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ELEOCHARIS MACROSTACHYA BRITTON WITH
SPECIAL REFERENCE TO THE KINETOCHORE.

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CHROMOSOMAL MORPHOLOGY AND BEHAVIOR IN
ELEOCHARIS MACROSTACHYA BRITTON WITH
SPECIAL REFERENCE TO THE KINETOCHORE

by

James V. Bernardini

A THESIS

Presented to the Faculty of
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1965

TITLE

CHROMOSOMAL MORPHOLOGY AND BEHAVIOR IN ELEOCHARIS MACROSTACHYA

BRITTON WITH SPECIAL REFERENCE TO THE KINETOCHORE

BY

JAMES V. BERNARDINI

APPROVED

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SUPERVISORY COMMITTEE

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DEDICATED TO

My late father, Camillo Victor Bernardini, M.D., and to
my mother, Audrey Annetta (Shipp) Bernardini, R.N.,
each of whom was devoted to the healing arts.

PREVIEW

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PREVIEW

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PREVIEW

CHROMOSOMAL MORPHOLOGY AND BEHAVIOR IN
ELEOCHARIS MACROSTACHYA BRITTON WITH
SPECIAL REFERENCE TO THE KINETOCHORE

PREVIEW

Chromosomal Morphology and Behavior in
Eleocharis macrostachya Britton with
Special Reference to the Kinetochore

Introduction:

Chromosomes are the vehicles of Mendelian inheritable factors, and the distributive migration of chromosomes is intimately related to the kinetochore and amphiastral or anastral achromatic figure during karyokinesis. The kinetochore is a highly specialized region of the chromosome, which manifests the intrinsic property of active mobility, organizes chromosomal spindle material and leads the chromosome to the metaphasic plate and daughter chromosomes in polar ascent.

The microscopical studies conducted by Lima-de-Faria (review, 1958a) on monokinetochoric chromosomes from selected plant and animal species revealed comparable, if not identical chromomeric and fibrillar patterns of kinetochores. The phase contrast observations of Gall (1954) on oöcytic lampbrush chromosomes of Triturus viridescens and Amblystoma tigrinum contributed additional evidence in support of kinetochoric morphology. Examination of the photomicrographs and line drawings published by Tjio and Levan (1950) of chromosomes from root cells of numerous plant species pretreated with

8-hydroxyquinoline reveals kinetochores with well defined chromomeric-fibrillar patterns.

Several observations have been recorded in which segments or regions of the arms of chromosomes with localized kinetochores have either organized chromosomal spindle fibers or exhibited activity on the spindle. The fibrillar organization and mobility of these loci on the spindle has been referred to as "neo-centric activity". Prakken and Müntzing (1942) and Östergren and Prakken (1946) observed this phenomenon in the terminal segments of a pair of meiotic chromosomes from an inbred line of Secale cereale L. Bajer and Östergren (1961) studied neo-centric activity in the chromosomal arms of Haemanthus katherinae Baker.

Dikinetochoric chromosomes have been observed in cells of many organisms, and as examples, the reader is referred to the studies in Narcissus pseudonarcissus L. by Darlington and Wylie (1953), in a rat tumor by Koller (1953) and in Zea mays L. by McClintock (1941).

Boveri (1904) demonstrated that the gonial progenitor cells of Ascaris megalocephala (= Parascaris equorum) contained undiminutive chromosomes. Bonnevie (1913) accurately figured "traction cones" with attached spindle fibers on the poleward surfaces of these long metaphasic chromosomes. Lima-de-Faria (1949b) and White (1936) considered these chromosomes

multikinetochoic, while Schrader (1935) considered them composed of many small localized kinetochoic chromosomes joined end to end.

An extensive literature has accumulated on organisms whose cells were reported to have non-localized or diffuse-kinetochoic chromosomes. The metaphasic orientation, anaphasic separation and migration of these chromosomes differed markedly from monokinetochoic chromosomes. Non-localized or diffuse-kinetochoic chromosomes oriented their entire lengths, or essentially so, on the metaphasic plane; i.e., perpendicular to the spindle axis. Anaphasic separation of daughter chromosomes, univalents or their cytological equivalents were reported as parallel or nearly parallel to one another. These chromosomes became crescent-shaped as the poles were approached; viz., their extremities were bent in the direction of the respective poles. The reader is referred to the works of Hughes-Schrader (1948), Lima-de-Faria (1949b), Bernardini (1959), Hughes-Schrader and Schrader (1961) and Nordenskiöld (1963) for references and discussions pertinent to organisms with multi-, non-localized-, or diffuse-kinetochoic chromosomes.

Hughes-Schrader and Ris (1941) were apparently the first investigators to test the hypothesis of the reality of non-localized or diffuse-kinetochoic chromosomes by the X-irradiation of the coccid

Steatococcus tuberculatus Morris. All irradiation-induced chromosomal fragments were reported to behave in the usual manner of non-fragmented chromosomes through several cell generations, regardless of size. Hughes-Schrader and Schrader (1961) X-irradiated the pentatomids Euschistus servus, E. tristigmus and Solubea pugnax and reported the majority of induced chromosomal fragments were kinetic and they attributed the behavior and presence of the occasional akinetic fragments to disturbances resulting from the irradiation treatment.

Godward published a series of papers (1950a, 1950b, 1954a, 1954b) on the cytology and cytotaxonomy of species of Spirogyra Link (Conjugatae). Relationships between species reported with localized, non-localized and multikinetochoic chromosomal systems were advanced. He now has favored, however, the non-localized or diffuse-kinetochoic chromosomal system, although the presence of a multikinetochoic chromosomal system has not been discounted altogether.

Malheiros, Castro and Camara (1947) were the first to report the occurrence of an apparent non-localized or diffuse-kinetochoic chromosomal system in specimens of the higher plant species Luzula purpurea Link (Juncaceae). Castro, Camara and Malheiros (1949) tested the hypothesized non-localized kinetochoic chromosomal system by exposing living spikelets of Luzula purpurea to X-rays. Irradiation-

induced chromosomal breakage resulted in the production of small akinetic fragments, interpreted as chromosomal "matrix", and large kinetic fragments. They interpreted their data to uphold the non-localized or diffuse-kinetochoric chromosomal system.

La Cour (1953) repeated the work of Castro, et al. (1949) and obtained similar results. However, the non-migrating fragments were interpreted as chromatic substance, and he suggested that a multiple-kinetochoric chromosomal system was operative instead of a non-localized chromosomal system.

Håkansson (1954) examined the chromosomes from X-rayed and untreated spikelet material from specimens of Eleocharis palustris ssp. vulgaris Walters (Cyperaceae) and suggested the presence of a diffuse- or multikinetochoric chromosomal system. Håkansson (1958) reinvestigated irradiation-induced chromosomal breakage in Eleocharis palustris (L.) R. and S., Eleocharis palustris ssp. microcarpa Walters and Eleocharis mamillata Lindb. fil. Håkansson considered these species had diffuse-kinetochoric chromosomes. His interpretation was based on the migration of chromosomal fragments.

Bernardini (1959) examined American material from specimens of a related species, Eleocharis macrostachya Britton, and suggested the presence of a multikinetochoric chromosomal system. The basis of

interpretation was the observation of traction cones on the poleward surfaces of chromosomes during the metaphasic-anaphasic transition of root tip and microspore mitosis.

Small akinetic chromosomal fragments have been reported frequently in studies from X-irradiation of cells of organisms reported having non-localized or diffuse-kinetochoric chromosomal systems. To the best of my knowledge Godward (1954a) was the first to demonstrate repeated stainable chromosomal structure in multikinetochoic chromosomes. The remarks of Hughes-Schrader (1948, p. 132) and those of Hughes-Schrader and Schrader (1961) and Nordenskiöld (1963) concerning our lack of knowledge of the architecture of the non-localized or diffuse-kinetochore appear apropos.

The objective of this investigation was to elucidate the morphology and behavior of the chromosomes, with special reference to the kinetochore, of Eleocharis macrostachya Britton.

Methods and Materials:

Living rhizomes of Eleocharis macrostachya Britton, collected in the summer of 1957 from San Diego, California, have been cultivated continuously in the greenhouse. Periodically, herbarium sheets of this clonal material have been prepared for deposition in the following herbaria: University of Minnesota (MIN), University of Nebraska (NEB), Academy of Natural Sciences of Philadelphia (PH) and Lund Botanical Museum (LD). This material previously bore the hyphenated number 57-15 on my label, and now has been changed to the serial number 90.

A different collection from Mills County, Iowa was kindly supplied to me by Mr. Murray E. Duysen (my label, No. 295). This plant appears closely referable to Eleocharis calva Torr. Eleocharis calva and Eleocharis macrostachya are closely related species according to Svenson (1947). This particular plant warrants inclusion in this report, because the kinetochore observed at metaphase I of meiosis was strikingly clear.

I am indebted to Dr. Henry K. Svenson for his annotations.

From the beginning of these studies in 1957, centering on available American species of Eleocharis R. Br. sub-series Palustres, except Eleocharis kamtschatica (C. A. Meyer) Kamarov and Eleocharis