CYTOLOGICAL STUDIES OF *PIRICULARIA ORYZAE* CAV.[1]

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In 1964 the laboratory of Microbiology, Institute of Botany, Academia Sinica started to induce biochemical mutants of *Piricularia oryzae* by using various dosages of ultraviolet light so as to study the relationship of these biochemical mutants with their pathogenicity on rice host plants. In order to understand the internal structure of this fungus fully, a detailed cytological study of this fungus is a prerequisite for further investigation. The detailed observation of this will be reported in this paper.

Review of Literature

In the past, voluminous literature could be found with cytological studies in many other fungi. These included Phycomycetes, Ascomycetes, Basidiomycetes and Fungi Imperfecti. It seems that except *Basidiobolus* (Robinow 1963) which was mononucleated, all other fungi studied were multinucleated (Olive 1953) in their vegetative hyphae. There were divergent views concerning the manner of nuclear division in the somatic mycelia. On the one hand, the division was taken place in a classical mitotic division with distinct chromosomes. Fungi such as *Helminthosporium* (Roan 1952, Knox-Davies and Dickson 1960), *Basidiobolus* (Robinow 1963). *Neurospora* (Cutter 1946, Singleton 1953, Somers *et al* 1656, 1960, Dowding 1960, Ward and Ciurysek 1962, Weijer and Dowding 1960), *Phytophthora* (Graham 1954), *Kabatiella* (Cole 1959), *Lipomyces* (Robinow 1961), *Sordaria* (Carr and Olive 1958), *Puccinia* (Pavgi *et al* 1960), Basidiomycetes (Ward and Ciurysek 1961), *Cyathus* (Lu 1962, 1964), *Alternaria* (Stall 1958, Hartmann 1964), *Monilinia* (Hall 1963) and others were studied. All these investigations came to the same classical mitosis conclusion. On the other hand, few investigators such as Bakerspigel (1958, 1959, 1960) and Saksena(1961) insisted that the nuclear division in the somatic hyphae was done

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amitotically.

Of the multinucleated type of mycelium in any fungus, all the nuclei seemed to divide synchronously and rapidly. With *Basidiobolus* (Robinow 1963) the formation of the septum was somewhat similar to that of the higher plant. However, there was no investigation of how the septa were formed in the multinucleated type hyphae. Some information was reported on the fine structure of the septum of *Rhizoctonia* (Braker and Butler 1963) and *Rhizopus* (Hawker and Abbott 1963).

Concerning the presence of the spindle apparatus and centriole in the vegetative hyphae of fungi, even though the nuclei were so small, there was still some information reported in *Neurospora* (Somers *et al* 1960), *Helminthosporium* (Knox-Davies and Dickson 1959). Zalokar (1959) and all came to the same conclusion that the growth of hypha was acropetal.

With *P. oryzae* Suzuki of Japan seemed to be the only worker who had done a lot of cytological work with this fungus. He presented a paper at the Symposium on Rice Blast Fungus Disease held at Los Banos, Philippines in 1963 (Suzuki 1963 unpublished). He maintained that this fungus divided in a classical mitotic manner with 4 chromosomes in most of the observations made. But aneuploidy was also in existence. Nuclei with 2, 3 or 5 chromosomes were also observed. Most of the hyphae were multinucleated and this fungus was a perpetuated heterokaryon. However, one homokaryotic strain was isolated.

Material and Methods

In this study the fungal isolate used was the isolate 2T-82S which was obtained from the Taiwan Provincial Agriculture Research Institute of Taipei.

Preparation of material for staining: The modified cellophane method was used (Roan 1952, Carmichael 1956). Instead of cellophane, cover glass was used on which a tiny drop of albumen was smeared. The culture of 7-days old PDA plate was used. The cover glass was inserted onto the plate with gentle pressure exerted. Then the cover glass was taken out gently to be immersed into the fixative. Fixation was done overnight in a 3:1 mixture of absolute ethanol and acetic acid with the addition of 0.5% ferric cholride. Carmine, Giemsa, and basic fuchsin etc. were used for staining, after fixative was washed off by 70% alcohol.

Staining: 1. HCl-Giemsa according to the schedule of Goss (1959).

2. Carmine stain. In addition to the use of aceto-carmine (Lima-de-Faria, A. *et al* 1954), propiono-carmine, and succino-carmine were also used. The results obtained from all methods employed were about the same.

3. Leuco-basic fuchsin was used in the Feulgen reaction (De Lamater 1948).

For phase contrast microscopic observation, spore suspension was made by using Tanaka's A medium (Tanaka 1963) to wash off the spores from 7-days old PDA plate. This suspension was incubated for 15–20 hours at 26°C. Then the suspension was mounted and was sealed by sealing wax. Preferably the culture medium used for this suspension was shaken for 15 minutes or more before washing was executed. This would provide better aeration for the growth of this fungus subsequently. The sequential series of photomicrographs were taken by a Leica M-3 camera and Leitz ortholux phase contrast microscope with the Heine condenser, Pv Apo Oil 90/1.15 objective. The stained material was observed under the Olympus FFE-Bi-III microscope, Plan achromat HI 100 N. A. 1.25 objective.

Results

I. Nuclei in the hypha: As general rule, majority of hyphae was multinucleated. According to the diagramatic sketchs made by Zalokar (1959) the different types of hyphae of *Neurospora* grown in different types of culture media would vary greatly from each other. Accordingly, with *P. oryzae*, the multinucleated condition of hyphae would vary also as they were found at different levels in the culture or different treatment of the culture medium. As in Fig. 36 which represents a hypha tip grown in continuous shaking culture, It was found to be with dense cytoplasm and very much compacted and dense nuclei which were very few in number as compared with hypha grown in PDA plate with limited aeration. As in Fig. 35, it is a segment of hypha with many vigorously dividing nuclei and free chromosomes. Possibly this type of hypha was found from the surface of the PDA culture medium with more aeration. While the hypha was grown in the intermediate or deep level of the PDA medium, the hypha would be filled by giant nuclei of various sizes as shown in Fig. 34 (phase-contrast-microscopy) and Fig. 66, 67 (Feulgen stain). It seems that if these giant nuclei could be well stained by leuco-basic fuchsin they were really chromatic (DNA) in nature (positive Feulgen reaction) and were not fat globules as being depicted by Zalokar (1959) from observation of living hyphae in *Neurospora*.

II. Mitosis: A typical single nucleus at prophase consisted of a prominent nucleolus, as shown by phase-contrast-microscopy in Figs. 22, 24, 40, 41 by Giemsa stain in Figs. 47; but with a tiny nucleolus with carmine stain as shown in Figs. 57, 60, 61. With leuco-basic fuchsin, the nucleolus was not stained whatsoever as shown in Fig. 70, for the nucleolus was not

DNA in nature. At this time nucleus was surrounded by a nuclear membrane. However, the chromatic material was only faintly visible inside this membrane, either by phase-contrast-microscopy or by different kind of stains used. Perhaps this was due to the fact that the size of the chromosomes were too small to make the observation possible at a dispersed state during early prophase. After nuclear division started a gradual change in the size of the nucleolus as well as its stainability took place. The nucleolus became smaller and fainter with the advancement of mitotic division. The chromatic material seemed to be visible at the periphery of the nucleus making the nuclear membrane to be very much thickened, as shown in Fig. 22 (phase-contrast-microscopy or PCM), Fig. 47 (HCl-Giemsa). As division proceeded further, gradually the nuclear membrane disappeared and the 4 chromosomes were greatly contracted to be distinctly visble (Fig. 48). Finally the nucleolus vanished also and the 4 different sized chromosomes linked together in a ring-like-structure on the plate (Figs. 20, 37 PCM; Figs. 54, 58, 59 carmine stain; Figs. 71, and 72 Feulgen stain). Sometimes a metaphase plate could be observed. Perhaps, the spindle apparatus seemed to be present as shown in Figs. 20, and 22 (only from plase-contrast-microscopy). This spindle apparatus was called "halo" by Suzuki (personal communication) but preparations made from different stains could not sustain its presence among all dividing figures, even though heavy dosage of ferric ion was incorporated in the fixative before staining. Anaphase occurred rather quickly, the 4 differerent sized chromosomes started to divide and separated from the ring like structure just as that in typical higher plants. In case there was the presence of the spindle apparatus, the movement of the daughter chromosomes to their respective poles would be the function of the spindle apparatus chiefly. Early anaphase figures could easily be observed especially in the stained preparations, as in Fig. 37 (PCM), Figs. 62, 68, and 69 (Feulgen stain). Late anaphase with stained preparations also were found in Figs. 20, and 26 (PCM), Fig. 47 (HCl -Giemsa), Figs. 55. 56. and 57 (carmine) and Figs. 65, 70, and 72 (Feulgen stain). It seemed that with phase-contrast-microscopy, the movement of the daughter chromosomes to the two poles was very fast. Perhaps soon after the chromosomes reached the poles, they were dispersed again with the gradual reorganization of the nuclei. At the same time a transverse nuclear membrane was formed at the middle of the oval apparatus bissecting and separating the original nucleus into two daughter nuclei. Possibly this was the telophase stage (Figs. 27, 38, and 39 PCM), Fig. 49 (HCl-Giemsa), Figs. 56, 57 (carmine), and Figs. 62, 63, 64, 69, and 73 (Feulgen stain).

From phase-contrast-microscopy, it seemed that it would take about 15 or more minutes from metaphase to telophase when the living material was on the slide and sealed and examined at 26°C. How long it would take from the prophase to metaphase, no timing was ever made so far.

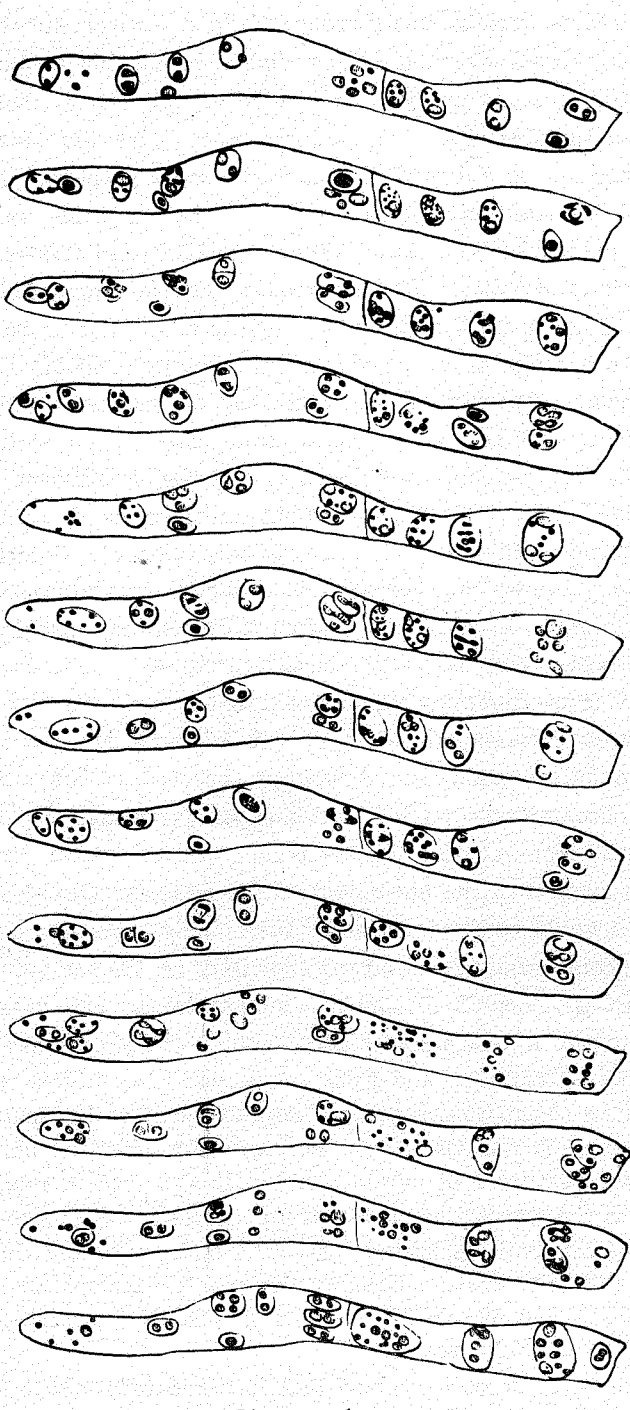
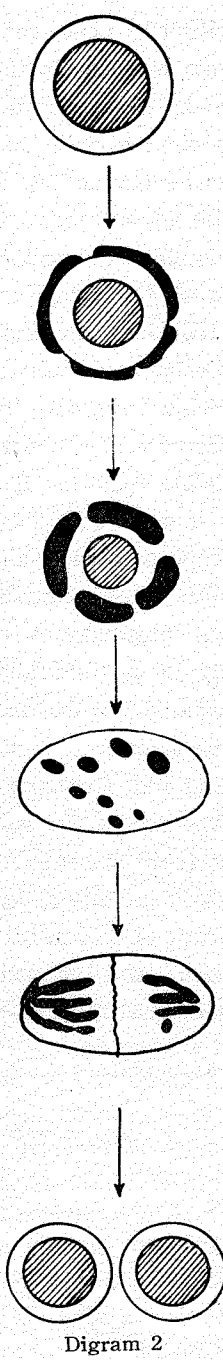


Diagram 1



Digram 2

Diagram 1: See description being made in connection with Figs. 1-11.
Diagram 2: Probable sequence of mitotic division of a single nucleus.

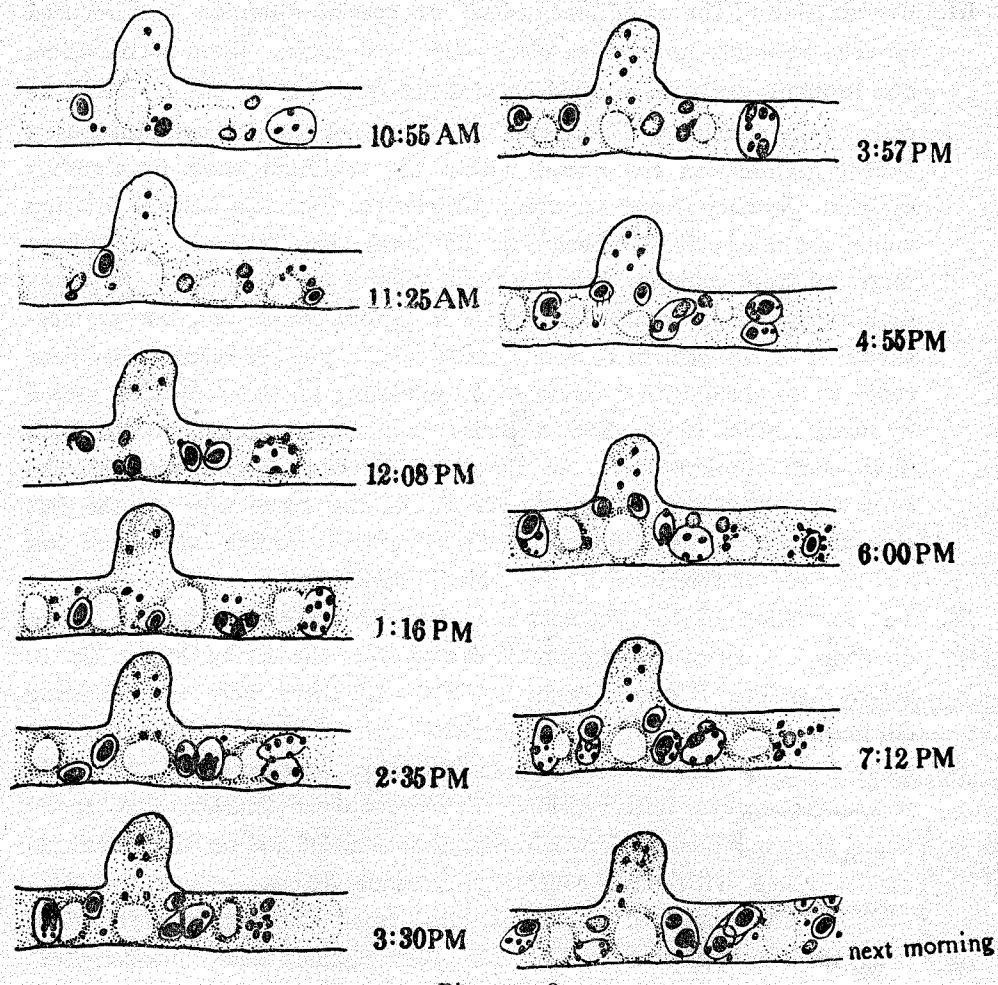


Diagram 3

Diagram 3: A series of diagrams drawn at intervals as indicated by the time when the diagrams were made. These were rough sketches which represented the major events in this hypha tip with actively dividing nuclei, showing repeated divisions of the nuclei at the "nuclear-sites" and finally leading to the formation of giant fused nuclei. Just behind the tip there were dividing nuclei with individual chromosomes usually in a group of four showing vigorous Brownian Movement. Since these chromosomes moved around so fast, as a consequence, actual and exact identification from the unflattened preparation was very difficult. At the end of this very observation however, "nuclear-sites" already laid down would lead to the formation of giant fused nuclei.

III. Germination of the conidium: Since no special attention was focused on this, therefore no detailed observation was made. Upon germination, one to many germ tubes could be sent out from one to all of the three cells of the conidium. But these tubes were not sent out simultaneously. During germination the nuclei within the conidium were in vigorous division, showing strong Brownian Movement. But this kind of division would not take place if there was no germ tube sent out. Cytoplasm migrated from conidium into the germ tube or tubes with one to many dividing nuclei going also along with it. From casual observation measurement of the growth of young germ tube, about 150 microns per hour could be obtained with a living hypha mounting on the slide and sealed by wax. Figs. 30–33 show a series of photomicrographs taken with phase-contrast-microscope. In these conidia upon germination all the cells were actively dividing. At the tip of the germ tube on the left side, there were free chromosomes in vigorous Brownian Movement but not confined or restricted by any spindle apparatus.

IV. The formation of the "nuclear-site" and its further expansion: From diagram 1, a series of diagrams is drawn from the living hypha tip in active division. It can be seen that at the tip there were free dividing chromosomes. Gradually a "nuclear-site" was formed a little distance away from the tip. It seems that generally, "nuclear-sites" were laid down acropetally. With repeated division at these "nuelear-sites" many nuclei were obtained as shown in diagrams 1 and 3 and serial photomicrographs taken with phase-contrast-microscope (Figs. 1–11 and 12–19). Finally these nuclei at a "nuclear-site" fused together to form giant fused nucleus as shown in Fig. 19. Why should these "nuclear-sites" expand to form giant fused nuclei and what is the function of this particular process, there is no explanation at this time. Since this kind of hyphae was found in deeper level of the cultural medium, some physiological changes, i. e. the lack of oxygen might be the inducing agent for these "nuclear-sites" to expand.

V. The nuclear division in hyphae grown on surface level of the culture: There were multitudinous nuclei in this types of hyphae. There was apparently no clear nuclear membrane surrounding every nucleus and simultaneously they would engage themselves in continuous division. In observing living material, since they had rapid Brownian Movement, detailed observation of this kind of nuclei was really difficult. When division began to take place, each nucleus apparaently consisted of 4 chromosomes. However no anaphase figure was ever observed, perhaps this stage was too abrupt to make the observation possible. Then finally

two newly formed daughter nuclei were observed. Sooner or latter one or both would divide again, so as to make the nuclei and chromosomes packed in the hypha look like the stars in the Milky Way as in Fig. 35 (phase-contrast-microscopy), Figs. 51, 52, and 53 (carmine stain).

VI. Aneuploidy: Suzuki (unpublished) made an assertion that besides 4 as a typical number of chromosomes in a single nucleus, 2, 3 or 5 chromosomes were also present. In our observation the typical number was four, since the living material could not be observed in a more flattened preparation (about 0.05 mm thick), numbers of chromosomes fewer than 4 could be asserted to be the result of faulty observation. Furthermore the chromosomes could migrate to quite a distance from the place of origin, one or two was possible to be included and counted with another nuclear group. In other words aneuploidy seemed to be not definitely observed in our experiments so far.

VII. Anastomosis: Fig. 46 shows the anastomosis to occur between two hyphae. This was the only case observed through-out our whole experiment.

Discussion

P. oryzae is classified in the order of Fungi Imperfecti. Its sexual stage has never been found so far. Since the hypha as well as cells of the conidium are multinucleated, it seems that no genetic study of this fungus can be obtained either from the sexual stage or by parasexual method. It is known that there is no diploidization and haploidization in the life cycle of this fungus. Genetic studies by the parasexual method seems to be precluded therefore.

From our observations, it is found that mitosis proceeds in a classical manner with distinct chromosomal division even though the spindle apparatus is not stained whatsoever with different kinds of stains used in our experimentation on top of the addition of ferric ion in the fixative used before staining. However, indications are that the spindle apparatus does exist especially in the photomicrographs taken with phase-contrast-microscope and by observation of living material. During metaphase the "halo" (Suzuki unpublished) is oval shaped instead of being roundish as in prophase (Figs. 20, 22). This oval shaped apparatus is maintained through anaphase to telophase. After the daughter nuclei are separated, the nucleus will resume the round shape again. This change of shape would tie up the resolving of the nuclear membrane with the formation of spindle apparatus in mitosis closely together. Since spindle apparatus is observed in several fungi such as *Basidiobolus* (Rabinow 1963), *Neurospora* (Singleton 1953, Somers *et al* 1960), and many other fungi, we are sure that if the staining technique can be improved, the spindle apparatus in stained preparations can be observed later.

Explanation of Plate Figures

Plate I. Figures 1-45 are from phase-contrast-microscopy Ca. 1280 X.

Figs. 1-II: These serial photomicrographs taken about 30 minutes interval showing the division of "nuclear-sites". Some of these photomicrographs are diagramatically presented in diagram 1 with the time when each diagram was made and is indicated at the right of each diagram. It is very interesting to note that the "nuclear-sites" were rather simple at the time when the observation started. Nuclei were dispersed with large sized vacuoles in between them. Only a portion of intercalary segment of the hypha is presented in the diagrams. During the observation both nuclei and chromosomes divided actively and moved vigorously with Brownian Movement. But these phenomena stopped altogether the next morning. It can be seen from the photomicrographs that nuclei of these expanding "nuclear-sites" were dividing and multiplying, finally leading to the formation of the giant fused nuclei.

Figs. 12-19: This series of photomicrographs were taken about half an hour interval showing the division of the "nuclear-sites" at the tip of a hypha. Arrows at the right point to the "nuclear-sites" in which repeated division of nuclei from 2 nuclei (Fig. 12), finally 4 (Fig. 17) were obtained, and finally all of them fused together to form a giant fused nucleus (Fig. 19). Arrows at left of the photomicrographs showing the expansion of the "nuclear-sites"by repeated divisions of the nuclei within this "nuclear-site".

Fig. 20: Showing a germinating 3-celled conidium. Arrows point to the dividing nuclei in the branched germ tube. From top to bottom: T, telophase; A, anaphase; M, metaphase, chromosomes arranged in the middle of the spindle apparatus.

Fig. 21: Conidium with giant fused nuclei, one for each cell.

Fig. 22: A segment of hypha showing: P, a dividing nucleus at prometaphase with nucleolus diminishing in size and chromosomes greatly contracted and lined up at the periphery of the nuclear membrane; SA, nucleus showing an oval "halo" indicating the presence of spindle apparatus. The oval apparatus is constricted in the middle indicating this nucleus is at telophase with daughter nuclei out of focus. The "halo" is oval in shape.

Figs. 23-27: A series of photomicrographs was taken at an interval of approximately 45 minutes appart, showing mitotic division of a nucleus as pointed out by arrows. I, interphase; P. prophase with a distinct nucleolus; A, anaphase, but the chromosomes in each of the two daughter nuclei are not very distinct. T, telophase with 2 daughter nuclei. The oval spindle apparatus is rather distinct.

Fig. 28: A young germinating conidium with a solitary nucleus near the tip of the germ tube.

Fig. 29: Two germ tubes were sent out by a germinating conidium. The nuclei inside the conidium were in vigorous division.

Figs, 30-33: A set of sequential photomicrographs which was taken at about 20 minutes interval, showing a germinating conidium with emerging germ tube.

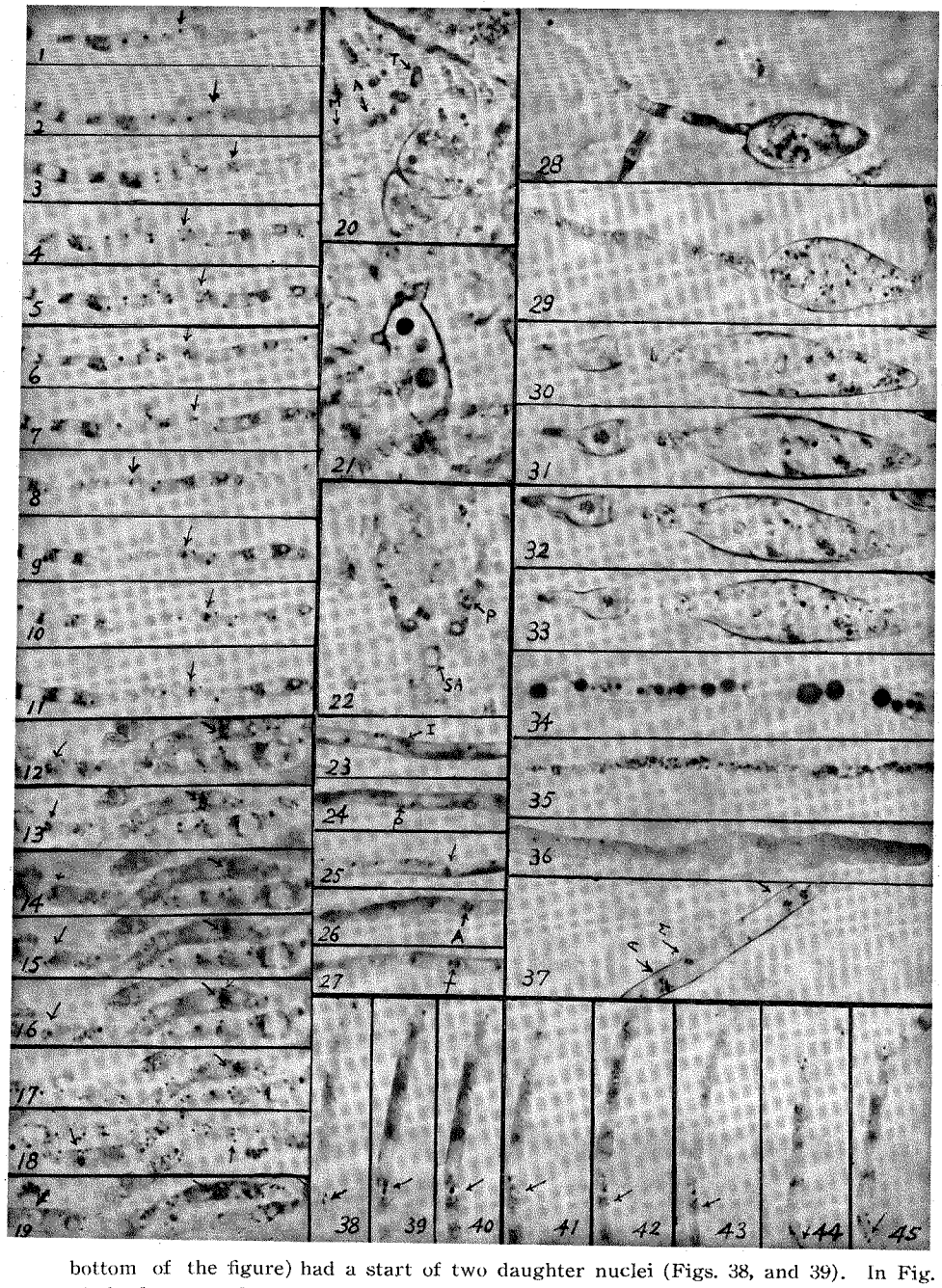
Fig. 34: A segment of hypha with numerous giant fused nuclei of various sizes.

Fig. 35; A segment of hypha with multitudinous and synchronously dividing nuclei, simulating the stars of the Milky Way.

Fig. 36: Tip of hypha grown in continuously shaking culture for 36 hours. Note the presence of dense cytoplasm, and the very sparsely placed compact nuclei of small size but dark and dense in appearance. This kind of hyphae grows rapidly and divides vigorously in spite of the sparsely placed nuclei.

Fig. 37: An old hypha with few nuclei. Arrows point to nuclei, M, metaphase; A, anaphase. Incidentally this hypha was under observation for almost 5 days. No further progress in the process of mitotic division was ever obtained.

Figs. 38-45: This is a series of photomicrographs, showing a segment of hypha with a young branch at the upper portion. The "nuclear-site" (pointed by arrows at the



bottom of the figure) had a start of two daughter nuclei (Figs. 38, and 39). In Fig. 40 the lower nucleus started to divide and the upper nucleus also divided later as shown in Fig. 42 and fianlly 4 daughter nuclei were obtained from successive divisions of the upper nucleus, but with only two daughter nuclei obtained from the division of the lower nucleus making a total of 6 nuclei in all for this "nuclear-site". Further division would mean more reduplication and accumulation of more nuclei, finally leading to the formation of a giant fused nucleus. At the upper segment of the hypha where the branch was located, the nuclei (3) were placed originally in the main hypha (Fig. 38) and gradually they migrated to the young branch (Figs. 44, 45).

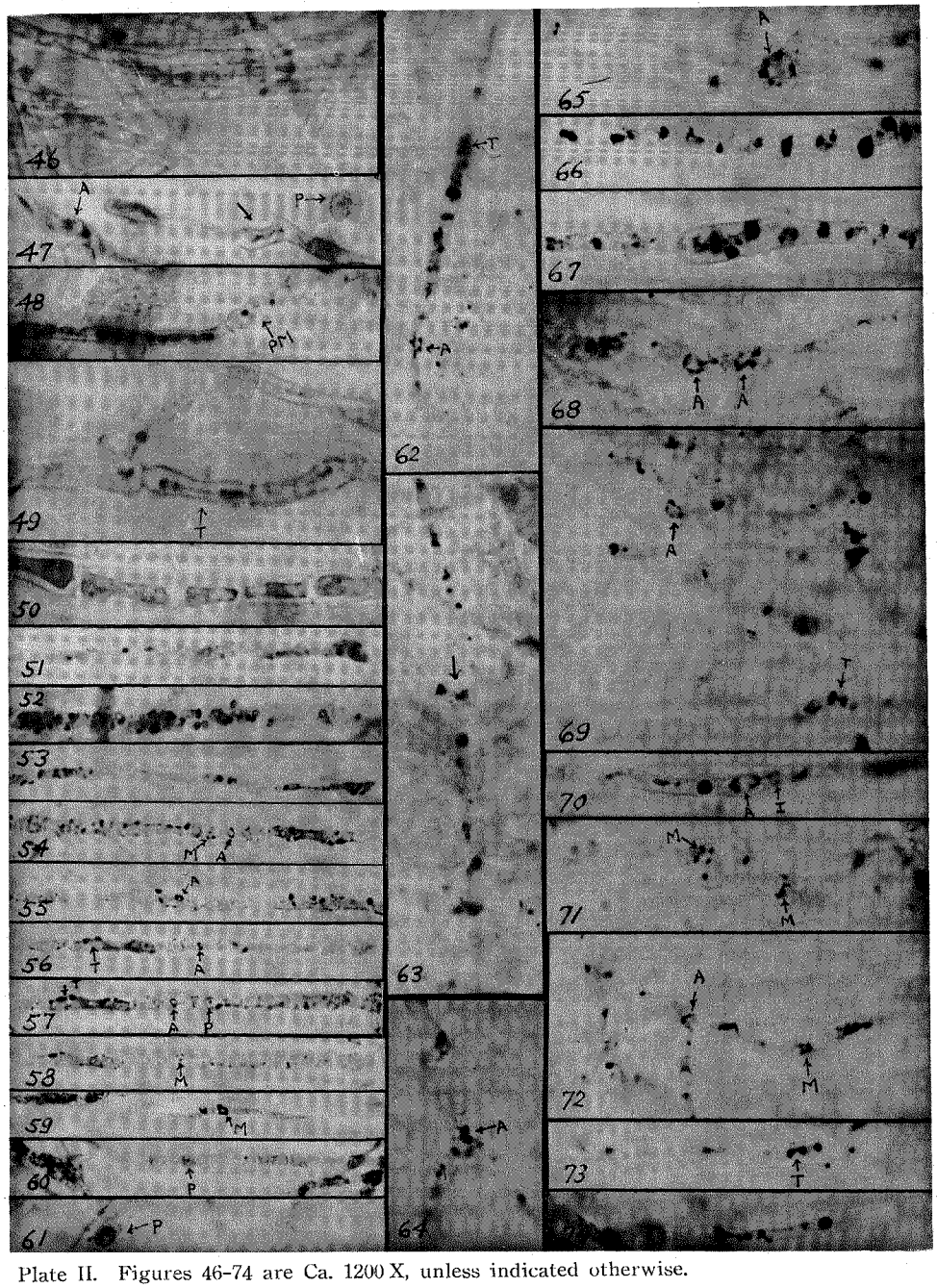


Plate II. Figures 46-74 are Ca. 1200 X, unless indicated otherwise.

Figures 46-50: HCl-Giemsa stain.

Fig. 46: Anastomosis. This was the only case observed through out the whole experiment.

Fig. 47: Nuclear migration indicated by an arrow. A, late anaphase figure on the left; P, arrow points to prophase with a nucleolus in the middle with chromatic material placed in juxtaposition to the nuclear membrane.

Fig. 48: prometaphase showing a nucleolus diminishing in size and fading in color in the middle of the nucleus. The four chromosomes were distinctly visible, two larger

It seems that the "nuclear-sites" are laid down near the tip of the hyphae which are grown in a submerged culture. From whatever meager observation obtained, the nucleus or nuclei moves into the germ tube together with the cytoplasm from the conidium which is in vigorous division with chromosomes in vigorous Brownian Movement. It seems that the daughter nuclei obtained would not be delimited by a nuclear membrane. One or both daughter nuclei may resume nuclear division again soon after the daughter nuclei are formed. However a nucleus with nucleolus and a distinct nuclear membrane, a typical nucleus, is formed behind these dividing free nuclei one

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and two smaller but they were all of different sizes.

Fig. 49: Telophase.

Fig. 50: Hypha with shorter cells separated by many septa. Each cell seemed to be multinucleated also.

Figs. 51-61: carmine stain.

Fig. 52: Hypha with multitudinous nuclei. Many of them were dividing synchronously.

Figs. 51, 52: Tip of hyphae. There were several nuclei near he tip dividing synchronously.

Figs. 54-58 Photomicrographs were taken of a single hypha at different foci showing different division figures.

Fig. 54: Arrows point to: A, anaphase; M, metaphase with 3 distinct chromosomes and the other one only faintly shown.

Fig. 55: A, anaphase.

Fig. 56: T, telophase; A. anaphase.

Fig. 57: T, telophase; A, anaphase; P, prophase, nucleolus appeared to be rather small with carmine stain as compared with that obtained from phase-contrast-microscopy or by HCl-Giemsa stain.

Fig. 58: M, metaphase, only 3 distinct chromosomes were visible at this focus.

Fig. 59: Metaphase figure showing 4 chromosomes attached themselves together in a ring--like-plate.

Fig. 60: Late prophase showing a small nucleolus in the middle with the contracted chromatic material at the periphery.

Fig. 61: Early prophase with nucleolus and chromatic material surrounded by distinct nuclear membrane.

Figs. 62-74: Leuco-basic fuchsin (Feulgen stain).

Fig. 62: A: Early anaphase, the daughter chromosomes beginning to move to their respective "polar" region. T, telophase.

Fig. 63: Migration of chromatic material from hypha to newly formed branch.

Fig. 64: Late anaphase.

Fig. 65: Anaphase figures, all the chromosomes were not clearly visible in this focus.

Figs. 66, 67: Hypha with giant fused nuclei, some "nuclear-sites" were dividing.

Fig. 68: Two neighboring anaphase figures as pointed by arrows.

Fig. 69: T, telophase; A, anaphase, all chromosomes being just divided.

Fig. 70: A, late anaphase; I, interphase, note the absence of nucleolus.

Fig. 71: M, mataphase, one on the left showing the 4 chromosomes lined up at the equator, while one on the right showing the 4 chromosomes rounded up like a ring.

Fig. 72: M, metaphase, showing 4 chromosomes rounded up like a ring. Note the chromatic connections among themselves. A, anaphase.

Fig. 73: Telophase.

Fig. 74: A giant fused nucleus near the tip of a hypha.

by one acropetally as shown in diagram 1, Figs. 30–33. These nuclei are the "nuclear-sites" laid down. Consequently, the growth of the hyphae is limited at the tip. Zalokar (1959) found that the growth of the hyphae of *Neurospora crassa* was localized a short distance from the tip of each hypha. Perhaps, the observation so obtained may offer the explanation for this.

After these "nuclear-sites" are laid down, these sites would resume repeated nuclear division again as shown in diagram 1 and 3, and finally these nuclei would fuse together to form giant fused nuclei of different sizes and various forms. Consequently, the end result of the repeated division at the "nuclear-sites" would lead only to the expansion or enlargement of these "nuclear-sites" but not direct to the lengthening of the hypha in which these "nuclear-sites" are located.

As both hyphae and conidia are multinucleated, and many giant fused nuclei are also multinucleated, the heterokaryotic condition of *P. oryzae* would be perpetuated if it has started as a heterokaryon. Furthermore, anastomosis is observed. This would initiate heterokaryosis even if it starts out from a homokaryon.

Zalokar (1959) working with *Neurospora* asserted that these giant fused nuclei are fat globules. Since they take up leuco-basic fuchsin readily, they must contain DNA. Therefore, they are chromatic in nature.

Summary

P. oryzae was multinucleated in both hyphae and conidia. The nuclear condition would vary when the hyphae were obtained from different levels of the culture. Or it would also vary if the culture was grown in an aeriated, continuous shaking liquid medium. Each nucleus had 4 chromosomes; two larger and two smaller but all of different sizes. They would divide with classical manner of mitosis. Upon germination, the conidium sent out one to several germ tubes and the nuclei in the dividing cell or cells of this conidium seemed to divide actively and synchronously. Cytoplasm as well as one to several nuclei from each cell were transmitted to its germ tube. "Nuclear-sites" were laid down acropetally. Further nuclear division of this "nuclear-sites" would lead finally to the formation of the giant fused nuclei. Anastomosis was observed.

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Piricularia oryzae Cav. 的細胞研究

朱 名 玉 李 先 聞

Piricularia oryzae 的 hypha 和 Conidium 都是多核的，由 culture 不同深度取出的 hyphae 細胞核的組成亦各不相同。或者細胞核的組成也會因它們長在一直不斷搖動的液體培養基中而有所變異。每個細胞核有四個染色體：兩個較大和兩個較小的，不過它們每個的大小都不一樣，這些染色體的分裂是與平常體細胞分裂相同的。在孢子發芽的時候，會由 Conidium 發出一個或數個 germ tubes。細胞核在這時分裂得快而且一致，細胞質也同時由 conidium 分別的流傳到這些 germ tubes 裏面，向心地產生了 "nuclear-sites"，這些 "nuclear sites" 細胞核的繼續分裂最後將導致 giant fused nuclei 的生成。Anastomosis 現象也曾觀察到。

Literature Cited

BAKERSPIGEL, A. The structure and mode of division of the nuclei in the vegetative spores and hyphae of *Endogone sphagnophila* Atk. Amer. Jour. Bot. **45**: 404-410. 1958.

BAKERSPIGEL, A. The structure and manner of division of the nuclei in the vegetative mycelium of *Neurospora crassa*. Amer. Jour. Bot. **46**: 180-190. 1959.

BAKERSPIGEL, A. The structure and manner of division of the nuclei in the vegetative mycelium of the Fungi Imperfecti I. *Phyllosticta* spp. Cytologia 24: 516-522.1959.

BAKERSPIGEL, A. Nuclear structure and division in the Fungi Imperfecti II. *Scopulariopsis brevicaulis*. Cytologia **25**: 344-351. 1960.

BAKERSPIGEL, A. Nuclear structure and division in the vegetative mycelium of the Saprolegniaceae. Amer. Jour. Bot. **47**: 94-100. 1960.

BRACKER, C. E. Jr. and E. E. BUTLER The ultrastructure and development of septa in hyphae of *Rhizoctonia solani*. Mycologia **55**: 35-57. 1963.

CARMICHAEL, J. W. The cellophane technique for studying morphlogy and hyphal fusion in fungi. Mycologia **48**: 450-453. 1956.

CARR, A. J. H. and OLIVE, L. S. Genetics of *Sordaria finicola* II. Cytology. Amer. Jour. Bot. **45**: 142-150. 1958.

COLE, H. and COUCH, H. B. Cytological investigations of *Kabatiella caulivora*. Amer. Jour. Bot. **46**: 12-16. 1959.

CUTTER, V. M. The chromosomes of *Neurospora tetrasperma*. Mycologia **38**: 693-698. 1946.

DARLINGTON, C. D. and L. F. LA COUR The handling of chromosomes. London, Georger Allen & Unwin Ltd. 1960.

DELAMATER, E. C. Basic fuchsin as a nuclear stain for fungi Mycologia **40**: 423-429. 1948.

DOWDING, E. S. and J. WEIJER Mitosis in *Neurospora*. Nature **188** (Oct. 22): 338-339. 1960.

EL-ANI, A. S., L. J. KLOTZ and W. D. WILBAR Heterothallism, heterokaryosis and inheritance of brown perithecia in *Ceratostomella radicicola*. Mycologia **49**: 181-187. 1957.

FUERST, R. and T. C. HSU Cytology of vegetative growth in *Neurospora crassa*. Genetics **42**: 371. 1957.

GOSS, R. D. Spermatium-trichogyne relationship in *Gelasinospora calospora* var. autosteria. Mycologia **51**: 416-428. 1959.

GRAHAM, K. M. Nuclear behavior in *Phytophthora infestans* (Mont.) de Bary. Phytopathol. **44**: 490. 1954.

HALL, R. Cytology of the asexual stages of the Australian Brown Rot Fungus *Monilinia fructicola* (Wint.) Honey. Cytologia **28**: 181-193. 1963.

HARTMANN, G. C. Nuclear division in *Alternaria tenuis*. Amer. Jour. Bot. **51**: 209-212. 1964.

HAWKER, L. E. and P. MCV. ABBOTT Fine structure of vegetative hyphae of *Rhizopus*. J. Gen. Microbiol. **30**: 401-408. 1963.

HSU, T. C. and R. FUERST Biology of *Neurospora crassa*. Genetics **41**: 648. 1956.

KNOX-DAVIES, P. S. and J. G. DICKSON Cyology of *Helminthosporim turcicum* and its ascigerous stage, *Trichometasphaeria turcica*.. Amer. Jour. Bot. **47**: 328-339. 1960.

KOENIG, R. and F. L. HOWARD Nuclear division and septum formation in hyphal tips of *Fusarium oxysporum*. Amer. Jour. Bot. **49**: 666. 1962.

LIMA-DE-FARIA, A. and BOSE, S. Spectrophotometric analysis of aceto-carmine solution. Hereditas **40**: 419-424. 1954.

LU, B. C. A new fixative and improved propionocarmine squash technique for staining fungus nuclei. Can. Jour. Bot. **40**: 843-847. 1962.

LU, B. C. and H. J. BRODIE Chromosomes of the fungus *Cyathus*. Nature **194**: 606. 1962.

LU, B. C. Polyploidy in the Basidiomycetes: *Cyathus stercoreus*. Amer. Jour. Bot. **51**: 343-347. 1964.

OLIVE, L. S. The structure and behavior of fungus nuclei. Bot. Rev. **19**: 439-586. 1953.

PAVGI, M. S., COPPER, D. C. and DICKSON, J. C. Cytology of *Puccinia sorghi*. Mycologia **52**: 608-620. 1960.

RATTENBURY, J. A. A rapid method for permanent acetocarmine squash preparation. Nature **177**: 1185-1186. 1956.

ROAN, C. W. A method of preparing fungi for cytological studies. Phytopathol. **42**: 480. 1952.

ROAN, C. W. Nuclear division and morphologic variation in *Helminthosporium carbonum*. Phytopathol. **42**: 480. 1952.

ROBINOW, C. F. Mitosis in the yeast *Lipomyces lipofer*. J. Biophys. Biochem. Cytol. **9**: 879-892. 1961.

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

SAKSENA, H. K. and VAARTAJA, O. Taxonomy, morphology and pathogenicity of *Rhizoctonia* species from forest nurseries. Can. Jour. Bot. **39**: 627-674. 1961.

SAKSENA, H. K. and VAARTAJA, O. Nuclear phenomena in the basidium of *Ceratobasidium praticolum* (Kotila) Olive. Can. Jour. Bot. **39**: 717-725. 1961.

SAKSENA, H. K. and VAARTAJA, O. Nuclear structure and division in the mycelium and basidiospores of *Ceratobasidim praticolum*. Can. Jour. Bot. **39**: 749-756. 1961.

SINGIETON, J. R. Chromosome morpnology and the chromosome cycle in the ascus of *Neurospora. crassa*. Amer. Jour. Bot. **40**: 124-144. 1953.

SOMERS, C. E., R. P. WAGNER and T. C. HSU Mitosis in vegetative nuclei of *Neurospora crassa*. Genetics **45**: 801-810. 1960.

STALL, R. E. An investigatien of nuclear number in *Alternaria solani*. Amer. Jour. Bot. **45**: 657-659. 1958.

SUZUKI, H. (unpublished) The origin of variation in *Piricularia oryzae* Car. Symposium on Rice Blast Disease Fungus (*P. oryzae*) at International Rice Research Institute, Los Banos, Laguna, Philippines. July 7-12, 1963.

TANAKA, S. Nutrition of *Piricularia oryzae* Cav. in vitro. Ibid.

TURIAN, G. and E. C. CANTINO A study of mitosis in the mold *Blastocladiella* with a ribonuclease, aceto-orcein staining technique. Cytologia **25**: 101-107. 1960.

WARD E. W. B. and K. W. CIURYSEK Somatic mitosis in a basidiomycetes. Can. Jour. Bot. **39**: 1497-1503 1961.

CIURYSEK WARDE, E. W. B. and K. W. CIURYSEK Somatic mitosis in *Neurospora*. Amer. Jour. Bot. **49**: 393-399. 1962.

WEIJER, J. and E. S. DOWDING Nuclear exchange in a heterokaryon of *Neurospora crassa*. Can. Jour. Genet. Cytol. **2**: 336-343. 1960.

ZALOKAR, MARKO Enzyme activity and cell differentiation in *Neurospora*. Amer. Jour. Bot. **46**: 555-559. 1959.

ZALOKAR MARKO Growth and differentiation of *Neurospora* hyphae. Amer. Jour. Bot. **46**: 602-610. 1959.