CYTOLOGICAL STUDIES IN *ASCOBOLUS IMMERSUS*[1]

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Introduction

Dr. Clare C. C. Yu-Sun of College of St. Joseph, New Mexico, presented two papers on the nutritional studies of *Ascobolus immersus* (Yu-Sun, 1964a and b). By that time, three linkage groups were found. Dr. Yu-Sun came to our laboratory in spring of 1966. She told us that there was only one paper published on the cytological studies on *A. immersus* by two Polish workers, J. Zuk and Z. Swietlinska (1965). From this paper it was found that the chromosome number of the species was suggested to be eight or nine. However, since the meiotic studies done by sectioning and photomitograph were not so clear Dr. Yu-Sun suggested to us to do more cytological studies of the fungus. By sending us the stock culture of the fungus as well as the fixed apothecia later, this work was made possible. We are very grateful to Dr. Yu-Sun kindness and for her help. Result of our studies are published briefly in this paper.

Materials and Methods

Stocks—The original stocks were supplied by Dr. Yu-Sun. She also supplied us with fixed apothecia. Fixation was done in a mixture of three parts of absolute alcohol and one part of glacial acetic acid. Another fixative used was: 6 parts absolute alchohol; 1 part glacial acid; 1 part lactic acid. The latter one was to induce the homologous chromosomes of the pachytene bivalent to remain rather widely separated. Fixed material was left at room temperature for one day, then stored at 5°C.

Culture—(1) For production of apothecia: The medium consisted of 2% agar, 1% glucose, 0.3% yeast extract and 0.2% cellulose powder. It was important that the cellulose powder should be well spaced in the culture medium by shaking when the agar medium was cooling down. We had repeated failures of not geting any apothecia just because all the cellulose sank to the bottom of the medium. (2) For germination of the ascospores: According to Dr. Yu-Sun, spore germination of this species would be quite natural without any

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pretreatment. Nontheless, we did not get any germination at all. Finally, when the spores were treated with 0.68% NaOH for 20 minutes, then the germination was quite satisfactory.

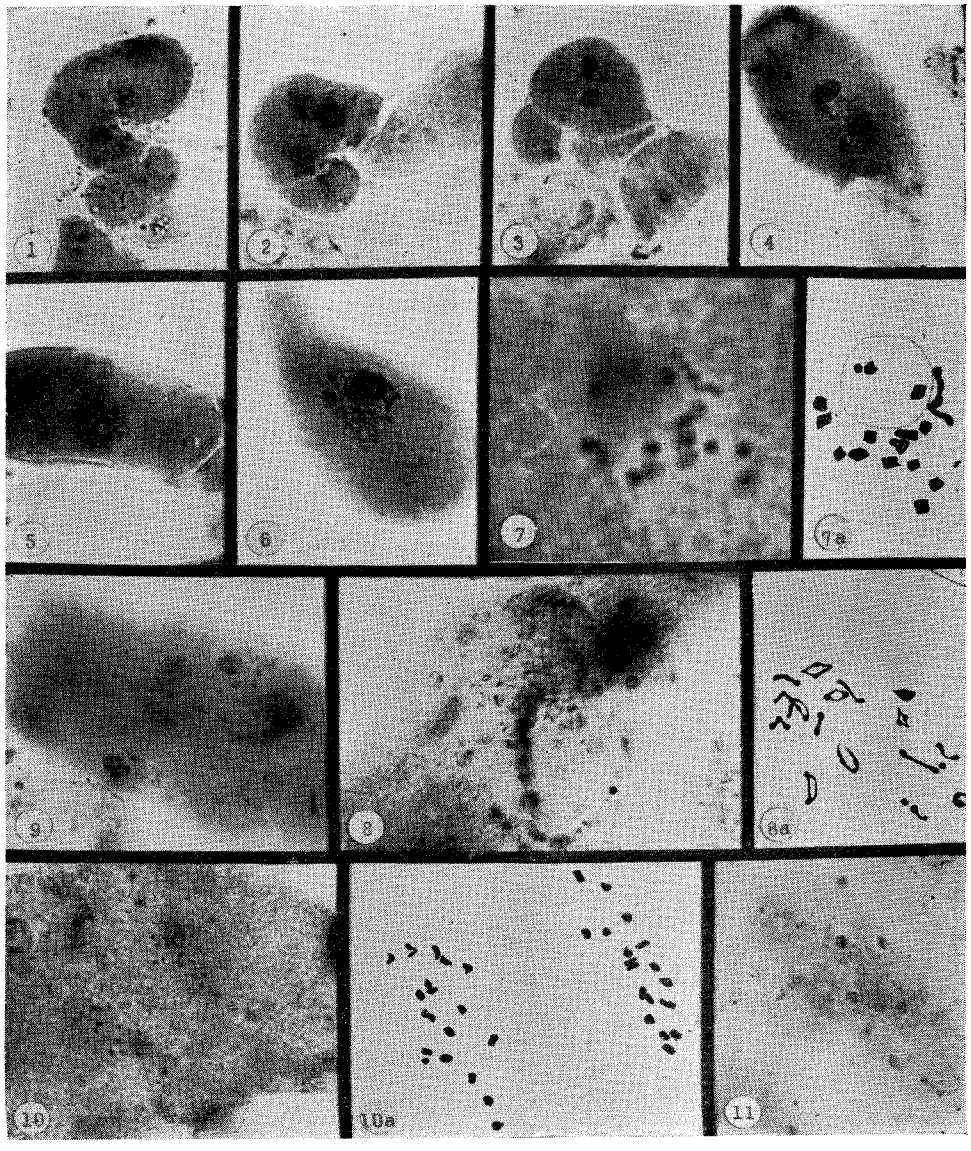
Staining—Apothecia were pretreated for 10 minutes at 60°C in 1N HCl. Staining was done with aceto-carmine with the addition of a trace of ferric chloride. Few apothecia were squashed with needle on a slide and stained for about 30 minutes. Destaining was done with 45% aceteic acid with light heating. To our suprise, we saw only deeply stained nucleolus in the prophase and later stages, but chromosomes were only very faintly stained. However, these slides were kept in the box for three months, then the chromsomes could be seen distinctly, showing that the penetration of carmine through the thick ascal wall into the ascus was really a slow process indeed. Luckily, one of us tried cytase digestion to remove the ascal wall by soaking the apothecia in the diluted cytase extracted from snail stomach overnight (Farberge 1945). The ascal wall of the asci was completely removed, thereby the chromosomes as well as the nucleoli could be stained readily. The African giant snail found locally were used to best advantage. The stomach were ground up and centrifuged. The final supernatant was diluted by addding three times the volume of water to the original extract.

Result

1. Crozier formation—Conjugate division of the two haploid nuclei of the crozier was not observed. However, the two nuclei stage (Fig. 1), 4 nuclei stage (Fig. 2), and the cross wall formation cutting off the ultimate and basal cells from the binucleate perultimate cell (Fig. 3) were found.

Explanation of Plate Figures

Fig. 1. Two nuclei stage of crozier. (1500×)
Fig. 2. 4 nuclei stage of crozier. (1500×)
Fig. 3. Cross walls formation in the crozier. The ultimate and basal cells being cut off from the penultimate cell whose nuclei are not yet fused. (1500×)
Fig. 4. Young ascus with the fusion nucleus. (1500×)
Fig. 5. Early pachytene stage. (1500×)
Fig. 6. Late pachytene stage with big nucleolus. (1500×)
Fig. 7. 18 chromosomes can be seen distinctly in metaphase I. (2000×)
Fig. 7a Camera lucida drawing of metaphase I of the same ascus showing 18 pairs of chromosomes. Two of these are yet to be condensed. (2000×)
Fig. 8. Diakinesis, 18 pairs of chromosomes. (1800×)
Fig. 8a Carmra lucida drawing of diakinesis of the same ascus showing 18 pairs of chromosomes. (2000×)
Fig. 9. Prophase of second division. (1800×)
Fig. 10. Metaphase II. (1800×)
Fig. 10a Carmra lucida drawing of the ascus. Showing each with 18 chromosomes. (2000×)
Fig. 11. 4 nucleated stage. (450×)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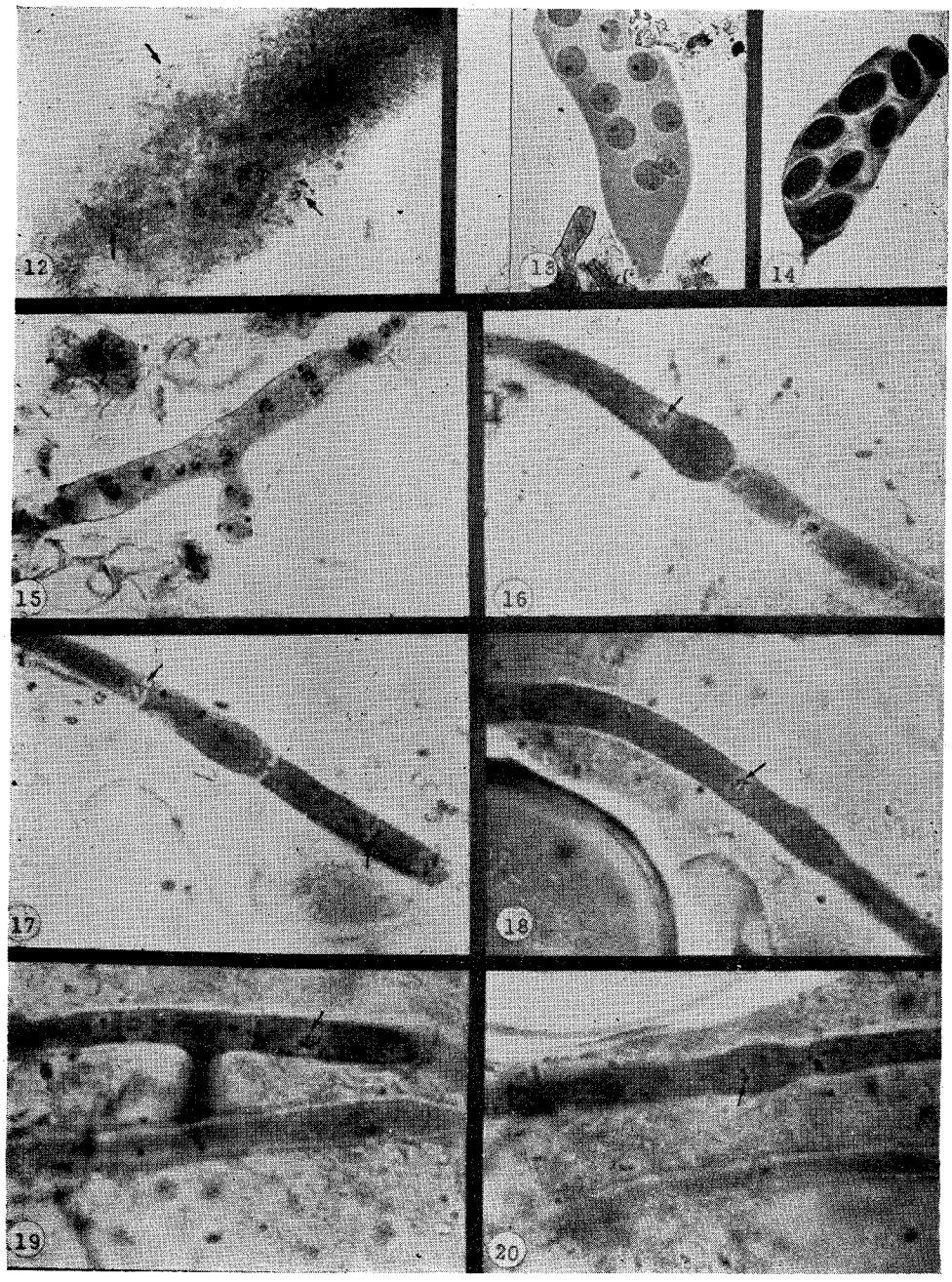
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2. Meiotic divisions—After fusion of these two nuclei, the young ascus was ready to proliferate. A predominant nucleolus and chromosomes in the early prophase stage were observed (Fig. 4). Since the chromosomes were so entangled, the identity of any individual chromosome was made impossible. As the ascus grew in length and width, the chromosomes contracted in length (Fig. 8 and 8a), showing *Ascobolus immersus* to possess 18 pairs of chromosomes instead of 8 or 9 as Zuk *et al* estimated (Zuk and Swietlinska, 1965). Several clear figures in metaphase stage were observed (Fig. 7 and 7a) showing 18 distinct bivalents. Most frequently, two of these chromosomes were yet to be condensed. The nucleolus did persist at this stage, the same as *Sordaria fimicola* (Carr and Olive, 1958). As many as five apothecia were used in the squash preparation, the debris remained would prohibit the complete flattening of the asci. Thus, the 18 chromosomes were naturally on different planes. The second division is shown in Fig. 9, 10 and 10a. In the latter photomicrograph, 18 chromosomes were counted in each metaphase plate. The chromosomes in the third division were too tiny to make counting possible, let alone the study of the karyotype possible (Fig. 11 and 12). Finally, ascopspres were mononuleated. They were not arranged in the linear order but most of them in two rows, with one slightly below the other.

3. Mitotic divisions—Young hyphae produced was multinucleated (Fig. 15). Since there were 18 chromosomes in each nucleus and the chromosomes were rather small, therefore, counting of chromosomes in mitotic figures was impossible. From Fig. 16 to 20 mitotic division from prophase, metaphase, anaphase and finally telophase are shown.

Summary and Conclusion

The ascus development of *Ascobolus immersus* was very similar to *Neurospora crassa* (McClintock 1945, Singleton, 1963) and *Sordaria fimicola* (Carr and Olive 1958). There were 18 pairs of chromosomes in the species which number was very different from 8 or 9 as had been speculated by Zuk and Swietlinska

Explanation of Plate Figures

Fig. 12. Metaphase III, in 3rd division. Arrows point to the division figures. The 4th is not in focus. (1800×)
Fig. 13. 8 nucleates stage before wall formation of spores. (450×)
Fig. 14. Ascus with eight spores. (4500×)
Fig. 15. Multinucleated young hyphae. (4500×)
Fig. 16. Resting nucleus pointed by arrow. (1500×)
Fig. 17. Figures showing metaphase in side view in young hyphae. (1500×)
Fig. 18. Anaphase. (pointed by arrow) (1500×)
Fig. 19. Metaphase. (polar view) (1500×)
Fig. 20. Telophase. (pointed by arrow) (1500×)

(1965). Dr. Yu-Sun (personal communication) told us that she found 9 linkage groups already in her genetic studies of this species. Even with pretreatment and enzyme digestion of the ascal wall, the staining of spindle as well as centriole was very difficult. Only very rarely was the stained spindle observed, but never was the centriole observed.

Mitotic division was observed but again the chromosomes were too small to make counting possible.

Ascobolus immersus 之細胞學研究

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Ascoborus immersus 子囊之形成過程與 *Neurospora crassa, Sordaria fimicola* 相同其染色體數爲18，與1965年波蘭的 Zuk 及 Swietlinska 所發表的8或9染色體大不相同。其子囊壁雖以 Cytase 加以處理，然 Spindle 及 Centriole 的染色仍極困難，尤其是 Centriole 從未染上過。

菌絲無絲分裂 (mitotic division) 時染色體的變化，因太小而無法加以觀察與計算。

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