Bothalia 13, 3 & 4: 415–429 (1981)

Sexual nuclear division in *Neocosmospora**

K. T. VAN WARMELO**

ABSTRACT

The process of sexual nuclear division in authentic isolates of *Neocosmospora vasinfecta* E. F. Smith, *N. africana* Von Arx and a related species isolated from soybean was studied. No significant differences were found. Six chromosomes were counted in each isolate. There was no evidence of aneuploidy or irregular reconstitution of daughter nuclei. The divisions leading to the formation of binucleate, homokaryotic ascospores were typically meiotic.

RÉSUMÉ

DIVISION NUCLÉAIRE SEXUELLE CHEZ LE NEOCOSMOSPORA

Le processus de division nucléaire sexuelle dans des isolats authentiques de Neocosmospora vasinfecta *E. F. Smith,* N. africana *Von Arx et une espèce apparentée isolée du soya a été étudié. Aucune différence significative n'a été trouvée. Six chromosomes ont été comptés dans chaque isolat. Il n'y eut aucune évidence d'aneuploïdie ou de reconstitution irrégulière des noyaux filles. Les divisions conduisant à la formation du binucleate, d'ascospores homokariotiques furent typiquement meiotiques.*

INTRODUCTION

A fungus isolated from soybean stem material showed great similarity in morphology and dimensions to two existing species of *Neocosmospora*, i.e. *N. vasinfecta* and *N. africana*. In fact, the local culture could be placed almost equally well in either species. It was further noticed that there were very few morphological differences between the two existing species. A study was, therefore, undertaken to examine the differences and similarities in the various processes and structures of the local isolate and authentic cultures of the two similar species. This paper reports on the nuclear divisions leading to ascosporogenesis.

REVIEW

The genus *Neocosmospora* was described in 1899 (Ferry, 1900). The type, and only species at that time, was *N. vasinfecta* (Atk.) Smith, derived from *Fusarium vasinfecta* Atk. (Seaver, 1909), which had been isolated from soil and had caused damping off of several types of cultivated plants.

Neocosmospora remained monotypic until Von Arx (1955) described a new species, *N. africana* Von Arx, which had been isolated from soil under grassland plots near Johannesburg. *N. africana* has not yet been implicated as a plant pathogen (Udagawa, 1963).

According to Von Arx (1955) *N. africana* differed from *N. vasinfecta* only in possessing a smooth epispore which was fairly regular in thickness. Von Arx proposed that, despite the differences in external spore texture, his new species should be included in the genus *Neocosmospora*. The surface texture of ascospores from authentic culture of both species of *Neocosmospora* have been reported to show very little differences under scanning microscopic examination (Van Warmelo, 1976).

Doguet (1956) made a detailed examination of the development of the perithecia of both *N. vasinfecta* and *N. africana* and found that it followed the same sequence in both species. This supported the statement by Von Arx (1955) that *N. africana* was similar to *N. vasinfecta* except for spore surface.

The process of meiosis in the fungi has received much attention and the mechanisms of division have been well described in a large variety of fungi (Olive, 1953 & 1965). Further significant contributions were made by Rogers (1964, 1965, 1967, 1968a, 1968b, 1968c & 1971), Lu (1966, 1967a & 1967b), Aldrich (1967), Barry (1967), Furtado (1970), Huguenin & Boccas (1970) and Wells (1970).

Division of the diploid nucleus follows a pattern basically similar to that established for the higher plants (Lu, 1966; Swanson, 1968; Woo & Partridge, 1969; Wells, 1970). There are, therefore, the same stages and processes during prophase 1 which lead to a recombination and segregation of chromatin after completion of the meiotic division. There are, however, some differences between the meiotic divisions in fungi and higher plants.

The nuclear membrane remains intact throughout the division (Huffman, 1968) or becomes discontinuous at the poles only (Aldrich, 1967). In these cases the spindles are fully intranuclear. Degeneration of the membrane also occurs, however, during the division, for example during anaphase (Wells, 1970), during metaphase (Olive, 1965; Lu, 1967b) and even as early as pachytene (Uecker, 1967).

The formation of a metaphase plate, which is infrequent in the fungi (Olive, 1965; Wells, 1970), was reported by Furtado (1970) and Rao & Mukerji (1970).

Centriolar plaques (also described as centrioles and centrosomes) occur in some organisms (Knox-Davies & Dickson, 1960; Lu, 1967a & 1967b; Uecker, 1967; Wells, 1970), but are absent in others (Aldrich, 1967).

Two types of fibres are found in the spindle, viz. chromosomal fibres which are attached to the chromosomes, and spindle fibres which are continuous between the centriolar plaques (Aldrich, 1967; Lu, 1967b; Wells, 1970). It is important to note

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

**Rand Afrikaans University, P.O. Box 524, Johannesburg, 2000.

that the spindle fibres may form in the absence of the centriolar plaques (Aldrich, 1967), in which case the fibres converge at the discontinuity of the nuclear membrane.

Asynchronous disjunction of chromosomes was frequently reported (Rogers, 1964, 1965, 1967; Olive, 1965; Uecker, 1967; Huffman, 1968; Furtado, 1969; Wells, 1970) and seems to be general in the fungi.

In the Pyrenomycetes the second meiotic division is commonly followed by a third division to give rise to eight haploid nuclei around which the ascospores then form (Singleton, 1953; Olive, 1965). Further division may take place within the delimited ascospores to give rise to multinucleate ascospores (Van Warmelo, 1966). Ascospores may, however, be delimited at the 16-nucleate stage (Furtado, 1970; Rao & Mukerji, 1970), or delimitation may occasionally be highly irregular (Rogers, 1967).

As a result of the frequency with which the regular processes of ascosporogenesis have been found in the Pyrenomycetes, this pattern of development is generally applied to all Ascomycetes, even to species in which few details are available. However, extrapolated data can be misleading, as was shown by Rajendren (1967), who found that in *Hemileia vastatrix* the nuclear cycle did not agree entirely with that expected from earlier work on the Uredinales.

According to Doguet (1956) the ascogonium of *Neocosmospora vasinfecta* arises by somatogamy and gives rise to numerous ascogenous hyphae. Moreau & Moreau (1950), however, stated that dikaryotization occurs by means of a 'short antheridial filament', which associates closely with the coiled ascogonium. Doguet (1956) did not observe karyogamy in the penultimate cell of the crozier. The presence of nuclei with what appeared to be two nucleoli was, however, taken as an indication that karyogamy had occurred.

According to Doguet (1956) nuclear divisions in the ascus follow the normal pattern leading to the formation of eight binucleate ascospores. Unfortunately only very few drawings of the various divisions were published. Doguet also stated that the mechanism of ascosporogenesis in *N. africana* is identical to that found in *N. vasinfecta,* implying that an ascogonium is formed by somatogamy and that the ascospores are binucleate. None of these stages was, however, illustrated.

MATERIALS AND METHODS

Three cultures were examined in this investigation:

1. *Neocosmospora vasinfecta* E. F. Smith; CBS 237.55.
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'.

Cultures were maintained on 1,5% Difco malt agar and yeast-starch agar (Cooney & Emerson, 1964) at 25°C under intermittent illumination, 12 h/d, by near ultra violet light (NUV) fluorescent tubes (GEC F15T8/BLB). Isolate P was also maintained on Difco oatmeal agar.

The nuclear divisions leading to ascosporogenesis were examined in all three isolates studied. Identical techniques were used for similar stages in the different isolates to ensure absolute comparability.

Nuclei were stained using the HCL-Giemsa technique (Van Warmelo, 1971) and examined and photographed under bright field illumination with a yellow green filter.

RESULTS

Unless otherwise stated, all the following observations apply equally to all three examined specimens.

The earlier sexual stage seen was the multinucleate ascogonium (Figs 1, 2, 40, 83 & 84). The ascogonia varied in size and form from the highly nucleated, simple spherical ascogonium (Fig. 1) to the elongated, cylindrical ascogonium (Figs 83 & 84) and the complex convoluted ascogonium (Figs 2 & 40). The nuclei were small, densely stained and associated in pairs. At no stage was the mechanism of dikaryotization observed. Ascogonia were found in very young perithecia and were identified as ascogonia on the basis of the usually multinucleate condition with commonly, associated pairs of nuclei.

The only ascogenous hyphae seen were found in *Neocosmospora africana* (Fig. 41). These were short, indistinct and typically binucleate. After a very restricted growth they showed signs of crozier formation.

Crozier and young ascus formation

Binucleate pre-croziers were found in *N. africana* (Fig. 42) and isolate P (Fig. 85). These were rounded and did not yet show the characteristic curvature of the crozier. The binucleate condition was, however, an indication of their origin.

Typical binucleate croziers were found (Figs 3, 43 & 86). Their development into young asci followed the typical pattern (Figs 4, 44, 45, 46, 47, 87 & 88). Occasional large croziers were found (Fig. 5), but were not of unusual significance.

The young ascus enlarged and became cylindrical after karyogamy (Figs 48, 49 & 89) which gave rise to the diploid nucleus (Figs 6, 50 & 90).

The nuclei showed marked variations in appearance during karyogamy and the subsequent diplophase. At the conclusion of the conjugate division in the binucleate crozier the nuclei were small and intensely and uniformly stained. During karyogamy the nuclei no longer stained uniformly and showed fragments of linear bodies interpreted as partially condensed chromosomes. The nuclei in the terminal and basal cells remained unchanged. The diploid nuclei were less varied in appearance. A featureless uneven mass of chromatin, apparently surrounded by a nuclear membrane, was sometimes observed (Fig. 90). Older diploid nuclei showed differentiation (Figs 6 & 50).

Division 1

Nuclear divisions in the asci are numbered according to the system used by Singleton (1953).

During prophase of the first meiotic division the nuclei enlarged and were usually centrally placed in the ascus. There was very little differentiation of chromosomes at this stage (Figs 7 & 91) and the uninucleate basal and terminal cells of the crozier were often still present at the base of the ascus (Figs 50, 91 & 92).

At leptotene the chromosomes were readily distinguishable as long thin strands (Figs 8, 51 & 92). In Fig. 92 there is evidence of a nuclear membrane around the chromosome mass. Nucleoli were demonstrated in some leptotene nuclei (Fig. 51), but not in others (Fig. 8).

At zygotene the chromosomes were much more distinct, and thickenings and other details became visible. The nuclei also appeared to be larger than during leptotene, possibly due to a better spread of a less cohesive chromosomal mass during squashing of the asci.

Zygotene showed chromosomes which were identified as homologues by the similarities in the terminal chromomere patterns (Figs 9, 10, 52, 53 & 93). Chromosomes pairs were seen in which synapsis was apparently completed while other chromosomes were only partially associated (Figs 52 & 93).

At pachytene the synapsed homologues were closely associated and were no longer distinguishable as chromosome pairs (Figs 11, 54 & 94). Occasionally fully synapsed pairs were found in which the two component chromosomes of the bivalent could be seen (Fig. 14). Nucleoli were only occasionally present during pachytene (Fig. 15).

Pachytene stages in which the chromosomes could be counted were found in *N. vasinfecta* (Figs 12 & 13) and isolate P (Figs 95 & 96). No countable stages were found in *N. africana*.

In *N. vasinfecta* (Figs 12 & 13) six chromosomes were counted and their lengths estimated. Centromeres could not be distinguished.

The estimated lengths of the six chromosomes, numbered in descending order of length (Singleton, 1953), were:

1 = 13,5 μm 2 = 7,5 μm 3 = 6,0 μm
4 = 5,25 μm 5 = 1,75 μm 6 = 1,5 μm

The lengths of chromosomes 5 and 6 were probably underestimated as the chromosomes appeared to have been compressed during squashing of the preparation.

Six chromosomes were also counted in isolate P (Figs 95 & 96). As in *N. vasinfecta,* no centromeres were distinguishable and the nucleolus had disappeared. The estimated lengths of the six chromosomes were:

1 = 25 μm 2 = 15 μm 3 = 12 μm
4 = 11 μm 5 = 8 μm 6 = 7 μm

Although there was little agreement between the estimated chromosome lengths of *N. vasinfecta* and isolate P, the relative lengths as given below agreed closely.

Relative chromosome lengths

	N. vasinfecta	Isolate P	Difference
Chromosomes 1:2	1,80	1,67	0,13
Chromosomes 2:3	1,25	1,25	0,00
Chromosomes 3:4	1,14	1,09	0,05
Chromosomes 4:5	3,00	1,38	1,62
Chromosomes 5:6	1,17	1,14	0,03

Only in the case of the ratio of chromosomes 4 and 5 is there a marked difference between the two fungi. It was pointed out above, however, that the lengths of chromosomes 5 and 6 in *N. vasinfecta* were probably underestimated.

During diplotene the bivalents shortened and became fuzzy in outline (Fig. 97). Crossover points were seen as small loops in the contracted bivalents (Figs 16, 55 & 98). Late diplotene chromosomes were noticeably shorter than pachytene chromosomes (Fig. 99) and were also thinner.

In both *N. vasinfecta* and *N. africana* what appeared to be a ring chromosome was seen during diplotene (Figs 16 & 55). There was however, no evidence of a ring chromosome during pachytene.

Diakinesis showed no abnormal or unexpected features (Figs 17, 56 & 100). The chromosomes were short, highly condensed and almost spherical. They could not be counted.

Metaphase I chromosomes were highly condensed, almost spherical and very short (Figs 18, 57 & 101). They were not arranged in a metaphase plate across the equatorial plane of the spindle, but appeared as a dense central aggregation of chromatin in the spindle. Spindles were sometimes clearly seen (Fig. 101), but were more frequently indistinct, their presence and orientation being indicated by diffraction lines in the cytoplasm of the ascus (Figs 57 & 58). Spindles were orientated along the long axis of the ascus (Figs 18 & 58), or at right angles to it (Figs 57 & 102), or obliquely (Fig. 101).

Chromosome counts at metaphase I were unreliable due to the close association of the chromosomes. Occasionally, however, fairly reliable counts could be made. In polar view (Fig. 102) six chromosomes were distinguished in isolate P, while the same number was seen in *N. africana* (Fig. 58).

In isolate P the chromosome count at metaphase I agreed with that made at pachytene. Although no countable pachytene stages were found in *N. africana,* the chromosome number at metaphase I was the same as that found in both *N. vasinfecta* and isolate P. Important also was the observation that, in *N. africana,* there appeared to be four large chromosomes and two smaller ones (Fig. 58), a situation similar to that found at pachytene in *N. vasinfecta.* In isolate P (Fig. 102) there were also size differences between the chromosomes but they were less marked.

It was impossible to estimate the chromosome sizes from metaphase I figures.

Separation of the chromosomes during anaphase I appeared to be sequential rather than synchronous and a number of different chromosome arrangements were seen. In *N. africana* a series of stages illustrating sequential separation were found. A stage was seen of the transition from metaphase I to anaphase I (Fig. 59) in which the arrangement of the chromosomes was virtually identical with one at metaphase I (Fig. 58). The only differences was that one or two bivalents had separated before the other chromosomes. A further stage was seen (Fig. 60) in which separation of the homologues had occurred with only slight movement of the chromosomes on the spindle. An even later stage was seen (Fig. 61) in which movement of the chromosomes had begun whilst maintaining a similar chromosomal arrangement.

Sequential rather than synchronous separation of bivalents was also seen in *N. vasinfecta* (Fig. 19) and isolate P (Fig. 103). Precocious chromosomes were linked to the median unseparated bivalents by thin spindle strands.

At mid anaphase I the condensed chromosomes were spread in a band along the spindle (Fig. 62) and individual chromosomes could not be distinguished. At late anaphase I the chromosomes had started to form terminal groups (Figs 20 & 104).

Telophase I nuclei were usually small, spherical and homogeneous. At early telophase I there were occasional lagging chromosomes (Figs 63 & 106). However, no case was seen in which any lagging chromosomes failed to be incorporated in the daughter nuclei. The reconstituted daughter nuclei rounded off at the conclusion of the division, connected only by the remains of the spindle (Figs 64 &

105). Discarded nucleoli were occasionally seen (Fig. 107).

At the conclusion of telophase I the nuclei entered interphase I. Generally this process was asynchronous and asci were found in which one nucleus was in interphase I while the sister nucleus was still in telophase I (Fig. 21).

Interphase I nuclei were larger than at telophase, approximately spherical and heterogeneous (Figs 22, 65 & 108). Individual chromosomes could not be distinguished, although nucleolus-like bodies were sometimes seen (Fig. 22).

Division II

Prophase II nuclei were less diffuse than at interphase I and stained intensely (Figs 23, 66 & 109). The chromosomes of *N. vasinfecta* and *N. africana* were short, whereas those of isolate P were indistinct. In *N. vasinfecta* nucleoli were seen in the nuclei (Fig. 23). Nucleoli were not seen in *N. africana* and isolate P.

Metaphase II was indistinct. At early metaphase the deeply-staining chromosomes were arranged centrally between the two centriolar plaques (Fig. 67). The chromosomes were so closely associated that counts were impossible (Figs 24, 68 & 110). Centriolar plaques were not always visible although spindles were sometimes seen (Fig. 110).

Anaphase II followed the same general pattern as anaphase I. Spindles were either parallel with (Fig. 69), or across (Figs 111 & 112) the long axis of the ascus. Sequential disjunction of the chromatids was once again suggested by the linear arrangement of chromosomes with interconnecting interzonal or spindle fibres.

Occasionally chromosomes were clearly delimited at anaphase II (Fig. 113). Six chromosomes were seen at this stage.

Telophase II nuclei (Figs 25, 70, 114 & 115) were identical with the telophase I nuclei. No lagging chromosomes or spindle bridges were seen at this stage.

Interphase II nuclei (Figs 26, 71 & 116) resembled the interphase I nuclei described. The four interphase II nuclei were commonly arranged as two adjacent pairs arranged symmetrically around the centre of the ascus (Figs 26 & 116).

Division III

Prophase III nuclei resembled the prophase II nuclei described. Initially long and diffuse, the chromosomes shortened and became intensely stained (Figs 27, 72, 117 & 118).

Prometaphase III stages showed short dense chromosomes (Figs 28 & 73) and occasional centriolar plaques (Fig. 73).

Metaphase III nuclei (Figs 29 & 119) resembled the metaphase II nuclei described. The arrangement of the four nuclei followed no regular pattern.

Anaphase III was seen only in isolate P (Figs 120 & 121) and was similar to the previously described anaphase stages. Divisions of the nuclei were not fully synchronous (Figs 120 & 121) and alignment of the spindles was variable.

Lagging chromosomes at late anaphase III/early telophase III were seen (Fig. 30) but these became incorporated into the reconstituted telophase III nuclei. Evidence for this was the absence of lagging chromosomes at late telophase. These nuclei (Figs 74 & 122) were similar to the telophase stages described, but could also be larger (Fig. 123).

Interphase III nuclei (Figs 31, 32, 75 & 124) resembled the nuclei seen at interphase I and II.

The earliest signs of spore delimitation were seen at interphase III (Fig. 31). There were, however, interphase III stages with no signs of incipient cytoplasmic cleavage (Figs 75 & 124), whereas in some asci well developed cytoplasmic cleavages were seen (Figs 32 & 76).

Division IV

Prophase IV nuclei (Figs 33, 77 & 125) were similar to the prophase II and III nuclei described. In all isolates asci in prophase IV without any signs of incipient cytoplasmic cleavage were found (Figs 33, 77 & 125), whereas other asci also in prophase IV were found in which well defined spore initials were clearly visible (Figs 78 & 126).

Prometaphase IV (Fig. 127) was found in isolate P only and was similar to prometaphase stages of the preceding divisions. Metaphase IV and anaphase IV stages were not found in any of the isolates.

Telophase IV nuclei (Figs 34, 35, 79, 128, 129 & 130) were identical with the previously described telophase stages. Interphase IV nuclei (Figs 37 & 131) also resembled similar stages in the preceding divisions.

Neocosmospora vasinfecta differed from the other isolates with respect to spore delimitation. Whereas the spores in *N. africana* and isolate P were invariably delimited before telophase IV (Figs 79 & 129), occasional asci were found in *N. vasinfecta* which contained sixteen nuclei not separated by cytoplasmic cleavage planes (Figs 34 & 35). Such asci were large and commonly globose. Normal cylindric asci with cytoplasmic cleavage planes at this stage were common (Fig. 36). As the mature spores of *N. vasinfecta* were always found in asci of normal size, it is postulated that the large uncleaved 16-nucleate asci degenerated without delimiting spores. How, why and when this occurred could not be determined.

Following division IV the eight ascospores in an ascus were binucleate. Nuclei in spores of increasing age, as determined from the increasing thickness of the spore wall, showed a progressive condensation (Figs 38, 39, 80, 81, 82, 132, 133 & 134). Nuclei in mature spores were thus condensed and intensely stained.

Ascus proliferation

Fusion of the terminal cell of the crozier with the basal cell was commonly seen in *N. africana* and isolate P, but was less common in *N. vasinfecta*. A single ascogenous hypha could therefore give rise to further asci.

DISCUSSION

The mechanism of dikaryotization was not elucidated. As no signs of receptive hyphae or spermatium formation were seen, the most likely mechanism is random somatogamy. As all three isolates are homothallic this mechanism is not unlikely. Doguet's (1956) observation of defined ascogonial structures in *Neocosmospora vasinfecta* and *N. africana* was thus not confirmed.

It is well established that the sexual nuclear division in the ascus is an ordered process corresponding to gametogenesis in higher organisms. The division can therefore correctly be called meiotic.

Sequence of nuclear divisions in the ascus

The sequence of nuclear divisions leading to the formation of mature ascospores was normal in all three isolates. This confirms the statement by Doguet (1956) that ascosporogenesis in *N. vasinfecta* and *N. africana* follows the established pattern.

Homology of meiotic prophase chromosomes

Zygotene chromosome pairs were seen in all isolates in which synapsis was apparently completed while other chromosome pairs were only partially associated. Homology of certain chromosomes was deduced from the similarities in chromomere pattern. Synaptic failure could either indicate slow pairing or non-homology of chromosome pairs or areas. Only in *N. vasinfecta* was one configuration seen (Figs 12 & 13) which suggested incomplete synapsis. This could, however, have been due to a chromosome abnormality not necessarily indicative of non-homology.

Furthermore, non-homologous chromosomes in the karyotype would result in pachytene associations showing incomplete bivalent formation. No post-pachytene stages showing abnormalities were, however, observed. All the available evidence therefore indicates that, although synapsis during zygotene is rather slow, there is complete homology of all chromosomes pairs.

The chromosome number of each of the three isolates was determined at several stages during ascosporogenesis. Six chromosomes were counted in all cases. This number agrees with the counts made during mitotic divisions (Van Warmelo, 1977).

The lengths of the pachytene chromosomes were estimated in *N. vasinfecta* and isolate P. Although chromosome lengths at pachytene vary considerably with the structure of the bivalent and the degree of squashing during preparation of the material, it is reasonable to assume that all chromosomes in a single nucleus will be squashed to very much the same extent. Relative chromosome lengths are therefore probably more important than absolute lengths.

The relative lengths of the chromosomes of *N. vasinfecta* and isolate P are almost identical, even though their estimated absolute lengths are markedly dissimilar. If it is assumed that chromosomes 5 and 6 in *N. vasinfecta* (Fig. 13) became distorted during squashing, there is a close similarity between the karyotypes of these isolates.

Although no countable pachytene stages were seen in *N. africana,* it is significant that four long and two short chromosomes were present at metaphase I (Fig. 58). As the chromosomes were highly condensed the relative lengths could not be estimated. It is, therefore, postulated that the chromosome ratios of *N. africana* at least approximate the ratios determined for *N. vasinfecta* and isolate P.

Metaphase/anaphase

Even though spindles were not always seen, evidence for their presence was provided by the presence of centriolar plaques and also diffraction patterns in the cytoplasm around the chromosomes. When spindles were seen, their size and orientation agreed with the observed diffraction patterns. Spindles are presumably present during nuclear divisions, although they cannot always be demonstrated.

Metaphase separation is essentially similar in all the isolates. It is suggested that separation of chromosome pairs/chromatids is sequential rather than synchronous. These results are in accordance with the statement by Olive (1965) that asynchronous anaphase separation in the ascus appears to be the rule in the fungi.

Although anaphase separation was asynchronous, there was no evidence of irregular chromosome disjunction. This was deduced from the absence of lagging chromosomes at late telophase or interphase, indicating complete reconstruction of the daughter nuclei, the constancy of chromosome numbers and the phenotypic constancy of the cultures. It can, therefore, be assumed that the regularity of karyotype and genome replication is high.

Ascopore delimitation

The occasional absence of cytoplasmic cleavage planes at telophase IV and interphase IV might be regarded as an important feature of *Neocosmospora vasinfecta,* and an important difference between it and *N. africana* and isolate P. However, the fact that the 16-nucleate uncleaved asci were not seen to form ascospores and apparently degenerated, reduces the emphasis that should be placed on this characteristic as taxonomic criterion. Although it is a difference between *N. vasinfecta* and the other isolates, it does not seem to be an important one.

There are, therefore, no significant differences between *N. vasinfecta* E. F. Smith, *N. africana* Von Arx and 'isolate P' and the former two species cannot be distinguished on the basis of sexual chromosomal data.

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 sojaboonweefsel het duidelike ooreenkomste getoon met twee bestaande Neocosmospora *soorte, t.w.* N. vasinfecta *E. F. Smith en* N. africana *Von Arx. Die geslagtelike kerndelings in outentieke kulture van hierdie twee soorte en die plaaslike isolaat is ondersoek om vas te stel of daar verskille tussen die kulture was. Geen betekenisvolle verskille is waargeneem nie. Ses chromosome is in alle gevalle aangetref en hulle relatiewe lengtes was blykbaar dieselfde. Daar was geen tekens van aneuploiedie of onreëlmatige vorming van dogterkerne nie. Delings is dus tipies meioties en lei tot vorming van tweekernige homokariotiese askospore.*

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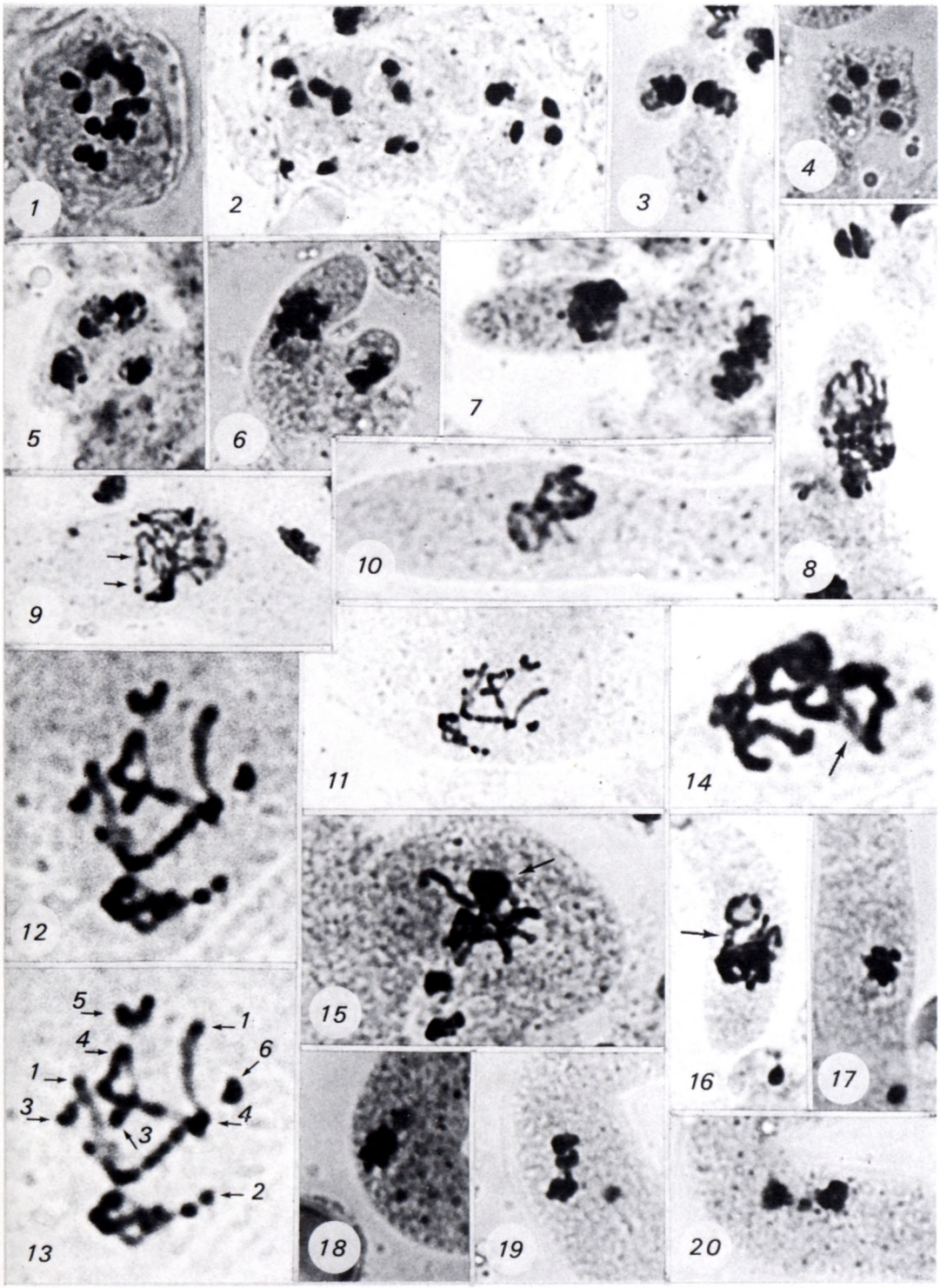
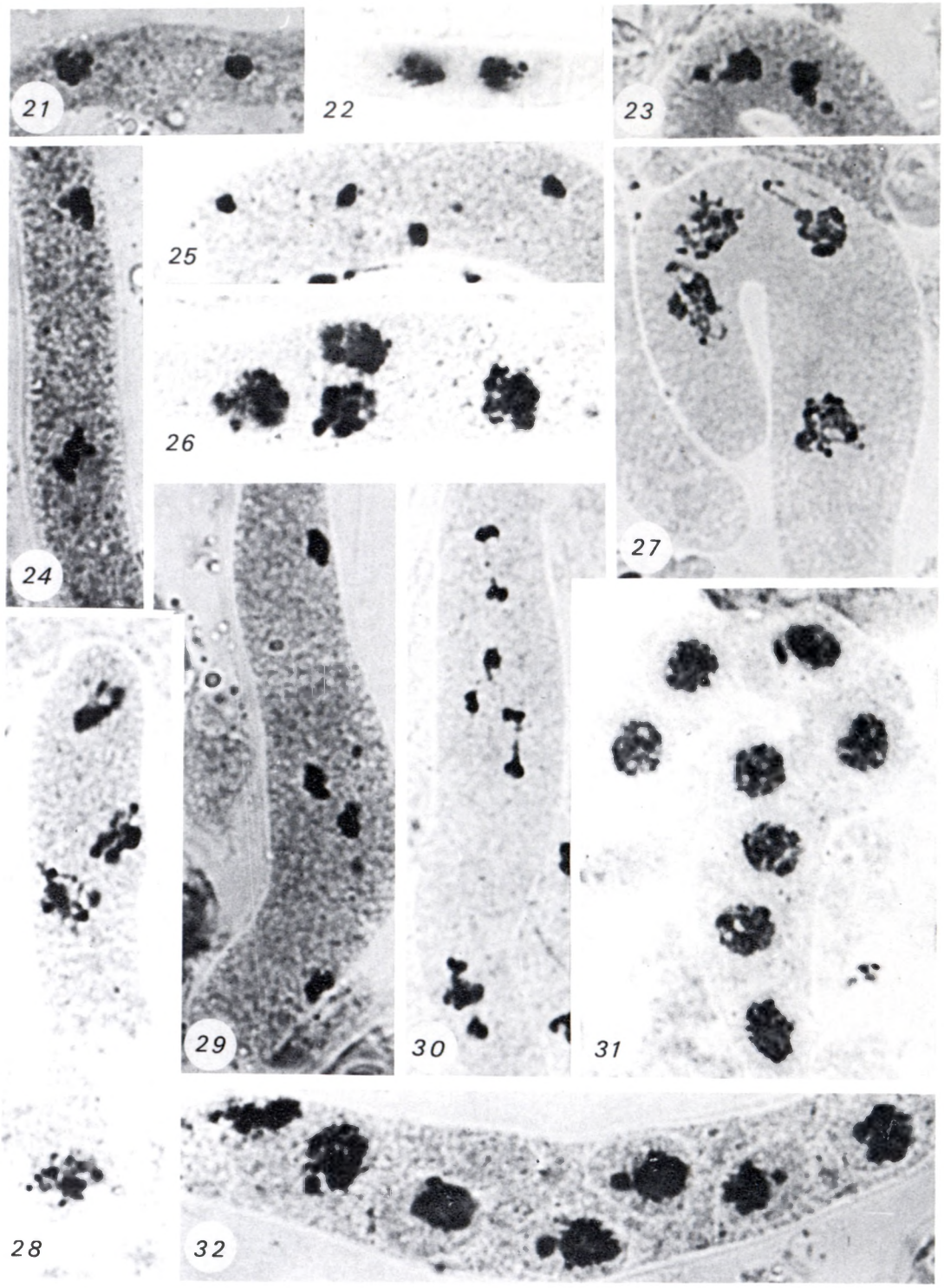
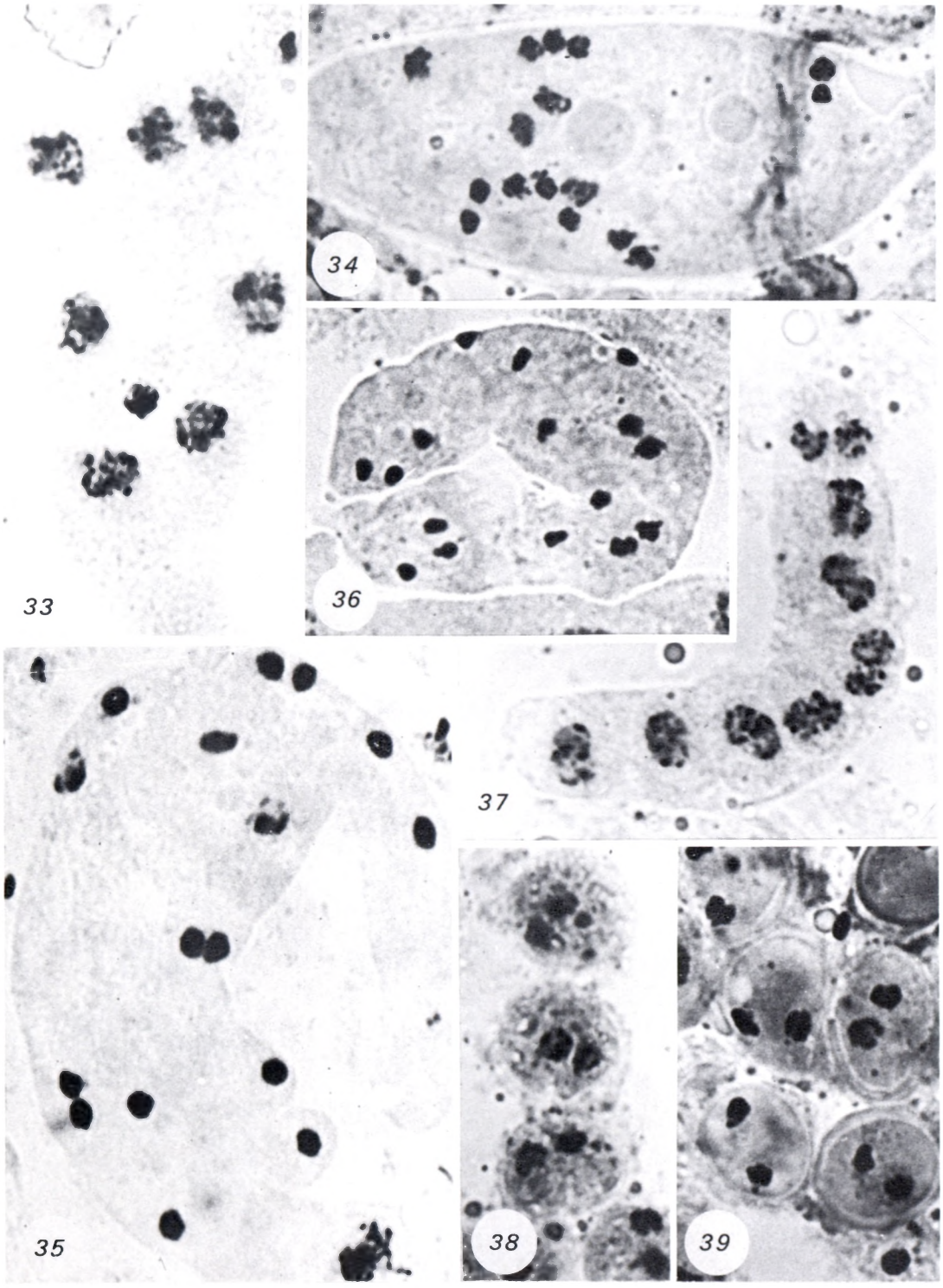


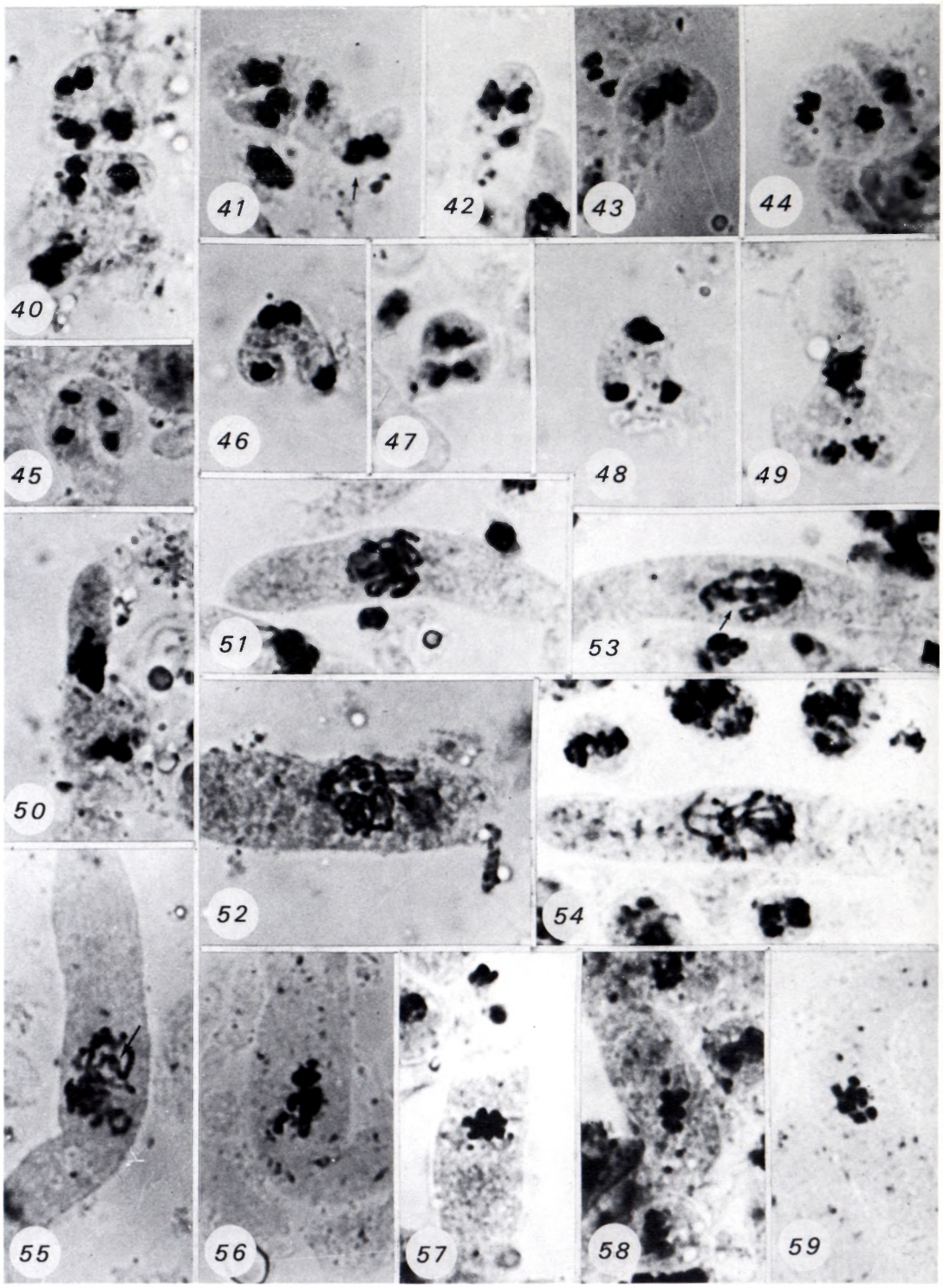
FIGS 1–20.—Meiosis in *Neocosmospora vasinfecta:* × 2 000 unless otherwise stated. 1, spherical multinucleate ascogonium; 2, morphologically complex multinucleate ascogonium; 3, binucleate crozier; 4, tetranucleate crozier; 5, tetranucleate crozier; 6, crozier after karyogamy; 7, beginning of prophase I; 8, leptotene; 9, zygotene; 10, zygotene/pachytene; 11, pachytene; 12, pachytene; 13, pachytene with numbered chromosomes, × 4 000; 14, pachytene showing bivalent structure, × 4 000; 15, late pachytene; 16, diplotene; 17, diakinesis; 18, metaphase I; 19, early anaphase I; 20, mid anaphase I.



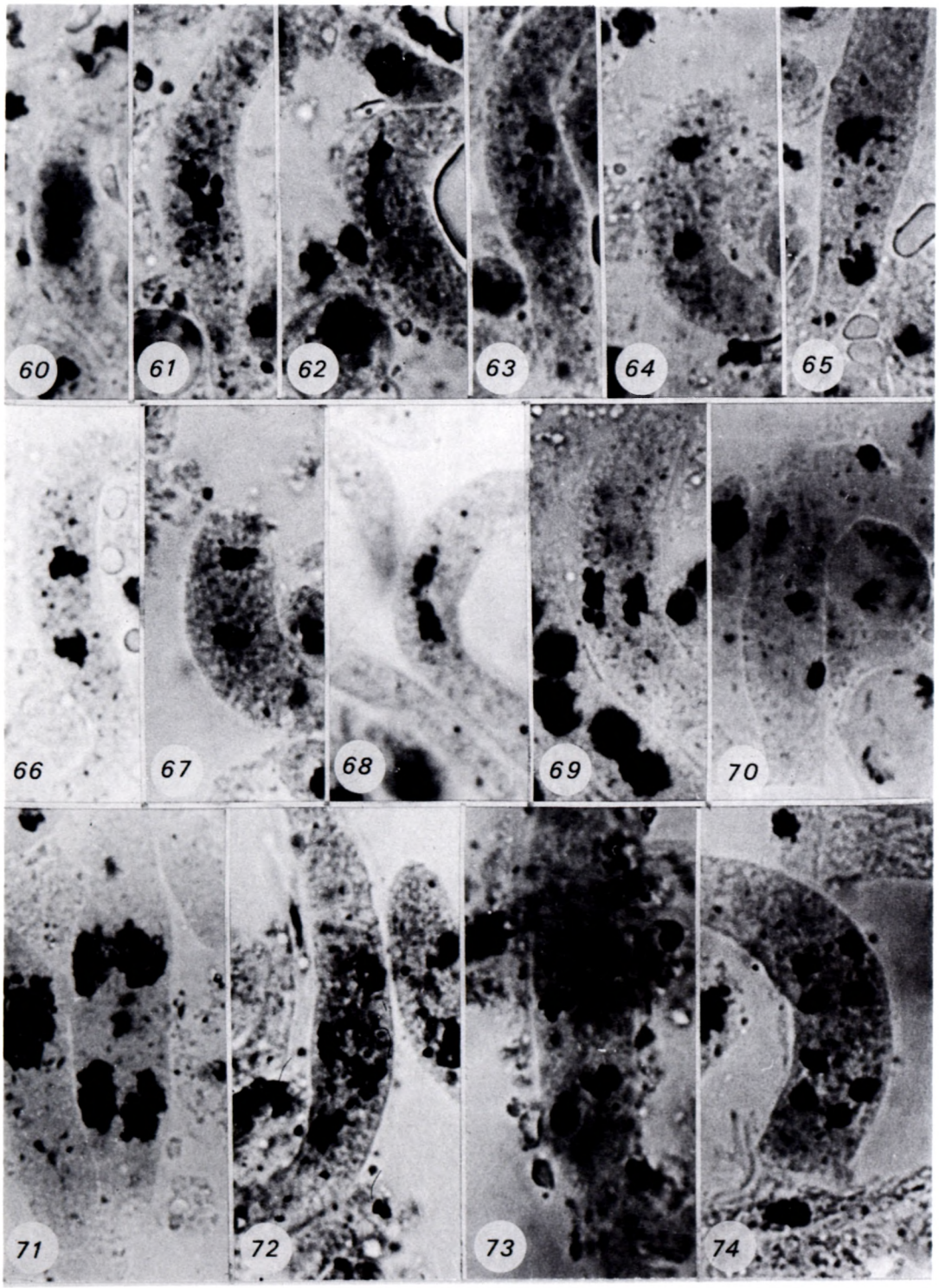
FIGS 21–32.—Meiosis in *Neocosmospora vasinfecta:* × 2 000. 21, telophase I; 22, interphase I; 23, prophase II; 24, metaphase II; 25, telophase II; 26, interphase II; 27, prophase III; 28, prometaphase III; 29, metaphase III; 30, anaphase/telophase III; 31, interphase III; 32, interphase III in delimited ascospores.



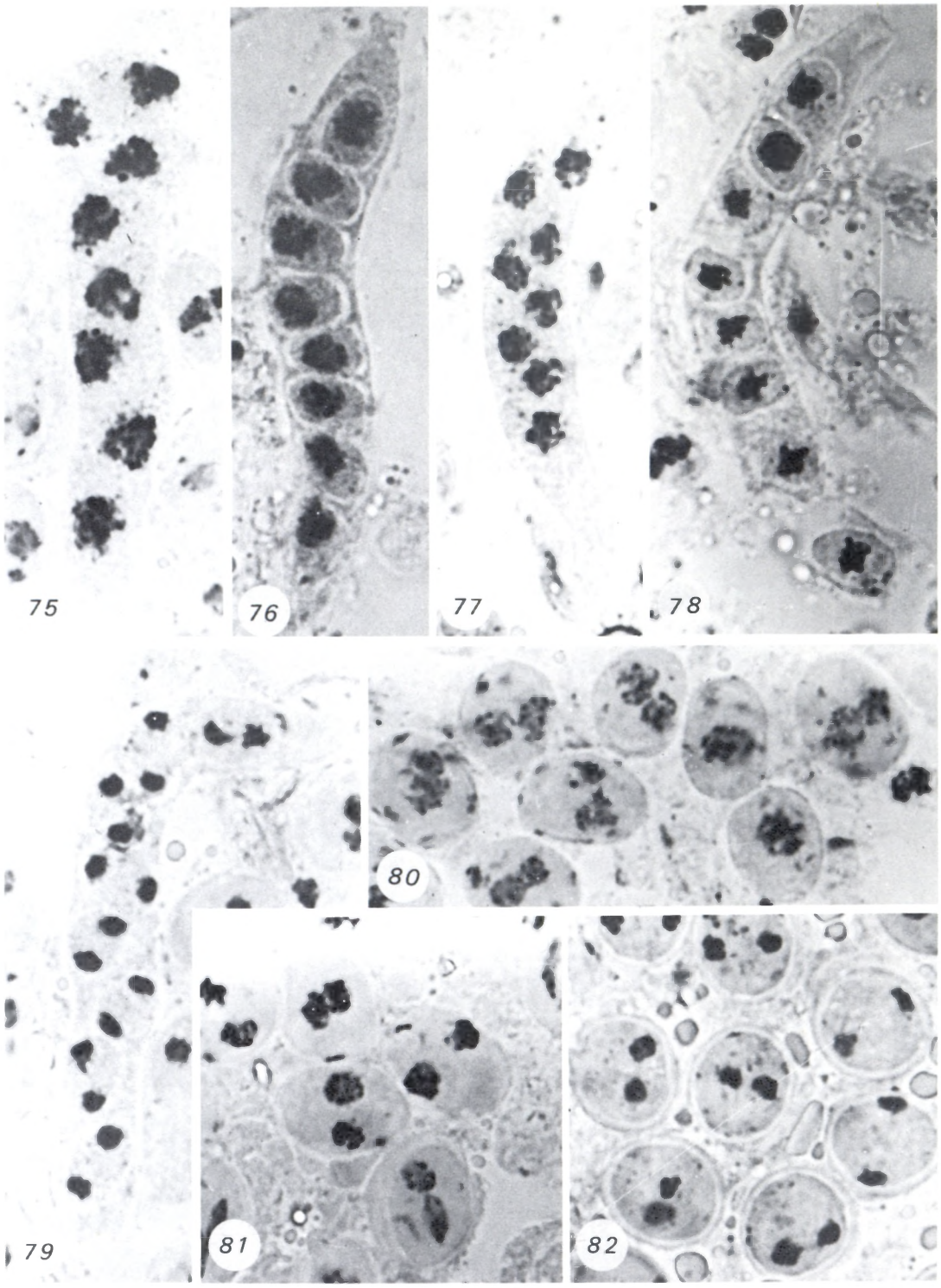
FIGS 33–39.—Meiosis in *Neocosmospora vasinfecta:* × 2 000. 33, prophase IV; 34, prophase IV without spore delimitation; 35, telophase IV; 36, telophase IV; 37, interphase IV; 38, mature binucleate ascospores; 39, mature binucleate ascospores.



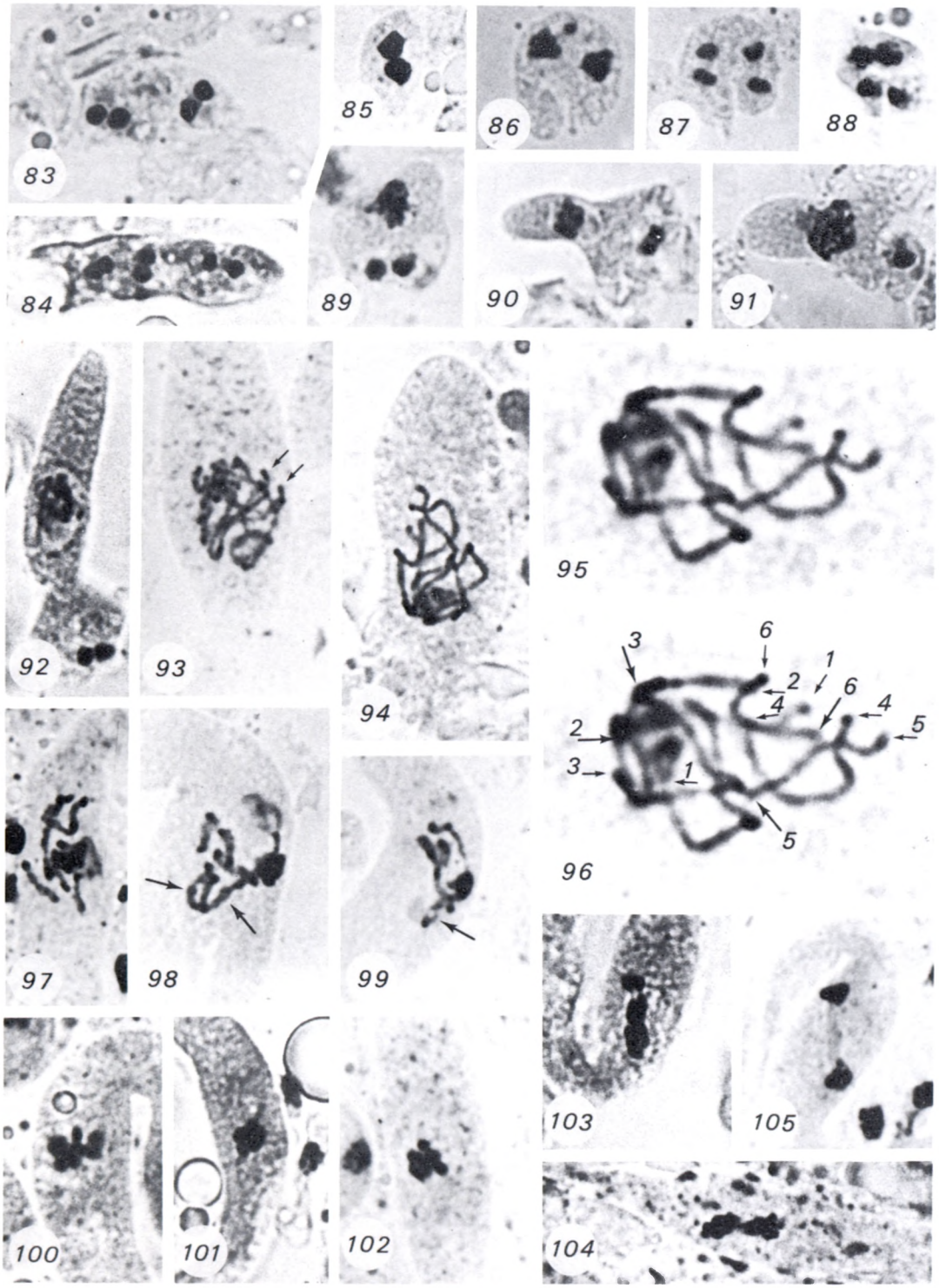
FIGS 40–59.—Meiosis in *Neocosmospora africana:* × 2 000. 40, ascogonium with paired nuclei; 41, ascogenous hypha; 42, binucleate pre-crozier; 43, binucleate crozier; 44, nuclear division in the binucleate crozier; 45, tetranucleate crozier after nuclear division; 46 tetranucleate crozier; 47, crozier after septum formation; 48, karyogamy in the young ascus; 49, diploid nucleus in the young ascus; 50, beginning of prophase I; 51, leptotene; 52, zygotene; 53, zygotene/pachytene; 54, pachytene; 55, diplotene; 56, diakinesis; 57, metaphase I with transverse spindle; 58, metaphase I with longitudinal spindle; 59, metaphase/anaphase I.



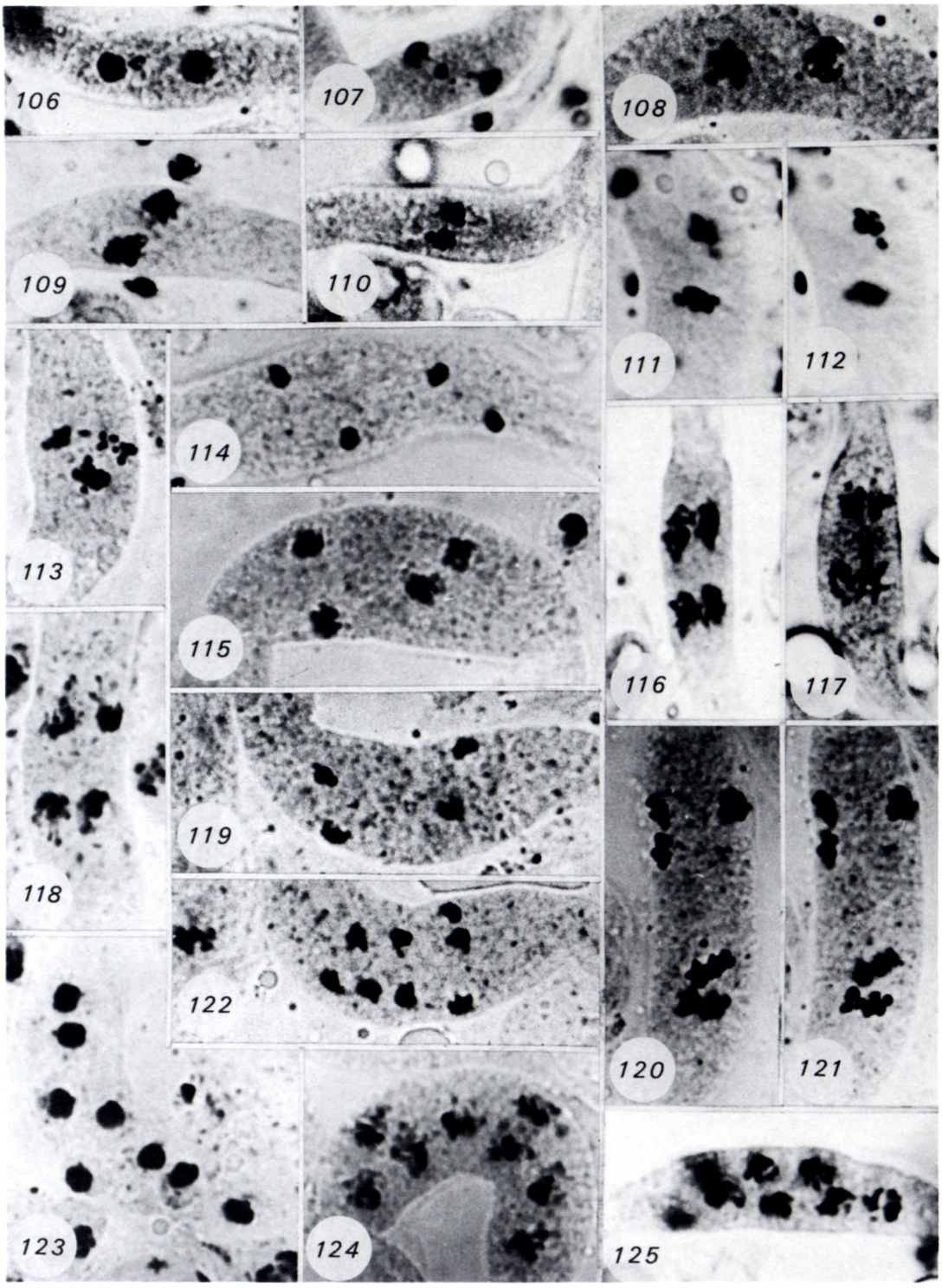
FIGS 60–74.—Meiosis in *Neocosmospora africana:* × 2 000. 60, early anaphase I; 61, anaphase I; 62, anaphase I; 63, anaphase/telophase I; 64, telophase I; 65, interphase I; 66, prophase II; 67, early metaphase II; 68, late metaphase II; 69, anaphase II; 70, telophase II; 71, interphase II; 72, prophase III; 73, prometaphase III; 74, telophase III.



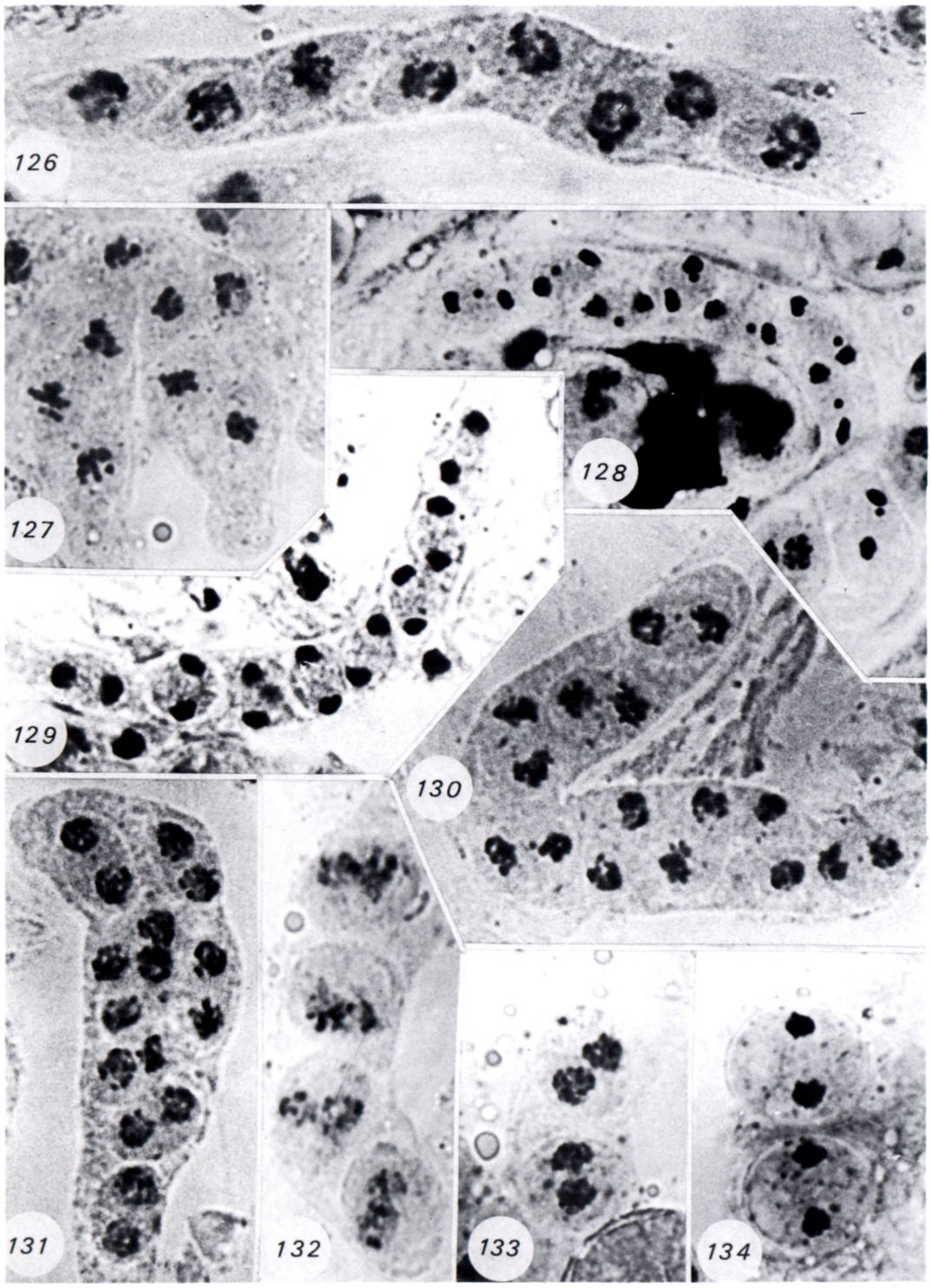
FIGS 75–82.—Meiosis in *Neocosmospora africana:* × 2 000. 75, interphase III before spore delimitation; 76, interphase III before spore delimitation; 77, early prophase IV: 78, prophase IV; 79, telophase IV; 80, interphase in binucleate ascospores; 81, interphase in binucleate ascospores; 82, interphase in binucleate ascospores.



FIGS 83–105.—Meiosis in *Neocosmospora* isolate P: × 2 000 unless otherwise stated. 83, ascogonium with two nuclear pairs; 84, ascogonium with three nuclear pairs; 85, binucleate pre-crozier; 86, binucleate crozier; 87, tetranucleate crozier after nuclear division; 88, tetranucleate crozier; 89, karyogamy in the young ascus; 90, diploid nucleus in the young ascus; 91, beginning of prophase I; 92, leptotene; 93, zygotene; 94, pachytene; 95, pachytene, × 4 000; 96, pachytene with numbered chromosomes, × 4 000; 97, pachytene/diplotene; 98, diplotene; 99, late diplotene; 100, diakinesis; 101, metaphase I; 102, metaphase I in polar view; 103, anaphase I; 104, late anaphase I; 105, telophase I.



FIGS 106–125.—Meiosis in *Neocosmospora* isolate P: × 2 000. 106, telophase I with slow chromosome; 107, telophase I with residual nucleolus; 108, interphase I; 109, prophase II; 110, metaphase II; 111, metaphase II; 112, anaphase II; 113, metaphase/anaphase II; 114, telophase II; 115, late telophase II; 116, interphase II; 117, early prophase III; 118, prophase III; 119, metaphase III; 120, anaphase III; 121, anaphase III; 122, telophase III; 123, large telophase III nuclei; 124, interphase III; 125, prophase IV.



FIGS 126–134.—Meiosis in *Neocosmospora* isolate P: × 2 000. 126, prophase IV with spore delimitation; 127, prometaphase IV; 128, telophase IV with residual median nucleoli; 129, telophase IV: 130, late telophase IV; 131, interphase IV; 132, interphase in binucleate ascospores; 133, interphase in binucleate ascospores; 134, interphase in binucleate ascospores.