preserved in bottom sediments. Sediments usually contain no intact Aulacoseira filaments; all that survive are pairs of linked sibling valves.

Electron microscopical studies of the frustules of Aulacoseira from ancient sediments and comparison of their structures with those of extant forms is important for palaeolimnological reconstructions and may also help to elucidate phylogenetic relationships within the Aulacoseira genus. However, it is first necessary to investigate morphological diversity within individual species. The present paper describes details of the fine structure of the A. baicalensis frustule and infraspecific variation as revealed by different electron microscopical techniques.
MATERIAL AND METHODS

*A. baicalensis* samples were taken by a Juday plankton net with mesh size 77 µm in Maloye More strait, Lake Baikal, on June 11, 1989. *A. islandica* samples were taken by Dr G. I. Popovskaya in Chivirkuy Bay, Lake Baikal. The samples were fixed in 4% formalin. Some of the preparations were boiled with 30% hydrogen peroxide for 5 min to remove organic components.

Light microscopy (LM) was performed with an MBI-15 microscope (LOMO, USSR) equipped with a photographic camera.

For transmission electron microscopy (TEM) of the fine structure of frustules, cleaned or uncleaned filaments were first placed in distilled water and crushed between two microscopic slides. The homogenates were then collected by pipette, centrifuged in micro-tubes in an Eppendorf 5414 centrifuge at 16,000 r.p.m. for 1 minute and frustules transferred to carbon-coated EM grids. The preparations were examined with a Philips EM 410 microscope at 60kV at magnifications ×2,000-×15,000.

For scanning electron microscopy (SEM) samples were washed to remove formalin, applied to stubs, dried in air, and coated with aluminium using a JEOL JEE 4C evaporator. The stubs were then examined using a scanning accessory EM-ASID in a JEM-100c (JEOL, Japan) at accelerating voltages of 20 and 40 mV and magnifications of ×1,000-×10,000. More detailed studies were performed with a Philips SEM 525M; samples for these studies were coated through evaporation of gold in argon, and examined using an accelerating voltage of 30 kV at magnifications of ×1,000 to ×20,000.

RESULTS

Cells of *A. baicalensis*, as seen in LM and SEM, are shown in Figs 1-3 (see also Popovskaya & Skabichevsky 1970). Typical species-specific linking sutures are present (Crawford 1979, Genkal & Popovskaya, in press).

Two types of areolae occur in *A. baicalensis* frustules (Figs 4-13). Areolae of type 1 are loculate, their internal openings covered by a vema (Fig. 4). They are similar to type 1 areolae in *A. granulata* (Florin 1970). Areolae of type 2 are present in some *A. baicalensis* frustules but are very rare; these areolae are small (56-63 nm) and non-loculate (Fig. 13).

The fine structure of the areolae was studied by means of TEM and found to be very diverse. Describing areolae, we used the nomenclature proposed by Ross et al. (1979). Areolae of type 1 had a vema (the structure occluding an areola) of the vema type (vema are radial bars or flaps extending from the sides of the areola, one to 6-7 per areola; Figs 5-9). “Double” areolae, having a greater number of vema, can also occur (Fig. 10) and the vema can be connected to each other by a small ring concentric with the areola.

The areolae shown in Figs 11, 12 have vela of the rota type, i.e. solid single bars crossing the pores, or multiple bars. These areolae are intermediate between types 1 and 2.

Areolae of type 2 – the simple, non-loculate type – may merge into groups of 2-6. Description is difficult: for example, a group of four areolae could be regarded as one areola, which would have to be considered as having a rota, with vema attached to it. Such an interpretation is supported by the fact that the vema are orientated radially, with a common centre (Fig. 13). The structure of areolae of type 2 is similar to that of the areolae of *A. islandica* (Fig. 14). However, increase in the width of a rota makes it indistinguishable from part of the frustule wall, so that each individual element with its vema can be regarded as an areola. Frustules with areolae of the various types shown in Figs 5-13 all had interlocking sutures composed of spines of the form typical of *A. baicalensis* (Crawford 1979, Genkal & Popovskaya, in press). Variation in the length of the spines (1.9 to 2.7 µm) did not correlate in any way with the form of the areolae.
Figs 1-4. *Aulacoseira baicalensis*. Figs 1, 2. Filaments in LM (Fig. 1) and SEM (Fig. 2). a = girdle band; b = valve; c = linking suture. Scale bar = 10 μm. Fig. 3. Linking suture, SEM. Scale bar = 2 μm. Fig. 4. Areolae of type 1, SEM. Scale bar = 1 μm.

The fine structure of the areolae and vela was studied by TEM in peroxide treated and crushed frustules. Cracks sometimes run along rows of areolae (Fig. 15) and, in areolae of type 1 (Figs 5-10), reveal additional fine membranes – thin perforated sheets (Figs 16-18) attached to the velae. The radii of the membranes varied between 0.27 and 0.54 μm. Figure 19 shows type 1 areolae with the vela disrupted to different extents. The weakest element is the fine membrane, followed by the central part of the velum, where the membranes and velae are linked. The strongest elements of the velae are where they are attached to the frustule wall.

Areolae of type 2 have very small membranes, with radii less than 0.1 μm (Fig. 20). The fine structure of these areolae is similar to that of areolae of *A. islandica* (Fig. 21).

SEM of frustules broken along the rows of areolae (Figs 22-25) reveals the depth at which the vela occur in type 1 areolae. The membranes attached to the velae are bell-shaped (Figs 23, 24) and touch the inner side of the cell walls (Fig. 25). The images shown in Figs 24-26 suggested the reconstruction of velum structure shown in Fig. 27.

SEM of uncleared frustules demonstrates the pattern of copula linkage (Fig. 28), which is similar to that reported for *A. italicca* (Kobayasi & Nozawa 1982). The copulae are perforated by fine pores (20-40 nm), which form regular rows. The rows on adjacent copulae are co-linear (Fig. 28), raising interesting questions about morphogenesis.
Figs 15-20. *A. baicalensis*, TEM. Structure of the velum as revealed in squashed filaments. Scale bar = 2 µm. Figs 16, 18. Structure of the fine membrane (viewed from above, from the outside of the frustule). Scale bar = 0.5 µm. Fig. 17. Fine membrane of a double areola. Scale bar = 0.5 µm. Fig. 19. Different stages of velum, dissolution or destruction. Scale bar = 1 µm. Fig. 20. Type 2 areolae. Scale bar = 1 µm. Fig. 21. *A. islandica* areolae, TEM. Scale bar = 1 µm.
The ornamentation on the copulae (Fig. 30) is different from that typical of *A. italica*. The narrower, partial overlapping ends of the copulae have 7-9 rows of pores in 1 μm, the density of pores within the rows being 11-13 per 1 μm. In the middle of the wider part the rows branch, and form a wedge in the region of ligula. Figure 31 represents an “unfolded” copula (pore rows not to scale).

**DISCUSSION**

Scanning and transmission electron microscopy of *A. baicalensis* frustules has revealed new features of their fine structure. The fine structure of vela is very diverse. Areolae of type 1 are similar to the areolae typical of *A. grandula* (Florin 1970). Areolae of type 2, on the other hand, have a fine structure like that of the areolae of *A. islandica*. Earlier descriptions based on SEM of *A. baicalensis* only mentioned type 2 areolae. The similarity of the two types of areolae in *A. baicalensis* to the areolae of *A. grandula* and *A. islandica* may give insights into the phylogeny of *Aulacoseira*, although the extent of velum disruption must be taken into account in comparisons with fossil forms. The two types of areolae exist along with many intermediate forms – one may trace continuous reduction of bell-like velum membranes, and “separation” of large areolae into smaller ones.
Figs 28-30. *A. baicalensis*: fine structure of copulae. Fig. 28. Part of girdle, showing linkage between copulae, SEM. Scale bar = 5 μm. Fig. 29. Detail of copulae, TEM. Scale bar = 2 μm. Fig. 30. Ornamentation on the copulae, showing the rows of fine pores, TEM. Scale bar = 2 μm. Fig. 31. Diagram of an “unfolded” copula (not to scale).
The results may also have implications for functional morphology, since Aulacoseira cells interact with the aquatic environment through the areolae and the vela. The velum may act not only as a passive sieve, but also as a valve, opening and closing areolae depending on the conditions; this possibility has been discussed by Nikolaev (1984) for other diatoms.

Our data confirm the importance of the form of the suture spines as a species-specific character (Crawford 1979, Genkal & Popovskaya, in press). The wide diversity of the fine structure of the areolae, on the other hand, indicates that this character may not be useful for species delimitation in Aulacoseira, contrary to some proposals (Ross & Sims 1973, Nikolaev 1984).

It is important to study variation in the above-mentioned characters in cells belonging to the same filament. Having studied such filaments to elucidate the pattern of connection between copulae, we also noticed that the areolae of different cells belonging to the same filament have different fine structure. However, these findings are only preliminary, and the contribution of “intra-filament” diversity to intra-species diversity will be a subject of future studies. Investigation of whole filaments is time-consuming, but will help to evaluate the diversity of phenotypes present in the genus and reveal any correlation of cell morphology with the age of single cells. Finally, it will become possible to interpret the dependence of morphology of Aulacoseira on the stage of the development of a population.

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