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Structure of Plant Cells with Special Reference to Lower Plants. II.

Feulgen's Nucleal Staining in Some Algae *

By

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While many investigators have reported on Feulgen's nucleal staining in algae, some of the results obtained by them do not always agree one another, especially in case of cyanophyta and conjugatae (cf. Kiesel and Doinikowa, 1937, Yamaha and Suematsu, 1938, Hil'ary, 1939).

It is the purpose of the present investigation to employ the Feulgen staining in various divisions of algae with a special attention to cyanophyta and conjugatae.

Material and Methods

Nuclei of algae which belong to seven divisions were used for the material. Materials were fixed with ethanol, corrosive sublimate-ethanol mixture, or corrosive sublimate-acetic acid mixture, followed by washing with water. After washing, they were hydrolyzed with N-HCl *in toto*, or, if necessary, in microtome sections, for 3, 5, 10, 15, and 20 minutes. After the hydrolyzed materials were stained with Schiff's reagent ** from 2 to 4 hours at room temperature (18–25°C), they were washed well with SO_2-water, then were observed with a microscope. In many species, however, the materials were sealed with canada balsam after dehydration and clearing.

Besides the nucleal staining, pyronin-methylgreen double staining was carried out to see the relation between the nucleal staining and the double staining.

Observations

Results of the observation are presented in the following table.

* This investigation was performed with the Grant for Fundamental Scientific Research.
** Schiff's reagent was prepared by reducing "Merck" basic fuchsin with natrium bisulfite.

	Species	Fixatives	Time of optimum hydrolysis	Feulgen staining	Preparations	Pyronin methlgreen staining
Cyanophyta	*Nostoc commune*	E,S-A,S-E		—	*in toto*	Cells are stained diffusely, but not differentially.
	N. carneum	〃		—		
	N. piscinale	〃		—		
	Oscillatoria Annae	〃		—		
	O. tenuis	〃		—		
	O. limosa	〃		—	*in toto* & microtome sections*	
	O. princeps	〃		—		
	O. putrida	〃		—	*in toto*	
	Cylindrospermum Michailovskoënse	〃		—		
	Brachytrichia balami	〃		—		
Rhodophyta	*Gelidium elegans*	S-A	5	+	tetraspore mother cells microtome sections *	Chromonemata and nucleoli are stained green and red respectively, but the color intensity is very faint.
	Nemastoma Nakamurae	〃	5	+		
	Grateloupia affinis	〃	5	+		
	Gloiosiphonia calmichael	〃	5	+	vegetative cells *in toto*	
	Corallina sp.	〃	10	+		
Chrysophyta	*Synedra ulna*	S-A	5	+	*in toto*	Chromonemata and nucleoli are well differentially stained green and red respectively.
	Gyrosigma sp.	E	5	+		
	Cymbella tumida	S-A, E	5	+		
	C. affinis	E	5	+		
	Surirella elegans	S-A,S-E	5	+		
	S. lanceolata	S-A	5	+		
	Dityrum Brightwelli	E	5	+		
	Planktoniella sol	〃	5	+		
	Biddulphia sinensis	〃	5	+		
	Coscinodiscus gigas	〃	10	+		
Pyrrophyta	*Dinophisis candina*	E-A, E	10	+	*in toto*	Chromonemata and nucleoli are differentially stained green and red respectively.
	Ceratium hirundinella	S-A	15	+		
	C. trichoceros	S-E, E	15	+		
	C. furca	〃	15	+		
	C. fusus	〃	15	+		
	Sargassum Horneri					
	egg	S-A		±	microtome sections	
	epid.		5	+		

Phaeophyta	*S. piluliferum*					
	egg	〃		±		
	epid.		5	+		
	S. Thunbergii					
	egg	〃		±		
	epid.		5	+		Egg nuclei are diffusely stained but chromonemata and nucleoli in a cell of conceptacle epidermis are differentially stained.
	S. tortile					
	egg	〃		±		
	epid.		5	+		
	S. hemiphyllum					
	egg	〃		±		
	epid.		5	+		
	Cystophyllum sisymbrioides					
	egg	〃		±		
	epid.		5	+		
	Hijikia fusiforme					
	egg	〃		±		
	epid.		5	+		
	Padina arborescens	〃	10	+	*in toto*	
	Colpomenia sinosa	〃	5	+		
Euglenophyta	*Euglena gracilis*	E, S-A, S-E	5	+	*in toto* & microtome sections*	Chromonemata and a nucleolus are well differentially stained green and red respectively.
	E. spirogyra	S-A	5	+	*in toto*	
	Facus sp.		5	+		
Chlorophyta	*Closterium sp.*	S-A		—	*in toto* & microtome sections *	
	Spirogyra setiformis	S-A, E		—		
	S. sp.	S-A, E		—		
	Zygnema sp.	S-A		—	*in toto* & microtome sections	
	Micrasterias sp.	〃		—		
	Nitella sp.	〃	10	+		

Abbreviations:
S-A; corrosive sublimate-acetic acid mixture. S-E; corrosive sublimate-ethanol mixture.
E; ethanol. epid.; conceptacle epidermis.
* Microtome sections were sealed by canada balsam after dehydration and clearing. Results of the Feulgen staining in permanent preparations were not different from those obtained by the observation immediately after washing with SO_2-water.

It is seen in this table that the nuclei of whole species in cyanophyta and conjugatae were negative with the nucleal staining.

Among Feulgen positive algae, it was observed in general that related species took nearly equal time of optimum hydrolysis and were stained with similar tone and intensity, and that chromonemata and nucleoli were stained differentially green and red respectively when they were stained by methylgreen and pyronin.

The nuclei of the algae belonging to pyrrophyta were colored most intensely with the Feulgen staining, while those of rhodophyta and phaeophyta were stained very faintly, so far as the material used in the present study concerns.

Generally speaking, marine chrysophyta seem to be stained more weakly than the species in freshwater.

Discussion and Conclusion

It has been reported by Poljansky and Petruschewsky (1929), Yamaha and Suematsu (1938), Hillary (1939), and Drawert* (1949), that cells of *Oscillatoria* are Feulgen positive. Other genera among cyanophyta have also been reported that they show positive nucleal staining by Poljansky and Petruschewsky (1929) in *Spirulina*, *Tolypothrix*, *Gloeotrichia*, and by Bringmann (1950) in *Lyngbya*. On the other hand, it has been stated by Kiesel and Doinikowa (1937) in *Oscillatoria* and *Tolypothrix*, by Herbst (1953, 1954) in *Oscillatoria*, *Aphanotheca*, *Pseudoanabaena*, and by Shinke (unpublished) and Oura (unpublished) in *Oscillatoria* and other blue green algae that the cells are Feulgen negative. In the present investigation, it was observed that many species of cyanophyta showed negative nucleal staining in sectioned preparations as well as *in toto* preparations. Bringmann (1950) has reported that cells of *Oscillatoria* are Feulgen positive when the cells are treated with lanthanum acetate before hydrolysis. In the present investigation, however, the cells of *Oscillatoria* and *Nostoc* did not show positive Feulgen staining though they were treated with lanthanum acetate.

Besides cyanophyta, conjugatae is also a problematic group. For instance, Geitler (1935), Yamaha and Suematsu (1938), and Hillary (1939) have reported that the nuclei of *Spirogyra* are Feulgen positive, while Shinke and Shigenaga (1933), Kiesel and Doinikowa (1937) have reported that *Spirogyra* is not stained by the Feulgen method. Besides *Spirogyra*, Yamaha and Suematsu (1938) in *Zygnema* and Hillary (1939) in *Closterium* have observed that these algae show positive nucleal staining. Four genera of conjugatae used in the present work, however, showed negative nucleal staining. It must be noted here that the nuclei of *Spirogyra setiformis* was Feulgen negative throughout whole mitotic cycle in permanent preparations (Ueda, in press).

Westbrook (1930, 1935), Kiesel and Doinikowa (1937), and Yamaha and Suematsu (1938) in a few species of rhodophyta, Kiesel and Doinikowa (1937) and Hillary (1939) in a few species of chrysophyta, Papenfuss (1934) and Kiesel and Doinikowa (1937) in a few species of phaeophyta have reported that these algae show positive nucleal staining. These results seem to be not disaccord with those of the present investigation.

In egg nuclei of *Sargassum*, the coloration was so faint that the present

* cited from Bringmann (1950)

author was not able to determine whether the Feulgen staining was positive or negative.

Summary

Feulgen's nucleal staining was carried out in forty eight species among seven divisions of algae.

Whole species of cyanophyta and conjugatae were Feulgen negative, and other species were positive so far as the material used in the present investigation concerns.

Related species seem to take an equal time of optimum hydrolysis and to stain similar tone and intensity.

Nucleoli were Feulgen negative in all species.

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