Journal of the Arkansas Academy of Science

Volume 20 Article 6

1966

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Arkansas Academy of Science Proceedings, Vol. 20, 1966

PROPOSED MECHANISMS OF ASEXUAL NUCLEAR DIVISION IN **NEUROSPORA CRASSA** SHEAR AND DODGE[1]

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INTRODUCTION

Perhaps no other biological phenomenon has as many different current explanations as does asexual nuclear division in **Neurospora crassa** Shear and Dodge. It is surprising that the nuclear behavior of an organism used so extensively in genetics would remain enigmatic. However, asexual nuclear behavior in the fungi is controversial and needs more study. In general, two schools of thought have developed concerning the mechanism of asexual nuclear division in **N. crassa.** One theory proposes that division occurs similarly to mitosis in higher plants (8, 9). The other contends that a mechanism basically different from classical mitosis is operative (3, 4, 6, 7, 10, 11, 12). Proponents of this latter theory have presented varied interpretations of this process (Fig. 1).

Somers et al. (8), and Ward and Ciurysek (9) propose that the chromosomes of **N. crassa** become arranged on a metaphase plate and divide by migrating to opposite poles. They failed to find a definite achromatic apparatus but maintained that the presence of a spindle was strongly suggested. The pictorial evidence presented by these workers is not convincing. This may be explained by the extremely rough treatment given their material before fixation. Ward and Ciurysek (9) state that in the preparation of their material "The light mycelial mat which developed was washed with distilled water to remove the culture medium and homogenized. Films of homogenate were spread over microscopic slides, air dried, . . ." All of this was done before the nuclei were fixed. Somers et al. (8) centrifuged their material twice before fixing it. It seems that homogenization, centrifugation, and drying prior to fixation should be avoided, since these procedures would be expected to disrupt living processes such as mitosis. One should also expect extreme physical distortion to result from drastic treatment.

Bakerspigel (4), Weijer (10), Weijer et al. (11), and Dowding and Weijer (7) contend that division in the vegetative hyphae of **N. crassa** differs from classical mitosis, but they disagree radically in their interpretation of what happens. In fact, J. Weijer and D. L. Weijer in three separate publications present what are apparently three different interpretations of division (7, 10, 11).

[1]Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

[2]Dr. Wilson is Associate Professor and Mr. Brushaber and Mr. Aist are undergraduate research participants in the Department of Plant Pathology.

Bakerspigel (4) interprets asexual nuclear division as follows: "This is another example of a fungus in which the vegetative nuclei do not appear to divide in the manner of classical mitosis. Instead, as division proceeds, the chromatin forms complexes of chromosomal filaments which then contract. At the end of division the contracted chromatin constricts and pulls rapidly apart without the aid of a visible spindle. Individually recognizable chromosomes were not observed to align themselves on a metaphase plate. In the vegetative Mycelium of **N. crassa** the central body elongates and divides by constriction at the mid-region. Thus at the end of division each of the sister nuclei is composed of a portion of the original chromatin and central body. It is suggested that both the elongated central body and the densely strained granule in the chromatin of these nuclei play significant roles during nuclear division."

Dowding and Weijer (7) studying stained material state that "We too have found that mitosis in **Neurospora** mycelium is unlike that in higher plants and animals and that there is no spindle; but we have observed also that the nuclei are filamentous and that they divide by splitting longitudinally."

Weijer et al. (11) observing living material under phase-contrast present a radically different interpretation of nuclear division. They do not attempt to consolidate this work with their previous interpretations of stained material. Prior to division the chromatin is arranged into a double-ring structure out of which protrudes a ballon-like membrane which eventually separates from the now compacted chromatin and disappears. The chromatin then changes to a bar-like structure with a nucleolus at one end. The chromatin bar splits longitudinally to form the daughter nuclei.

This paper presents still another interpretation of the asexual nuclear behavior of **N. crassa.** More detailed information on asexual nuclear behavior in **N. crassa** will be presented in additional papers, and it is anticipated that this information will help clarify the current dilemma.

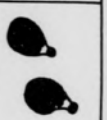
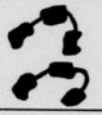
MATERIAL AND METHODS

Preparations for this study of **N. crassa** were made according to the HCL-Giemsa technique described by Aist and Wilson (1). Wild-type cultures of **N. crassa** were used.

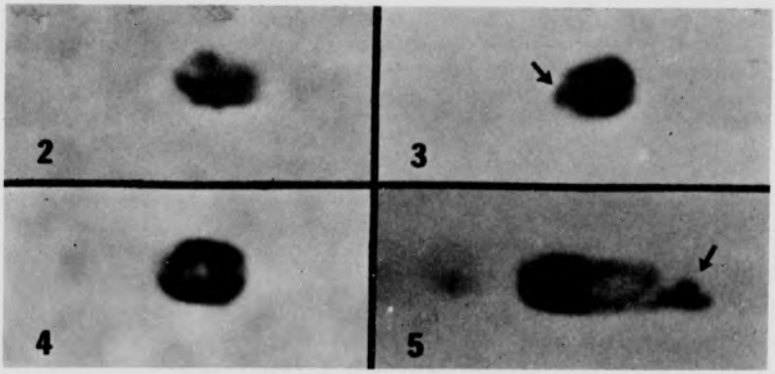
RESULTS

We have found that asexual nuclear division in **N. crassa** occurs in a manner not previously described for this organism. Nuclear division in the thallus of **N. crassa** is similar to that described for **Ceratocystis fagacearum** (Bretz) Hunt and other plant pathogens (1, 2, 5). It occurs perpendicular to the longitudinal axis of the hyphae (Fig. 3). Metaphase chromosomes are associated lineally to form a bar-shaped metaphase plate (Fig. 2). Anaphase movement is usually unilateral and not synchronized. Spindles are usually seen only between chromatids or groups of chromatids which have already separated.

FIG. 1 DIFFERENT EXPLANATIONS OF ASEXUAL NUCLEAR DIVISION IN NEUROSPORA CRASSA

AUTHORS	PROPHASE	DIVISION	DIVISION	SEPARATION	SEPARATION	DAUGHTER NUCLEI
WARD & CIURYSEK (1962) (CLASSICAL MITOSIS) SOMERS, WAGNER, HSU (1960)						
BAKERSPIGEL (1959)						
WEIJER KOOPMANS WEIJER (1963)						
WEIJER & DOWDING (1960)						
WILSON BRUSHABER AIST (1966)						

Asexual nuclear division in **N. crassa** occurs within an enlarged nuclear envelope (Figs. 2, 3, 4). After the nucleus divides within the envelope, a new membrane is formed around each daughter nucleus, and the daughter nuclei migrate out of the old envelope (Fig. 5). The old nuclear envelope eventually disappears (Fig. 1). Nuclei migrating out of the envelope may become considerably attenuated.



DESCRIPTION OF FIGURES

Figure 2. Metaphase with particulate chromatin. Nuclear membrane is visible in original preparation.

Figure 3. Anaphase within nuclear envelope. Division oriented perpendicular to longitudinal axis of hypha. Note point at which nuclear envelope extends beyond dividing chromatin (arrow). Only part of the chromatin is in focus.

Figure 4. Late anaphase or early telophase.

Figure 5. Nucleus (arrow) migrating out of nuclear envelope. The other daughter nucleus is still within the envelope.

DISCUSSION

We found no indications that vegetative nuclei of **N. crassa** divide by classical mitosis. Workers who propose this type of division have not presented clear pictorial evidence to support their contentions (8, 9).

The mechanisms of division proposed by Bakerspigel (4), Weijer et al. (11), and Dowding and Weijer (7) have features which are consistent with certain of our findings. We believe that the constricting and dividing nuclei reported by Bakerspigel may have resulted from a staining of the nuclear matrix within the envelope as well as the chromatin. When we overstain we get similar figures. As the daughter nuclei migrate out of the nuclear envelope, the envelope is stretched

and figures are produced comparable to those illustrated by Bakerspigel as divisional stages.

Weijer et al. (11) and Dowding and Weijer (7) have shown the chromosomes to be associated lineally into a filament of chromatin. They interpret division as the splitting of this filament into two strands of lineally associated chromosomes which then separate. We find the chromosomes associated into a filament of double stranded chromatin prior to division. A spindle then develops between separating chromatids and appears to push them apart.

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