Kezer, J. University of Oregon, Eugene, Oregon. Observations on salamander spermatocyte chromosomes during the first meiotic division.

Introduction: Salamanders have long been known as a source of superb material for the study of meiosis. This paper will be devoted to a presentation of the behavior of the chromosomes of salamander spermatocytes during the first meiotic division from leptotene through prometaphase, as seen in longitudinal sections through the testis and in squash preparations of spermatocytes. The chromosome preparations have been obtained from various species of the Plethodontidae, a family of salamanders widely distributed throughout the United States and Latin America.

The structure of the testis of plethodontid salamanders has been described by Kingsbury (1902) and Burger (1937). The latter paper contains an excellent description that should be consulted for detailed information. A typical plethodontid testis has a cylindrical structure and consists of a longitudinal duct surrounded by ampullae which are connected by short ducts to the main longitudinal duct. Primary spermatogonia are clustered about the short ducts of the ampullae and these cells, along with the duct system, constitute the persistent structures of the testis. The reproductive cycle is an annual event in the temperate zone plethodonts. After the ampullae have been emptied of their sperm, the testis is built up by proliferation from the persistent primary spermatogonia, so that at a particular time during the year, the ampullae become filled with secondary spermatogonia that are available for transformation into spermatocytes.

The meiotic divisions appear first at the posterior end of the testis and spread through the gonad during a period of about two months as a caudocephalic "wave". As a consequence, it is possible to secure salamanders in which the meiotic events are present in the testis as a sequentially ordered series: the ampullae at the extreme anterior end will contain spermatocytes in leptotene, slightly more posterior ampullae will be filled with zygotene spermatocytes, and all other stages of meiosis will follow in sequential order through spermatids to developing sperm in the extreme posterior ampullae. This orderly progression of events within the gonad makes possible interpretations and identifications that are difficult in the mixed-up cells of a squash preparation. To exploit such a situation, longitudinal sections of entire testes can be used to determine the sequence of meiotic events, supplemented with squash preparations of spermatocytes to reveal the contents of entire nuclei in a spread-out condition.

The accompanying plates of photomicrographs have been prepared with the above ideas in mind. Photos taken from longitudinal sections of the testis of Batrachoseps attenuatus (California slender salamander) are presented along the left margins of the first three plates. Extending from these to the right, are photomicrographs of squash preparations of spermatocytes corresponding in stage of development to those seen in the sections. The squashes were obtained from a variety of plethodontid species, identified in the legends that accompany the plates. The photos of sections illustrate the sequence of meiotic events present in the Batrachoseps testis beginning with the leptotene spermatocytes in the extreme anterior ampullae and ending with the ampullae about midway in the testis, in which the latest meiotic cells are in prophase of the second meiotic divisions.

Materials and Methods: Longitudinal sections were obtained from testes fixed in Sanfelice's fixative, paraffin embedded, sectioned at 10 micra and stained with iron hematoxylin. Acetic-orcein squashes were prepared by a technique modified from LaCour (1941). A small piece of fresh testis (about 2 mm in diameter) was dismembered in a drop of 2% orcein dissolved in 45% acetic acid. A coverglass coated with dried Mayer's egg albumen was placed over this suspension of stained cells, the slide inverted over absorbent paper and the cells forcefully squashed by pressure on the back of the slide. The coverglass, with cells embedded in the film of albumen, was soaked free from the slide in 15% acetic acid, dehydrated in absolute ethanol and mounted in Euparal. Testes for the Feulgen squashes were fixed in Clarke's fluid (3 parts absolute ethanol and 1 part glacial acetic acid), hydrolyzed for 10 minutes in N HCl at 60°C, stained for 30 minutes in the Feulgen reagent, dismembered into small pieces and gently squashed in 45% acetic acid. The Feulgen preparations were made permanent by the quick-freeze method of Conger and Fairchild (1953).

Observations and Discussion: Leptotene nuclei, as they appear in sectioned material, are shown in Fig. 1. Figs. 2 and 3 are Feulgen squash preparations of nuclei at this same stage. The severely squashed nucleus of Fig. 3 illustrates the leptotene chromosomes as elongate strands with a chromomeric structure. Centromeric heterochromatin is seen as deeply staining material in some of the sectioned spermatocytes.

Synapsis of the homologous chromosomes is illustrated with remarkable clarity in the zygotene nuclei of Figs. 4, 5, 6, 7, 8 and 9. Synapsis begins simultaneously at both ends of a pair of homologues and proceeds inward toward the middle of the pair, bringing homologous chromomeres together. This mode of synapsis has been observed in amphibians by Beçak, Beçak and Rabello (1967), in connection with their studies of polyploid frogs. The chromosome ends, at which the synapsis begins, are directed approximately toward a part of the cytoplasm in which the centrioles are located, producing the so-called bouquet arrangement of the bivalents that will persist through pachytene. These ideas are illustrated by the isolated zygotene bivalent shown in Fig. 9, in which synapsis has been completed along about two thirds of the length of the pair of homologues. In Fig. 5, synapsis is just beginning, as indicated by the short stretches of more deeply staining bivalent ends along the lower right where homologous pairing has been completed. Fig. 6 illustrates a nucleus in which synapsis is about half completed, and Fig. 8 is a zygotene nucleus so severely squashed that the contrast between the paired and unpaired portions of the homologues is accentuated. Exactly these same events can be seen in the sectioned material of Figs. 4 and 7, in which synapsed portions of the homologous pairs appear as the more deeply staining strands at a particular position within a zygotene nucleus. In Fig. 7, the five nuclei along the right margin of the photomicrograph have completed synapsis and are now in pachytene.

Pachytene nuclei are illustrated in Figs. 10, 11 and 22. Synapsis has brought the two members of a homologous pair into such an intimate association that they appear as a single strand. A salamander pachytene nucleus thus contains the haploid number of bivalent U-shaped loops with their ends oriented toward the portion of the nuclear envelope that is adjacent to the centrioles.

Pachytene is followed by a diffuse stage, illustrated in Figs. 12, 13 and 23. The chromomeres of the pachytene bivalents spin out into Feulgen-positive loops, well shown in Fig. 23, and this process continues until the bivalents disappear, producing a nucleus lacking clearly defined chromosomal strands. It seems that this is a stage during which the DNA that was folded into the chromomeres of the pachytene bivalents is completely spun out into the nuclear sap. Indeed, the spermatocyte diffuse stage may represent a situation comparable to the lampbrush condition of young amphibian oocytes, in which the nucleus is filled with pairs of elongate lateral loops that extend out from the chromomeres of bivalent axes (Callan and Lloyd, 1960).

Feulgen-positive loops can be seen protruding from the chromomeric axes of the zygotene bivalents of Figs. 5, 6, 8 and 9. Moreover, Donnelly and Sparrow (1965) have observed Feulgen-positive lateral loops at zygotene and pachytene in spermatocytes of the salamander, Amphiuma means tridactylum. Thus, loops of DNA extending out from the chromomeres of bivalent axes are present in salamander spermatocytes at least as early as zygotene. It is during the diffuse stage following pachytene that these loops reach their maximum extension, and because of this diffuse distribution of the DNA, it becomes impossible to resolve the bivalents as clearly defined structures. During the diffuse stage, centromeric heterochromatin remains condensed. In the diffuse nucleus of Fig. 23, there are 13 masses of centromeric heterochromatin, two of which are fused, and in the salamander from which the nucleus was obtained, n=13.

The existence of a diffuse stage between pachytene and diplotene would be difficult to determine if one had available only squash preparations with their mixtures of unordered nuclei. The tendency would be to classify as leptotene or pre-leptotene all large spermatocyte nuclei that lack clearly defined chromosome strands. It is when these diffuse nuclei are seen occupying a position in the testis just posterior to pachytene nuclei and just anterior to early diplotene nuclei that the reality of the diffuse stage becomes clearly apparent. And the existence of this stage becomes even more convincing when one can observe, in longitudinal sections, the gradual change from pachytene to diffuse and the gradual appearance of diplotene bivalents from the diffuse nuclei, as shown in Fig. 14, in which diffuse nuclei are at the top of the photo, and early diplotene nuclei in the lower half. A diffuse stage between pachytene and diplotene has been identified in the meiosis of many organisms. A review of the literature is given by Barry (1969).

To produce the diplotene nuclei shown in Figs. 14, 15, 16, 17 and 19, the greatly extended loops of DNA must again become folded into the chromomeres of the bivalent axes. As the diplotene bivalents emerge from the diffuse nuclei, the component bivalent halves, so closely associated at pachytene, now appear separated except at the positions of chiasmata. And as the diplotene stage progresses, the chromosomal duplication that took place during the interphase prior to the first meiotic prophase becomes visible, producing four-strand bivalents such as those shown in Fig. 21.

In Fig. 18, late diplotene nuclei are present at the upper left. The smaller nuclei in the lower portion of the photo are in prophase of the second meiotic division. In Fig. 20, late diplotene nuclei are shown in the upper left, nuclei in interphase between the first and second meiotic divisions are at the upper right and first meiotic metaphase nuclei are in the lower half of the photo.

Fig. 24 is a photomicrograph printed with high contrast to accentuate the short loops that invest the axes of these prometaphase bivalents. This situation is interpreted as indicating that the DNA loops of the diffuse stage are not completely folded back into their respective chromomeres, but remain as short lateral extensions from bivalent axes. Indeed, loops approximately similar to those seen in this photomicrograph can be demonstrated at diplotene, first meiotic metaphase and anaphase and at all stages of the second meiotic division. They appear to be a characteristic feature of salamander meiotic chromosomes.

The "fuzzy" and "whiskery" condition of animal spermatocyte chromosomes has been noted by many investigators, and this condition has been cited as possible evidence for a lampbrush stage in spermatogenesis. Moreover, in a study of pigeon spermatocyte chromosomes, using electron microscopy, Nebel and Coulon (1963) demonstrated lateral loops at pachytene and first meiotic metaphase. Thus, it is possible that the Feulgen-positive loops of salamander meiotic chromosomes are of general occurrence in animal spermatocytes. The big question that remains concerns the meaning of these lateral loops of DNA relative to the overall structure of meiotic chromosomes.

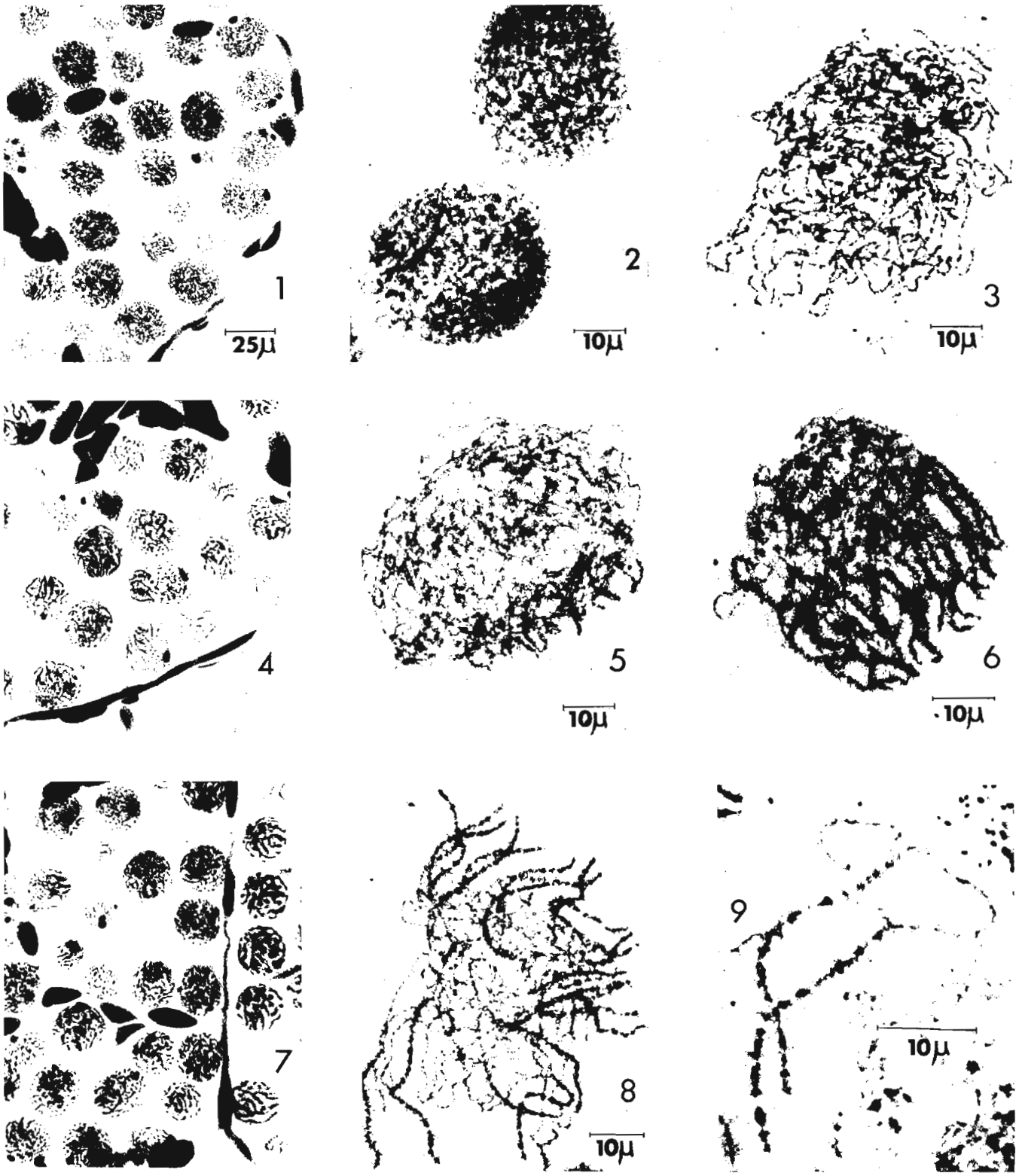
Summary: In salamander spermatocytes, synapsis of the homologous chromosomes begins at their ends and proceeds inwards, bringing the homologous chromomeres into such an intimate association that the resulting pachytene bivalents appear as single strands. The arrangement of the bivalents as U-shaped loops with their ends directed approximately toward the centrioles occurs at the very beginning of zygotene and persists through pachytene. Pachytene is followed by a diffuse stage in which the DNA of the bivalent chromomeres spins out so completely into enormously long lateral loops that the bivalent axes disappear. It is from these diffuse nuclei that the diplotene bivalents emerge.

Feulgen positive lateral loops, springing from chromomeres, can be seen in salamander spermatocytes at least as early as zygotene. The loops reach their maximum extension during the diffuse stage. The formation of diplotene bivalents involves a return of this extended DNA back into the chromomeres of the bivalent axes, but it does not fold in completely, since Feulgen-positive lateral loops can be demonstrated along the axes of salamander meiotic chromosomes until the conclusion of the meiotic divisions.

Acknowledgements: Many of the observations on which this paper is based were made at St. Andrews University, Scotland, during a 1968-69 sabbatical year, working as the guest of Prof. H.G. Callan and Dr. Herbert C. Macgregor. The photomicrographs of Batrachoseps testis sections were made from slides prepared by Prof. Callan for use in the general zoology course at St. Andrews University. The ideas presented here regarding synapsis and the diffuse stage resulted from conferences with Prof. Callan, who has generously given me permission to use them in this paper. The Feulgen squash preparations of Oedipina uniformis were prepared by Dr. Kathleen Church. I am indebted to Mr. Harrison M. Howard for the photomicrograph of Fig. 24.

Literature Cited: Barry, E.G. 1969, The diffuse diplotene stage of meiotic prophase in Neurospora. Chromosoma 26: 119-129. Beçak, M.L., Beçak, W. and Rabello, M.N. 1967, Further studies on polyploid amphibians (Ceratophrydidae). I. Mitotic and meiotic aspects. Chromosoma 22: 192-201. Callan, H.G. and Lloyd, L. 1960, Lampbrush chromosomes of crested newts Triturus cristatus (Laurenti). Phil. Trans. R. Soc. B243: 135-219. Burger, J.W. 1937, The relation of germ cell degeneration to modifications of the testicular structure of plethodontid salamanders. Journal of Morphology 60: 459-487. Conger, A.D. and Fairchild, L.M. 1953, A quick-freeze method for making smear slides permanent. Stain Technology 28: 281-283. Donnelly, G.M. and Sparrow, A.H. 1965, Mitotic and meiotic chromosomes of Amphiuma. Journal of Heredity 56: 90-98. Kingsbury, B.F. 1902, The spermatogenesis of Desmognathus fusca. Am. J. of Anat. 1: 97-135. LaCour, L. 1941, Acetic-orcein: a new stain-fixative for chromosomes. Stain Technology 16: 169-174. Nebel, B.R. and Coulon, E.M. 1962, The fine structure of chromosomes in pigeon spermatocytes. Chromosoma 13: 272-291.

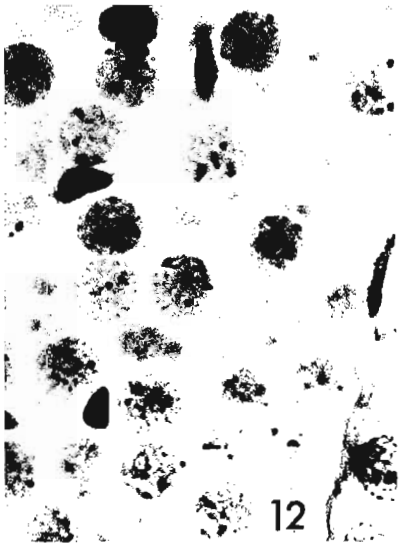
This investigation was supported by research grants from U.S. Public Health Service GB 12431 and National Science Foundation GB7767.



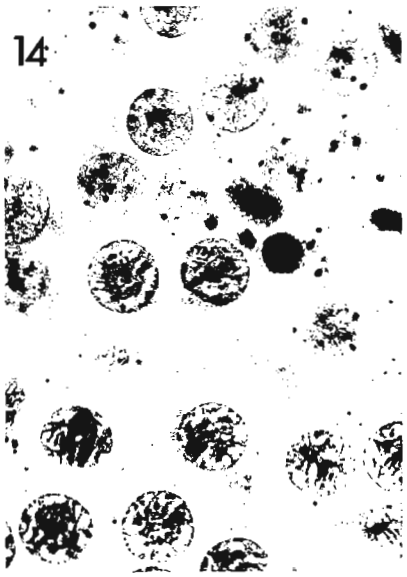
Figs. 1, 4, 7, 10, 12, 14, 16, 18 and 20. Batrachoseps attenuatus. 10μ sections. Iron hematoxylin stain. The scale on Fig. 1 applies to all the photos of this series.
Figs. 2, 3, 5, 6, 8 and 9. Oedipina uniformis. Feulgen squash.
Figs. 11 and 13. Batrachoseps attenuatus. Acetic-orcein squash.
Figs. 15, 17 and 19. Plethodon cinereus. Acetic-orcein squash.
Fig. 21. Oedipina uniformis. Acetic-orcein squash. Fig. 22. Thorius dubitus. Feulgen squash. Fig. 23. Pseudoeurycea werleri. Acetic-orcein squash. Fig. 24. Hydromantes platycephalus. Acetic-orcein squash.

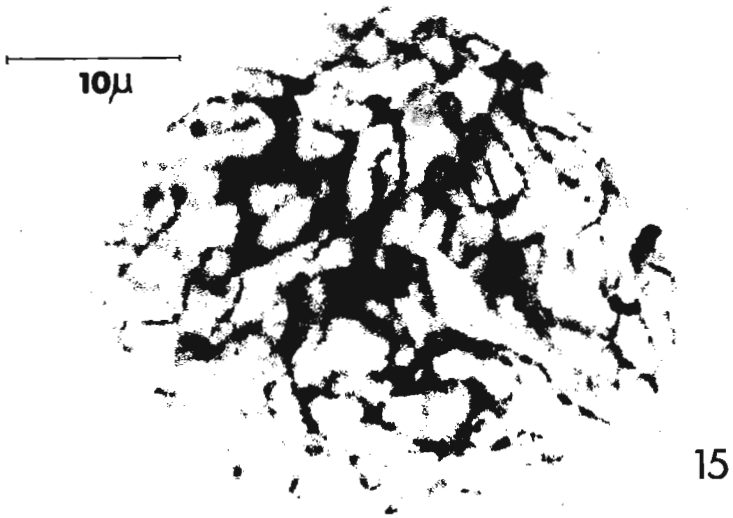

10

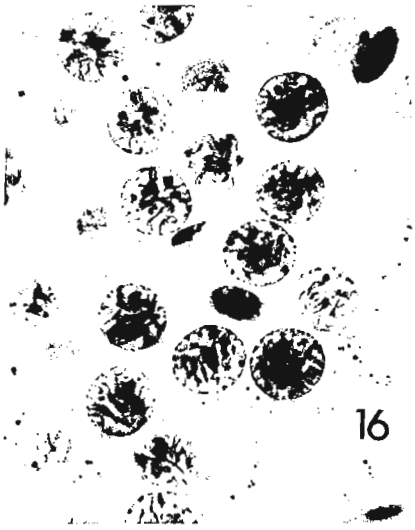

10μ
11

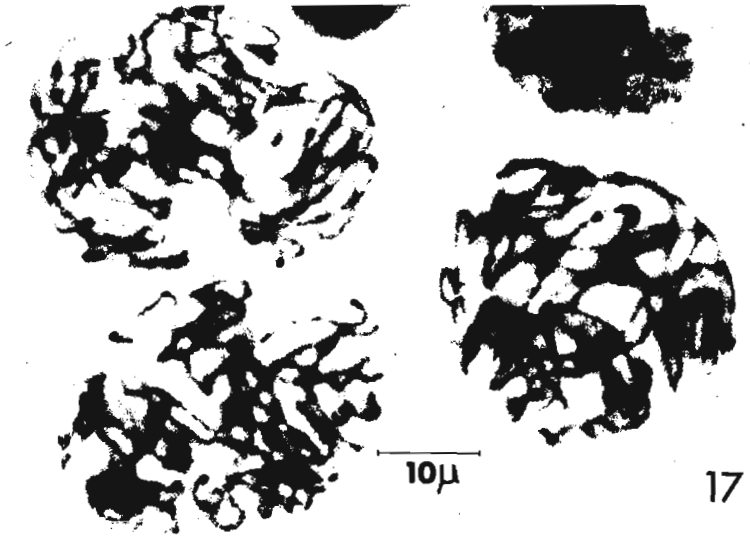

12


10μ
13

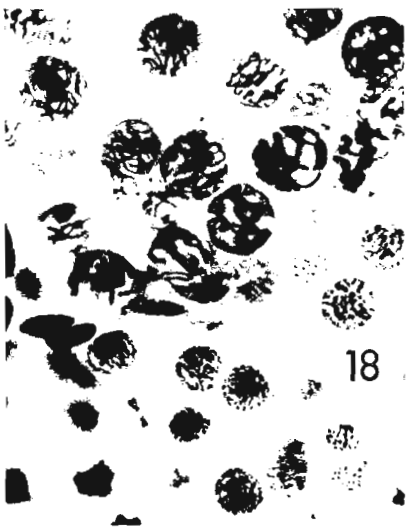

14

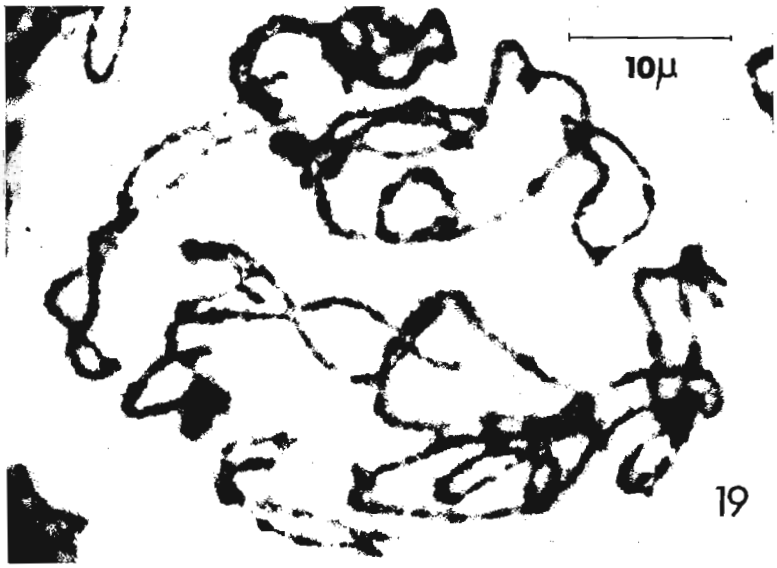

10μ
15


16

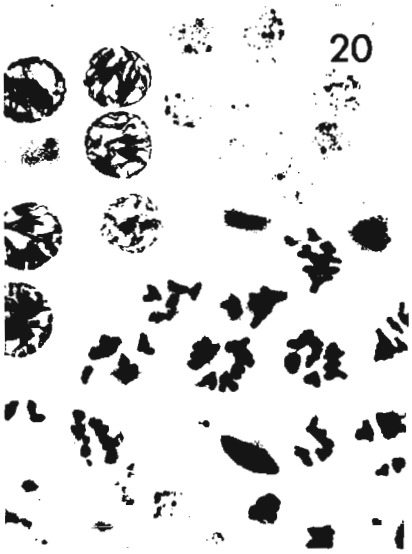


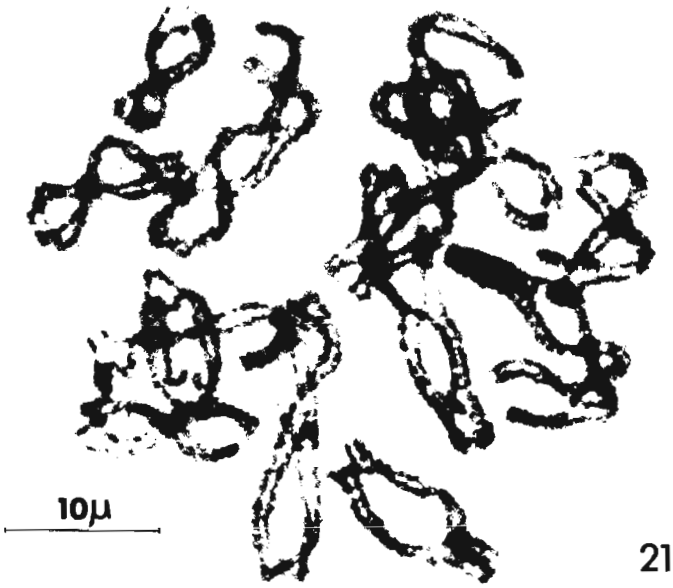
17


18



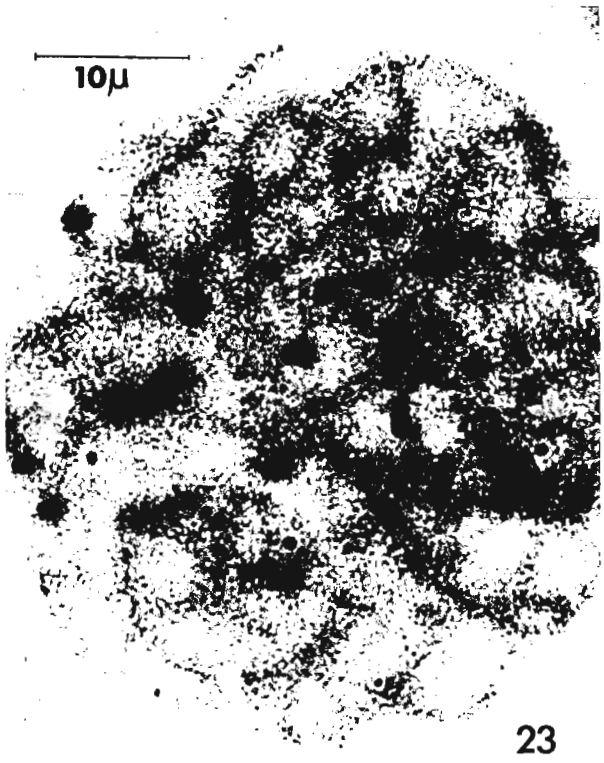
19

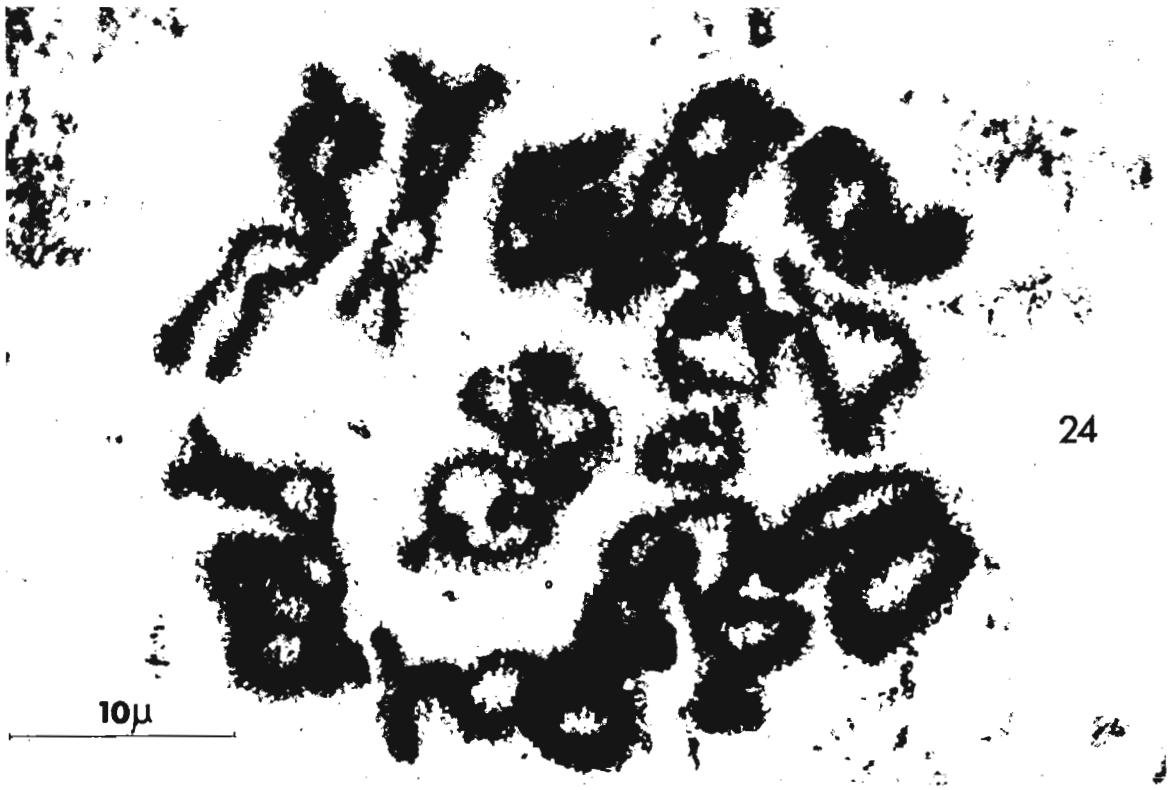

20



21


22
10μ


10μ
23


24
10μ