SIZE-VARIATION IN THE ANTHERIDIA AND OOGONIA OF *CHARA VULGARIS* UNDER DIFFERENT EXPERIMENTAL CONDITIONS

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ABSTRACT

Changes in sizes and shapes of sex organs – antheridia and oogonia – were studied in wild populations of *Chara vulgaris* collected during summer, autumn, and from samples cultured in the laboratory under varying L:D and temperature conditions. Morphometric measurements indicate that the size variations refer mainly to male sex organs, while those evidenced for oogonia are much lower. Our observations are discussed in terms of changing morphogenetic preferences for antheridia and oogonia within the life-span of a plant.

KEY WORDS: *Chara vulgaris*, antheridium, oogonium.

INTRODUCTION

Variation patterns of sex organs represent a common area of interest for biologists searching for fossil remnants of *Chara*, and those who became engaged in the present day forms of these plants. Biometrical studies of extinct populations, basing exclusively on gyrogonites (i.e. calcified parts of the fertilized female sex organs), have important value for systematic classification. However, a large scale studies of these fossils made by Soulie-Marsche (1989) clearly indicate that spread of sizes does not necessarily mean the presence of more than one species. Extinct forms can be divided into stable species which show similar biometrical characteristics, and unstable, polymorphic species which show considerable variation of shapes and sizes of gyrogonites, even those from plants occurring in local populations. For a physiologist, any change of size and shape may become a prominent value in his search for causal relations between external and internal environment of cells and organs he is going to study and describe.

Changes in antheridial sizes (Kwiatkowska et al. 1990) prompted us to undertake further studies concerning the morphological variations among reproductive organs in *Chara*. This short presentation gives a preliminary score of looking for interrelations between the size-variation in antheridia and oogonia of *C. vulgaris*. There is at least one reasonable cause, anatomic in character, which might account for such type of correlation. If we properly cut both sex organs, no matter how old they are, a distinct connection can be seen between their basal cells (Fig. 1). The lower sub-basal cell is their common property. Keeping this in mind, but without going too far into possible functional implications, we started our investigations of the dynamics of growth of antheridia and oogonia throughout their development.

Fig. 1. Schematic drawing illustrating a cross-section throughout a young antheridium and oogonium with a portion of thallus. A sub-basal cell adjoining both gametangia is indicated by an asterisk.

MATERIAL AND METHODS

Collections of *Chara vulgaris* L. were taken from the ponds located in the Rogów Arboretum (branch of SGGW, Warsaw) in the months of June (the control "summer" series) and September ("autumn" series). "Summer" plants, transferred to the laboratory were kept at about 18°C under a 14:10 h light:dark cycle using white fluorescent tubes (100 mmol m⁻²s⁻¹) and,

following 2 weeks of cultivation, they were divided into 4 variants:

(I): L:D = 14:10 (L at 18°C, D at 3°C);

(II): L:D = 16:18 (18°C);

(III): L:D = 3:21 (18°C);

(IV): L:D = 1:23 (18°C).

After 8-day cultivation plants were fixed in cold Carnoy's mixture of absolute ethanol and glacial acetic acid (3:1) for 2h, washed with 100% (3 times) and 70% ethanol and kept for further studies.

For Feulgen-staining, apical parts of thalli with whorls of lateral branches (pleuridia) carrying antheridia and oogonia were thoroughly washed with distilled water and hydrolysed in 4N HCl for 1 h at 18°C. Plants were then stained in Schiff's reagent (pararosaniline, Gurr) for 1h at room temperature and soaked in SO₂-water. For morphometry, dissected pairs of adjoined antheridia and oogonia were placed in a drop of distilled water on a microscope slide and measured using an ocular micrometer. In order to determine the stage of antheridial development, gametangia were placed in a drop of 45% acetic acid, squashed on gelatinized slides. After freezing with dry ice, cover slips were removed and the dehydrated dry slides were embedded in Euparal.

RESULTS AND DISCUSSION

Relationships between the average diameters of antheridia and the mean lengths of oogonia in *Chara vulgaris* are given in Fig. 2a. Successive stages of antheridial development are expressed as a number of germ-line cells which compose the antheridial filament, and then, during spermiogenesis, as A, B, and C, which indicates successive stages of sperm cell formation. The above data are plotted against average lengths of un-

Fig. 2a. Relationship between the mean diameter of an antheridium (solid line) and the average length of the oogonium (dashed line).

fertilized oogonia or oosporangia (after disruption of antheridia indicated as OG and OS, respectively), depending on stage within the ontogenetic time-scale of the generative organs. Up to the stage of the 16-cell filaments, the diameters of antheridia and the lengths of oogonia increase gradually and parallelly. After the 16-cell break-point, the antheridia stop growing while oogonia increase their lengths till the end of spermiogenesis, that is till the liberation of mature spermatozooids. A remarkably similar kind of relationship can be seen in Fig. 2b which, according to the same coordinates of the graph, correlates average diameters of antheridia and the mean widths of female sex organs. Relatively young but quite large antheridia are accompanied by long, yet thin oogonia (Fig. 3). Soon after passing the developmental break-point, characterized by the domination of the 16-cell filaments, the antheridia attain their upper size limits (Figs 4 and 5). At further stages of development, the oogonia keep growing to reach their maximum widths (Fig. 6).

It seems possible that a set of processes becomes engaged in this phenomenon, and to two of them we would like to turn more attention. Recent studies made by Kwiatkowska (1988) strongly imply that one of the most important factors governing the morphogenetic way towards spermiogenesis in *Chara* is connected with changes in intercellular junctions, or, more precisely, with the breakage of plasmodesmata. The experiments and observations carried out on control material revealed a spontaneous symplasmic isolation of antheridia at both sides of a basal cell that takes place shortly before the onset of spermiogenesis. The breakage of plasmodesmata connecting the antheridium with the thallus was found to be a signal which releases, in all antheridia, mechanisms that: (I) block endomitotic DNA synthesis in manubria, (II) restrict the growth rate and the divisions of antheridial filament cells, and

Fig. 2b. Relationship between the mean diameter of an antheridium (solid line) and the average width of the oogonium (dashed line).

Fig. 3. Young antheridium (a) neighbouring an elongated oogonium (o). x 200.
Figs 4 and 5. Further development of oogonia (o) and antheridia (a), after the 16-cell stage of antheridial filaments. x 200.
Fig. 6. Maximum size of oogonia after the disruption of antheridia and releasing sperm cells. Following fertilization, female gametangia become brown-coloured and transform into oosporangia (os). x 200.

(III) induce spermiogenesis in these antheridia, in which manubria attained the sufficient level of polyploidy. It seems reasonable to believe then, that the break-point coinciding with the 16-cell stage of filaments is the landmark which starts to favour thickening of oogonia. Moreover, it can be supposed that cessation of the symplastic transport from the thallus to the antheridium results in a decrease of the uptake

TABLE 1. Morphometric analysis of antheridia of *Chara vulgaris* in various environmental conditions

Experimental series	Diameter [μm]		
	minimum	maximum	mean
Autumn	212.5	281.3	242.1 ± 2.7
Summer (control)	306.3	518.8	376.9 ± 9.3
I – temp. ± 4°C	283.5	396.9	331.0 ± 4.8
II L:D = 6:18	213.5	465.4	342.0 ± 7.9
III L:D = 3:21	201.3	453.3	342.8 ± 7.8
IV L:D = 1:23	213.5	427.0	327.9 ± 7.0

of plant hormones (e.g. gibberellins; Kwiatkowska 1991, 1995) and/or nutritive substances. Such process may arrange efficient ways and means to supply the growing oogonium.
Results concerning the effects of environmental conditions on sizes of sex organs in *Chara* are given in Tables 1 and 2a, b. Comparisons referring to populations of plants taken in summer, autumn, as well as to those kept in cold and under different light regimes, show remarkable changes of the minimum, maximum and mean diameters of antheridia (Table 1). A considerable difference should be noted between the summer and autumn populations. On the other hand, as compared with the antheridia, the variation of lengths found in oosporangia from plants cultivated under the same conditions are relatively small and the differences are statistically insignificant (Table 2a). In general, the same holds true in case of the data referring to the widths of oosporangia (Table 2b).

TABLE 2a. Morphometric analysis of the oosporangia of *Chara vulgaris* in various environmental conditions

Experimental series	Length [μm]		
	minimum	maximum	mean
Autumn	487.5	587.5	522.4 ± 3.1
Summer (control)	475.0	575.0	523.3 ± 5.9
I – temp. ± 4°C	475.0	587.5	508.9 ± 3.7
II L:D = 6:18	437.5	537.5	495.8 ± 4.2
III L:D = 3:21	500.0	550.0	529.6 ± 2.6
IV L:D = 1:23	512.5	587.5	549.5 ± 4.4

TABLE 2b.

Experimental series	Diameter [μm]		
	minimum	maximum	mean
Autumn	337.5	400.0	374.4 ± 2.2
Summer (control)	300.0	450.0	373.3 ± 8.9
I – temp. ± 4°C	325.0	487.5	357.7 ± 3.5
II L:D = 6:18	337.5	387.5	363.6 ± 2.6
III L:D = 3:21	350.0	387.5	372.1 ± 1.5
IV L:D = 1:23	362.5	400.0	376.0 ± 2.7

The obtained results seem to indicate that various external conditions exert much more pronounced morphological effects on male sex organs of *Chara vulgaris* than on female oosporangia, where all of the evidenced alterations have been marked to a considerably lesser degree. It is striking, however, that the differences between antheridia growing on plants kept in the extreme experimental conditions are much lesser than the differences revealed between summer and autumn plants. It may be, thus, that environmental conditions at the final periods of a vegetation season influence the entity of a plant throughout a more complex system of relations acting via stimuli which affect a greater number of physiological targets of a plant.

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ZMIENNOŚĆ ROZMIARÓW ANTERYDIOSTANÓW I OOGONIÓW *CHARA VULGARIS* W RÓŻNYCH WARUNKACH EKSPERYMENTALNYCH

STRESZCZENIE

Zmiany wielkości i kształtów organów generatywnych – anterydiostanów i oogoniów – badano w populacjach ramienicy (*Chara vulgaris*) rosnącej w środowisku naturalnym latem, jesienią oraz w laboratorium, w różnych warunkach fotoperiodu i temperatury. Analizy morfometryczne wykazały, że znaczna zmienność rozmiarów charakteryzuje gametangia męskie, natomiast zróżnicowanie oogoniów jest mniejsze. Wyniki przeprowadzonych obserwacji omawiane są w kontekście zmieniających się preferencji morfogenetycznych anterydiostanów i oogoniów podczas cyklu życiowego rośliny.

SŁOWA KLUCZOWE: *Chara vulgaris*, anterydiostany, oogonia.