Bothalia 12, 2: 247–250 (1977)

Asexual nuclear division in *Neocosmospora**

K. T. VAN WARMELO†

ABSTRACT

A fungus isolated from soybean stem material showed marked similarities to two existing species of *Neocosmospora*, i.e. *N. vasinfecta* E. F. Smith and *N. africana* von Arx. The processes of somatic nuclear division in authentic cultures of these two species and the new isolate were examined to determine the nature of the mechanism of nuclear division and whether there were any differences among the cultures. No significant differences were observed. The divisions showed asynchronous anaphase disjunction but there was no evidence of aneuploidy or irregular reconstitution of daughter nuclei. Nuclear division was interpreted as being strictly mitotic.

RESUME

CARYOCINESE ASEXUEE CHEZ NEOCOSMOSPORA

Une moisissure isolée de tiges de soya a montré des ressemblances marquées avec deux espèces existantes de Neocosmospora, *soit* N. vasinfecta *E. F. Smith et* N. africana *von Arx. Les processus de caryocinèse somatique ont été examinés dans des cultures authentiques de ces deux espèces et dans le nouvel isolat pour déterminer la nature du mécanisme caryocinétique et noter les différences entre cultures s'il y en a. On n'a pas observé de différences significatives. Les divisions ont montré une disjonction asynchrone à l'anaphase, mais il n'y avait aucun signe d'aneuploïdie ou de reconstitution irrégulière de noyaux-fils. La caryocinèse a été interprétée comme étant strictement mitotique.*

REVIEW

Reported mechanisms of somatic nuclear division in the fungi seem to fall into two basic categories, i.e. divisions which are essentially mitotic, differing only slightly from the classical concept, and non-mitotic divisions in which completely different processes are operative.

Divisions in a large variety of fungi were shown to differ from mitosis in higher plants only in that the nuclear membrane was persistent throughout the division (Thyagarajan, Conti & Naylor, 1962; Moor, 1966; Robinow & Marak, 1966; Heath & Greenwood, 1968; McManus & Roth, 1968; Aist, 1969; Aldrich, 1969; van Winkle, Biesele & Wagner, 1971). The spindle was thus fully intranuclear (Ichida & Fuller, 1968; Motta, 1969; Zickler, 1970).

Persistence of the nuclear membrane was, however, not an invariable condition, as fungi were reported in which the membrane broke down during division (Robinow, 1963; Namboodiri & Lowry, 1967; Motta, 1969; Brushaber & Jenkins, 1971).

An additional difference between somatic divisions in fungi and higher plants was the variable presence of centrioles. Several fungi, representative of widely differing taxonomic groups, were reported as lacking centrioles (Namboodiri & Lowry, 1967; McManus & Roth, 1968; Aldrich, 1969) whereas their presence was demonstrated in others (Moor, 1966; Robinow & Marak, 1966; Brushaber & Jenkins, 1971; van Winkle *et al.*, 1971).

Despite the differences referred to above, however, the divisions in a large number of fungi have been described as essentially mitotic (Robinow, 1963; Hosford & Gries, 1966; Knox-Davies, 1966, 1967; Finley, 1970; Brushaber & Jenkins, 1971, van Warmelo, 1971).

The actual arrangement of chromosomes immediately prior to, and during, separation has been the subject of intensive investigation and a large number of different mechanisms of chromosomal separation have been proposed as a result.

The formation of typical metaphase plates was reported (Robinow, 1963; Finley, 1970) and also the separation of discrete chromatids on a well-defined spindle (Knox-Davies, 1966, 1967; Ichida & Fuller, 1968; McManus & Roth, 1968; Motta, 1969; Brushaber & Jenkins, 1971). Aist (1969), however, maintained that instead of forming a true metaphase plate the chromosomes became attached to the spindle at different points. During anaphase separation the chromatids thus became strung out on the spindle.

Instead of separate chromosomes, the presence of a single coherent strand, formed by the linked chromosomes has been reported (Weijer, Koopmans & Weijer, 1965; Dowding, 1966; Weijer & Weisberg, 1966; Brushaber, Wilson & Aist, 1967; Laane, 1967; Namboodiri & Lowry, 1967; Heale, Gafoor & Rajasingham, 1968; Brushaber & Jenkins, 1971). The single nuclear strand is thought to replicate to form a double strand which then separates in a number of ways. Movement of each replicated strand on a well defined spindle was reported by Heale *et al.* (1968). Spindles were, however, apparently absent in the other fungi showing filamentous nuclei. Shearing of chromatids by cytoplasmic streaming was suggested by Weijer *et al.* (1965) but, whereas this proposal received some support (Laane, 1967), it has not been generally accepted. In the absence of a spindle the exact mechanism of chromatid disjunction and movement is not yet fully understood.

Judging from the conflicting descriptions given above it seems possible, as suggested by Bracker (1967), that there may be a number of mechanisms of somatic nuclear division in the fungi, instead of one common process.

MATERIALS AND METHODS

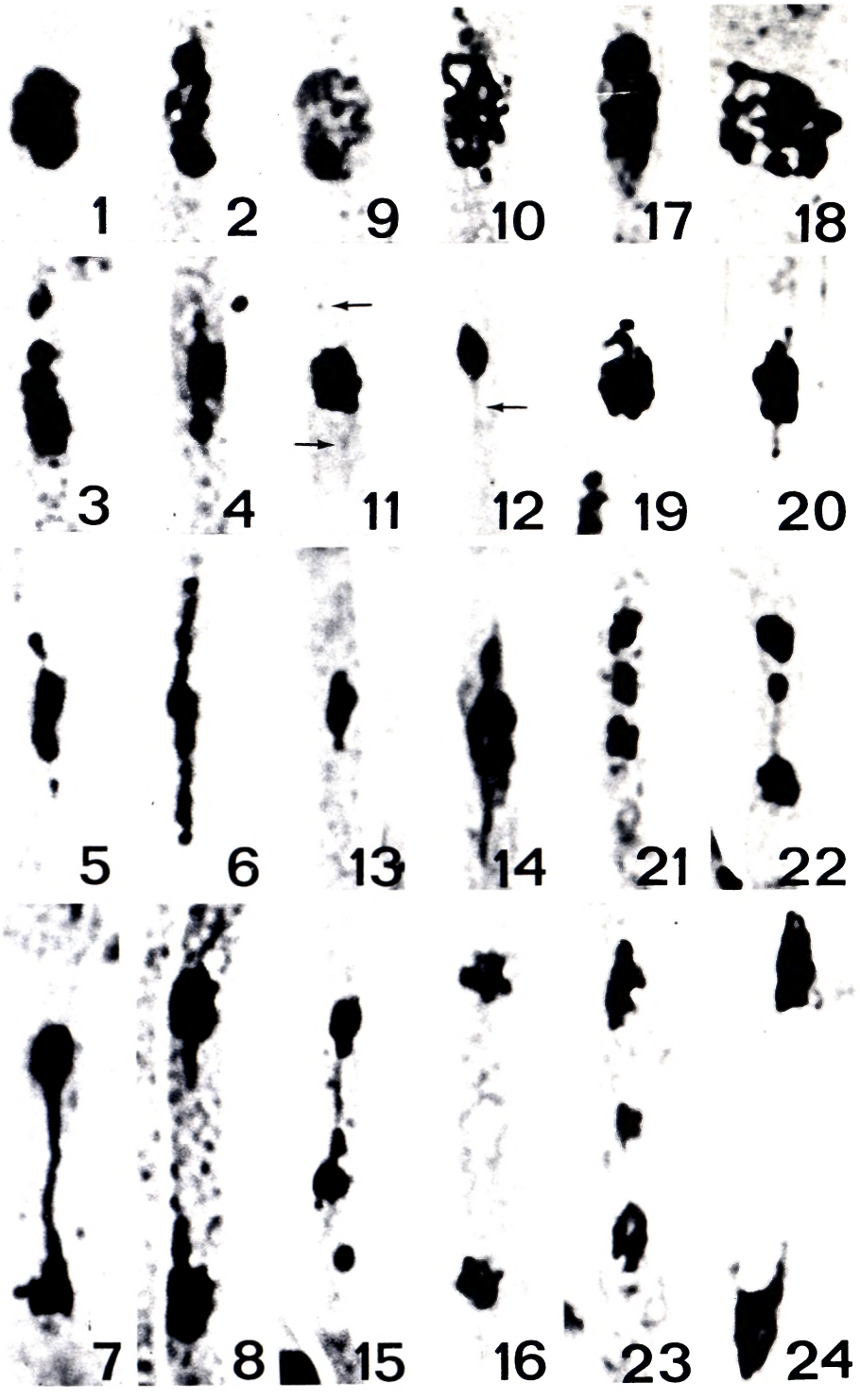
Three cultures were examined in this investigation:

1. *Neocosmospora vasinfecta* E. F. Smith; CBS 237.75.
2. *Neocosmospora africana* von Arx; CBS 237.
3. *Neocosmospora* sp. isolated from soybean stem, Pietermaritzburg, South Africa. Referred to in the following text as "isolate P."

Somatic divisions were studied using the macerated mycelium technique described by Ward & Ciurysek (1962). After being stained using the HCl-Giemsa technique (van Warmelo, 1971) the hyphae were flattened under a coverglass which was then sealed with paraffin oil.

* Based on a Ph.D. (Agric.) thesis submitted to the University of Stellenbosch, 1973. Promoter: Prof. P. S. Knox-Davies, Ph.D. (Wis.).

† Rand Afrikaans University, P.O. Box 524, Johannesburg.



FIGS 1–8.—Mitosis in *Neocosmospora vasinfecta*, ×3000. 1, early prophase; 2, prophase with clearly visible chromosomes; 3, late prophase with short chromosomes; 4, mid metaphase with six distinguishable chromosomes; 5, metaphase/anaphase; 6, mid anaphase showing chromosomes between clearly visible centriolar plaques; 7, early telophase with spindle bridge apparently enclosed within nuclear membrane; 8, early telophase.

FIGS 9–16.—Mitosis in *Neocosmospora africana*, ×3000. 9, early prophase with visible chromosomes; 10, prophase; 11, prometaphase in polar view with visible centriolar plaques (arrowed); 12, very early metaphase with forming spindle (arrowed); 13, early metaphase; 14, anaphase; 15, early telophase with spindle bridge; 16, telophase.

FIGS 17–24.—Mitosis in *Neocosmospora* isolate P, ×3000. 17, early prophase with visible chromosomes and nuclear membrane; 18, prophase in large hypha with clearly visible chromosomes; 19, prophase with shortened chromosomes; 20, mid metaphase with formed spindle; 21, early anaphase; 22, late anaphase with chromosome disjunction almost completed; 23, late anaphase showing residual interzonal fibres; 24, late telophase.

RESULTS

As the mechanisms of somatic division in the three cultures did not show any significant differences, the processes of division will not be described separately. Individual isolates will be highlighted only where necessary.

The interphase nuclei were large and typical. The onset of prophase was shown by the delimitation of chromosomes within the nuclei (Figs 1, 9 and 17). Initially long and thin, the chromosomes were shorter and more distinct at mid-prophase (Figs 2, 10 and 18). Nucleoli which were clearly distinguishable at early prophase, were usually absent at mid-prophase. The size of the somatic cell appeared to affect the size of the nucleus. In isolate P (Fig. 18) cells of large diameter were found in which the nuclei were much larger, and the chromosomes much clearer, than in the more common narrow hyphae. At late prophase the chromosomes were appreciably shorter than at early prophase, but could not be counted (Figs 3 and 19). Occasional persistent nucleoli were seen at this stage.

At prometaphase (Fig. 11) centriolar plaques were sometimes distinguishable.

During early metaphase the chromosomes had condensed further and were shorter than at late prophase (Fig. 12). At metaphase they were situated midway between the two centriolar plaques and incipient spindles were observed (Figs 12 and 13). At mid-metaphase the spindle was clearly distinguishable (Figs 5 and 20).

Occasionally individual chromosomes were distinguished at metaphase (Figs 4 and 20) and a count at this stage gave an estimated number of six.

Anaphase was a variable stage and chromosome separation appeared to be asynchronous, as a variety of nuclear figures were found (Figs 6, 14, 21 and 22). At late anaphase the interzonal fibres between the terminal chromosome aggregations were occasionally observed (Fig. 23).

At early telophase the two daughter nuclei became reconstituted but were frequently connected by a chromosome or spindle bridge (Figs 7 and 15) apparently still enclosed within the nuclear membrane. The bridge then broke and shortened (Fig. 8). The divisions thus appear to be entirely intra-nuclear.

Mid- and late-telophase nuclei (Fig. 16) were dense and smaller than post-division interphase nuclei (Fig. 24) which were identical with pre-division interphase nuclei.

At no stage were nuclear strands observed or stages seen which would have suggested linking-up of individual chromosomes.

DISCUSSION

No significant differences in nuclear division were found to occur in these three cultures. This agrees with their close similarity in other respects.

Due to the small diameter of most somatic hyphae there is probably insufficient space available for the formation of a typical metaphase plate, as found in higher organisms, and for the simultaneous disjunction and movement of chromatids on the spindle. The only mechanism which would ensure an equal distribution of chromatids under such conditions would be a sequential or asynchronous separation. Pairs of chromatids would then separate and move away from the central spindle area before separation of the next chromatid pair, thus ensuring non-interference by non-sister chromatids destined for different daughter nuclei.

Although anaphase separation was asynchronous there was no evidence of irregular chromosome disjunction or aneuploidy. This was deduced from the absence of lagging chromosomes or fragments at late telophase or interphase, indicating complete reconstitution of daughter nuclei, the constancy of chromosome numbers and the phenotypic stability of the cultures.

The consistent presence of observably separate chromosomes during nuclear division, coupled with the absence of stages showing linking of chromosomes, indicates that somatic nuclear division in *Neocosmospora* can be interpreted as strictly mitotic.

ACKNOWLEDGEMENTS

This study was supported by research grants from the Rand Afrikaans University, Johannesburg and the Council for Scientific and Industrial Research, Pretoria.

UITTREKSEL

'n Swamsoort afkomstig van sojaboon weefsel het duidelike ooreenkomstes getoon met twee bestaande Neocosmospora *soorte, nl.* N. vasinfecta *E. F. Smith en* N. africana *von Arx. Die somatiese kerndelingsprosesse in outentieke kulture van hierdie twee organismes en in die nuwe isolaat is ondersoek om vas te stel wat die delingsmeganismes was en of daar verskille tussen die kulture aanwesig was. Geen wesenlike verskille tussen die kulture is opgemerk nie. Die delings het asinkroniese anafaseskeidings getoon maar daar was geen tekens van aneuploiedie of onreëlmatige vorming van die dogterkerne nie. Die meganisme van kerndeling is suiwer mitoties.*

REFERENCES

AIST, J. R., 1969. The mitotic apparatus in Fungi, *Ceratocystis fagacearum* and *Fusarium oxysporum*. *J. Cell Biol.* 40: 120–135.

ALDRICH, H. C., 1969. The ultrastructure of mitosis in myxamoebae and plasmodia of *Physarum flavicomum*. *Am. J. Bot.* 56: 290–299.

BRACKER, C. E., 1967. Ultrastructure of fungi. *A. Rev. Phytopath.* 5: 343–374.

BRUSHABER, J. A. & JENKINS, S. F., (Jr.) 1971. Mitosis and clamp formation in the fungus *Poria monticola*. *Am. J. Bot.* 58: 273–280.

BRUSHABER, J. A., WILSON, C. L. & AIST, J. R., 1967. Asexual nuclear behavior of some plant pathogenic fungi. *Phytopathology* 57: 43–46.

DOWDING, E. SILVER, 1966. The chromosomes in *Neurospora* hyphae. *Can. J. Bot.* 44: 1121–1125.

FINLEY, D. E., 1970. Somatic mitosis in *Ceratobasidium flavescens* and *Pellicularia koleroga*. *Mycologia* 62: 474–485.

HEALE, J. B., GAFOOR, A. & RAJASINGHAM, K. C., 1968. Nuclear division in conidia and hyphae of *Verticillium albo-atrum*. *Can. J. Genet. Cytol.* 10: 321–340.

HEATH, I. B. & GREENWOOD, A. D., 1968. Electron microscopic observations of dividing somatic nuclei in *Saprolegnia*. *J. Gen. Microbiol.* 53: 287–289.

HOSFORD, R. M. & GRIES, G. A., 1966. The nuclei and parasexuality in *Phymatotrichum omnivorum*. *Am. J. Bot.* 53: 570–579.

ICHIDA, A. A. & FULLER, M. S., 1968. Ultrastructure of mitosis in the aquatic fungus *Catenaria anguillulae*. *Mycologia* 60: 141–155.

KNOX-DAVIES, P. S., 1966. Nuclear division in the developing pycnospores of *Macrophomina phaseoli*. *Am. J. Bot.* 53: 220–224.

KNOX-DAVIES, P. S., 1967. Mitosis and aneuploidy in the vegetative hyphae of *Macrophomina phaseoli*. *Am. J. Bot.* 54: 1290–1295.

LAANE, M. M., 1967. The nuclear division in *Penicillium expansum*. *Can. J. Genet. Cytol.* 9: 342–351.

MCMANUS, SISTER M. A. & ROTH, L. E., 1968. Ultrastructure of the somatic nuclear division in the plasmodium of the Myxomycete *Clastoderma debaryanum*. *Mycologia* 60: 426–436.

MOOR, H., 1966. Ultrastrukturen im Zellkern der Bäckerhefe *J. Cell Biol.* 29: 153–155.

MOTTA, J. J., 1969. Somatic nuclear division in *Armillaria mellea*. *Mycologia* 61: 873–886.

NAMBOODIRI, A. N. & LOWRY, R. J., 1967. Vegetative nuclear division in *Neurospora*. *Am. J. Bot.* 54: 735–748.

ROBINOW, C. F., 1963. Obervations on cell growth, mitosis, and division in the fungus *Basidiobolus ranarum*. *J. Cell Biol.* 17: 123–152.

ROBINOW, C. F. & MARAK, J., 1966. A fiber apparatus in the nucleus of the yeast cell. *J. Cell Biol.* 29: 129–151.

THYAGARAJAN, T. R., CONTI, S. F. & NAYLOR, H. B., 1962. Electron microscopy of *Rhodotorula glutinis*. *J. Bact.* 83: 381–394.

VAN WARMELO, K. T., 1971. Somatic nuclear division in *Stemphylium botryosum*. *Bothalia* 10: 329–334.

VAN WINKLE, W. B., BIESELE, J. J. & WAGNER, R. P., 1971. The mitotic spindle apparatus of *Neurospora crassa*. *Can. J. Genet. Cytol.* 13: 873–887.

WARD, E. W. B. & CIURYSEK, K. W., 1962. Somatic mitosis in *Neurospora crassa*. *Am. J. Bot.* 49: 393–399.

WEIJER, J., KOOPMANS, A. & WEIJER, D. L., 1965. Karyokinesis of somatic nuclei of *Neurospora crassa*. III. The juvenile and maturation cycles (Feulgen and crystal violet staining). *Can. J. Genet. Cytol.* 7: 140–163.

WEIJER, J. & WEISBERG, S. H., 1966. Karyokinesis of the somatic nucleus of *Aspergillus nidulans*. I. The juvenile chromosome cycle (Feulgen staining). *Can. J. Genet, Cytol.* 8: 361–374.

ZICKLER, DENISE, 1970. Division spindle and centrosomal plaques during mitosis and meiosis in some Ascomycetes. *Chromosoma* 30: 287–304.