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Nuclear Membrane Behavior during Mitosis in Normal and Heteroploid Myxomycetes

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With 35 Figures

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Summary

Photomicrographic evidence is presented of the difference in behavior of nuclear membranes during mitosis in amoebae, zygotes and plasmodia of Myxomycetes. One of the species was cultured on bacteria and possessed a normal cycle of plasmogamy and karyogamy between the amoebal and plasmodial phases. The second species was axenically grown in liquid media and had become highly heteroploid and lacked the ability to develop into plasmodia, existing only in the amoeboid form. The significance of the amoeboid form of mitosis in the heteroploid axenically cultured strain is discussed in relation to the difference in nuclear membrane behavior and the possible significance of such behavior.

1. Introduction

The progressive changes in cell form and behavior that occur during the Myxomycete life cycle have attracted the attention in recent years of those interested in cell differentiation and morphogenesis. Despite this interest, many phases of the Myxomycete life cycle and of the mechanisms that control the changes from one cell form to another are very imperfectly understood both descriptively and functionally (cf. ALEXOPOULOS 1966). One aspect that has not received a great deal of attention is the behavior of the nuclear membrane during mitosis. CADMAN (1932) indicated that the nuclear membrane disappeared during mitosis in the amoebae of *Didymium nigripes* but was retained throughout mitosis in the plasmodia. These observations have recently been confirmed in various Myxomycetes by several investigators using time-lapse and still photomicrography and electron microscopy (KOEVENIG 1964, SCHUSTER 1965, ALDRICH 1967, ROSS 1967 b and c, N. KERR 1967, S. J. KERR 1967). However, most of these observations were made in conjunction with life cycle and general studies and have been mentioned more or less in passing with the suggestion that such behavioral differences were useful in distinguishing between haploid and diploid mitosis in the Myxo-

mycetes. With one exception, the implication to be derived from the past work is that such nuclear membrane behavior is a function of the ploidy of the cells involved, but there has been little discussion of any possible further significance of the phenomenon. S. J. KERR (1967) refers to the possibility that the nuclear membrane behavior may not be directly related to ploidy, but since the origins of the plasmodia and the ploidy of the cells she examined are obscure it is difficult to arrive at a definitive conclusion from her study. According to MAZIA (1964) virtually nothing is known about the mechanisms controlling the breakdown of the nuclear membrane. It would seem, therefore, that the Myxomycetes, which appear to be unique in possessing two different patterns of nuclear membrane behavior in different vegetative phases of their life cycle, may be valuable organisms with which to investigate nuclear membrane behavior, once the conditions responsible for the differences are better understood.

2. Materials and Methods

The Myxomycetes chosen for study were *Didymium iridis*, a heterothallic species isolated from leaf debris on the UCSB campus, and *Badhamia curtisii*, a homothallic species isolated from oak leaves in Mt. Lake, Va. *D. iridis* was selected because its pattern of sexuality and plasmodium formation has recently been established (Ross 1967 c), it will complete a normal life cycle in a few days on a bacterial food supply, and since it is heterothallic, it can be maintained indefinitely in the amoeboid state. *B. curtisii* has been grown for several years in axenic culture on defined media and has undergone severe cultural changes leading to heteroploidy and an inability to form plasmodia (Ross 1966). The organisms were grown in microcultures, described in detail elsewhere (Ross 1967 c), which allow prolonged phase contrast observation without hindering normal development. Photographs were made through a Zeiss WL microscope equipped with phase contrast optics on Kodak High Contrast Copy film using a Leitz Mikroblitz electronic flash for illumination.

3. Observations

Mitosis in Myxomycete amoebae, zygotes, and plasmodia is very similar to that of other organisms at the light microscope level, with the exception of the behavior of the nuclear membrane. In the amoebae of *D. iridis*, mitosis

Fig. 1. Flagellate cell, interphase nucleus. ×1,800
Fig. 2. Amoeboid cell, interphase nucleus. ×1,800
Fig. 3. Amoeba, nucleus in prophase. ×2,000
Fig. 4. Same cell as in Fig. 3, 1 minute later. ×2,000
Fig. 5. Same cell as in Fig. 4, 5 seconds later, nuclear membrane has just disappeared. ×2,000
Fig. 6. Amoeba, nucleus in prophase. ×2,000
Fig. 7. Same cell as in Fig. 6, 30 seconds later. ×2,000
Fig. 8. Amoeba, nucleus in prophase. ×2,000
Fig. 9. Same cell as in Fig. 8, 2 seconds later. ×2,000
Fig. 10. Amoeba in metaphase, note conical spindle. ×1,800
Fig. 11. Same cell as in Fig. 10, 1 minute later, anaphase beginning. ×1,800
Fig. 12. Same, 30 seconds later, late anaphase. ×1,800
Fig. 13. Same, 2 minutes later, telophase and cytokinesis. ×1,800

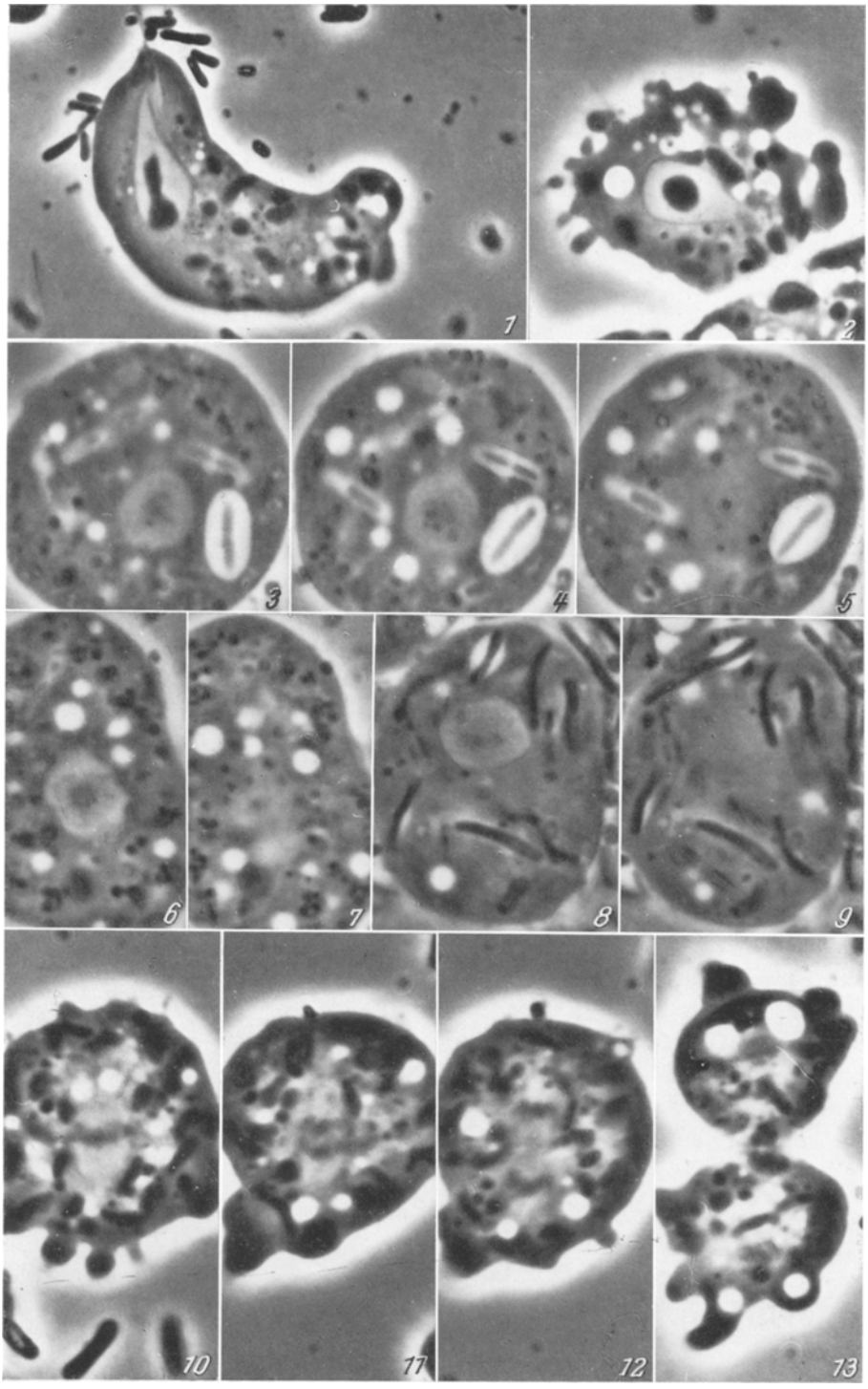

Plate I. Figs. 1–13. *Didymium iridis*

was relatively rapid, the duration varying from 5–15 minutes, while mitosis in zygotes and plasmodia required 15–30 minutes. The times given are from the first visible indication of the onset of mitosis, the rounding up of the nucleus and the appearance of chromatin strands surrounding the nucleolus, and consequently do not include any prophase events prior to those visible with the phase contrast microscope. Characteristic interphase nuclei of the amoeboid phase (flagellate and non-flagellate) are shown in Figs. 1 and 2 for comparison.

In *D. iridis* amoebae the nuclear membrane disappeared one to two minutes after the chromatin strands had become visible, concurrently with the final dissolution of the nucleolus (Figs. 3, 5, 6, 7, 8, and 9). The rapidity with which the nuclear membrane vanishes is indicated by noting that Fig. 5 was taken 5 seconds after Fig. 4 and Fig. 9 was taken 2 seconds after Fig. 8. Once the nuclear membrane had disappeared the nuclear area was no longer sharply separated from the cytoplasm, but gradually merged with it (Figs. 5, 7, and 9). In most cases chromatin strands that had been visible during early prophase became invisible immediately after the membrane vanished (compare Fig. 6 with Fig. 7 and Fig. 8 with Fig. 9), indicating a sudden change in the refractive index of the nucleoplasm. Metaphase plates were usually very indistinct in *D. iridis* and the course of amoebal mitosis could be followed mainly by noting the elongation of the gray nuclear area. The sequence shown in Figs. 10–13 was taken under excellent phase contrast conditions and shows the metaphase plate and separating groups of anaphase chromosomes far more clearly than were usually seen. Fig. 10 shows the characteristic double conic shape of the spindle at metaphase. After cytokinesis had begun the reorganizing nuclei remained indistinct and did not reappear clearly for another 10–15 minutes.

Mitosis in *B. curtisii* amoebae resembled that just described but with all events much clearer and of longer duration, lasting 30–45 minutes. The prophase chromatin was very clear (Figs. 14 and 16), which made it relatively easy to recognize the beginning of mitosis. Prophase lasted 10–15 minutes but terminated rapidly with the disappearance of the nuclear membrane (Figs. 14–15 and 16–17). Prometaphase chromosomes were indistinct (Figs 15, 17, and 18), but rapidly formed a clear metaphase plate with the

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Fig. 14. Amoeba with polyploid nucleus in prophase

Fig. 15. Same cell as in Fig. 14, 30 seconds later after nuclear membrane has just disappeared

Fig. 16. Amoeba with polyploid nucleus in prophase. Arrow points to part of a smaller nucleus in another amoeba

Fig. 17. Same cell as in Fig. 16, 2 minutes later. Arrow indicates another small nucleus

Fig. 18. Amoeba just after disappearance of the nuclear membrane

Fig. 19. Same cell as in Fig. 18, 3 minutes later, nucleus in metaphase

Fig. 20. Same, 30 seconds later, metaphase plate swinging

Fig. 21. Same, 2 minutes later, anaphase

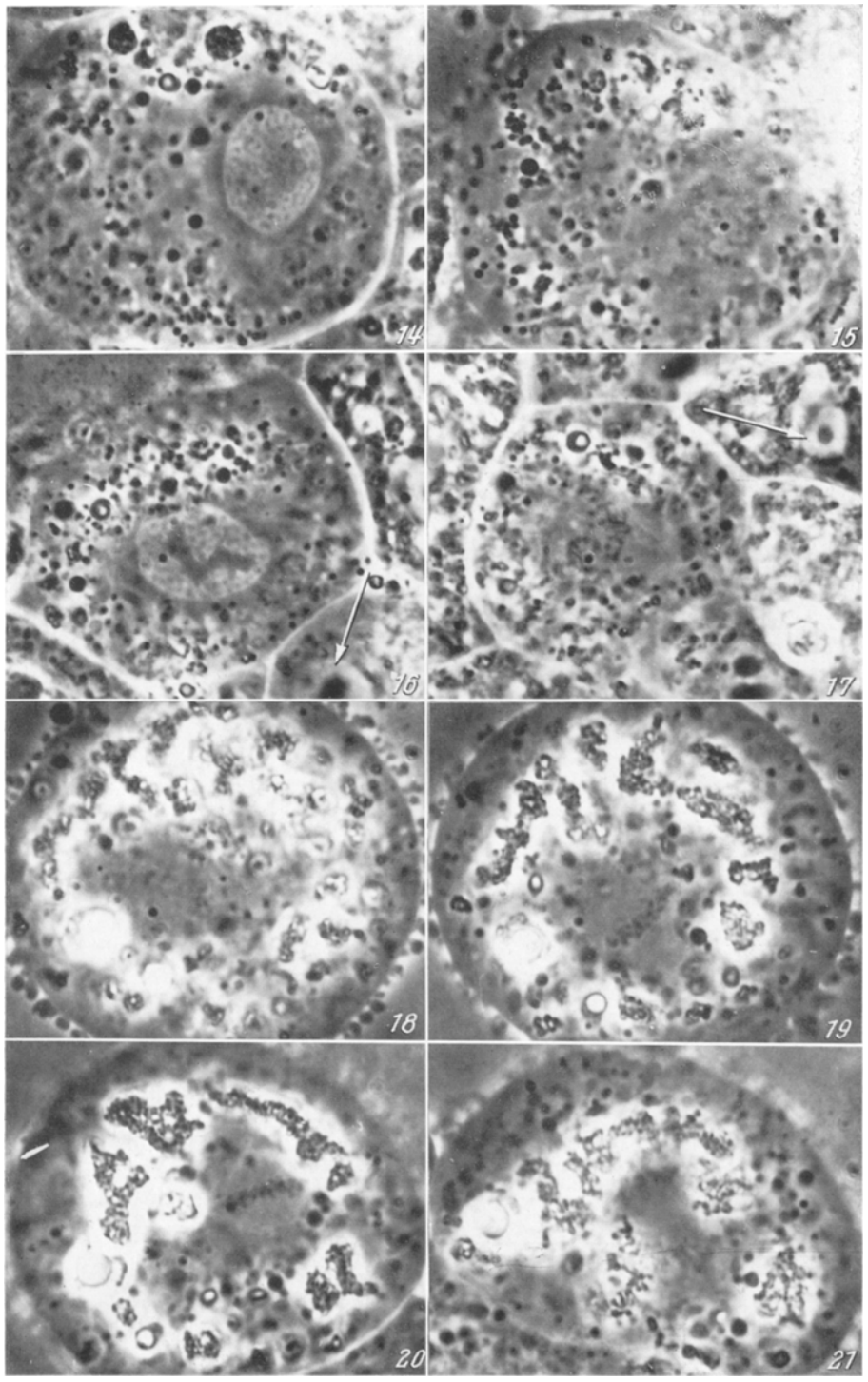

Plate II. Figs. 14–21. *Badhamia curtisii.* All ×1,600

characteristic spindle shape (Fig. 19). Movement of individual chromosomes on the plate could not be seen, but the plate as a whole swung slowly back and forth in a short arc (compare Figs. 19 and 20) before anaphase began (Fig. 21). Because of the heteroploid condition of these cultures amoebae with several different sizes of nuclei with chromosomes numbers ranging from 20–300 were always present (note nuclear sizes in Figs. 16 and 17). The pattern of membrane behavior just described was found in all cells of *B. curtisii* amoebae throughout the range of sizes and chromosome numbers in the cultures.

Occasionally in these cultures large cells can be found containing 2–8 nuclei. On two occasions I have seen mitosis in such multinucleate cells, the divisions in both cases being synchronous and all nuclear membranes disappeared at the end of prophase.

Mitosis in the zygotes and plasmodia of *D. iridis* is indistinguishable from that of the amoebae during early prophase (Figs. 27 and 32) but becomes recognizably different from prometaphase onward by the retention of the nuclear membrane (Figs. 22–26, 27–30, and 32–35). The continued presence of the nuclear membrane is indicated in light microscopy by the absence of any change in the periphery of the nuclear area, the persistence of a sharp delimitation of the nuclear area from the cytoplasm, and the presence under good phase contrast conditions of a distinct black line separating the nucleus from the cytoplasm (Fig. 30). Throughout metaphase the nuclear area remains spherical with no visible indication of a double conic spindle shape. The metaphase plates in both zygotes and plasmodia rotated completely within the nuclear membrane until anaphase (compare Figs. 22 and 23, 32, 33, and 34). The speed of rotation was not constant, but no pattern could be determined in the erratic pulsing of fast and slow rotation. Mitosis in the zygote (Figs. 22–25) is not followed by cytokinesis, but results in a bi-nucleate cell that develops into a multi-nucleate plasmodium by successive mitosis (Figs. 27–31) and/or coalescence with other zygotes (Ross 1967 c). The mitoses in larger multi-nucleate plasmodia (Figs. 32–35) were not in precise synchrony, but gradually spread from one end of the plasmodium throughout the rest so that anaphase in the early part (Fig. 35, bottom) began 15 minutes before the last nucleus in the plasmodium entered anaphase. This mitotic wave pattern was characteristic of all *D. iridis* plasmodia examined and has been illustrated elsewhere for the Myxomycete *Perichaena vermicularis* (Ross 1967 b).

Fig. 22. Zygote, nucleus in metaphase (arrow), *fv* = food vacuole with engulfed amoeba. ×2,000

Fig. 23. Same cell as Fig. 22, 30 seconds later, metaphase plate rotating. ×2,000

Fig. 24. Same, 2 minutes later, anaphase. ×2,000

Fig. 25. Same, 30 seconds later. ×2,000

Fig. 26. Same, 30 seconds later, late anaphase. ×2,000

Fig. 27. Bi-nucleate plasmodium, both nuclei in late prophase (arrows). ×1,800

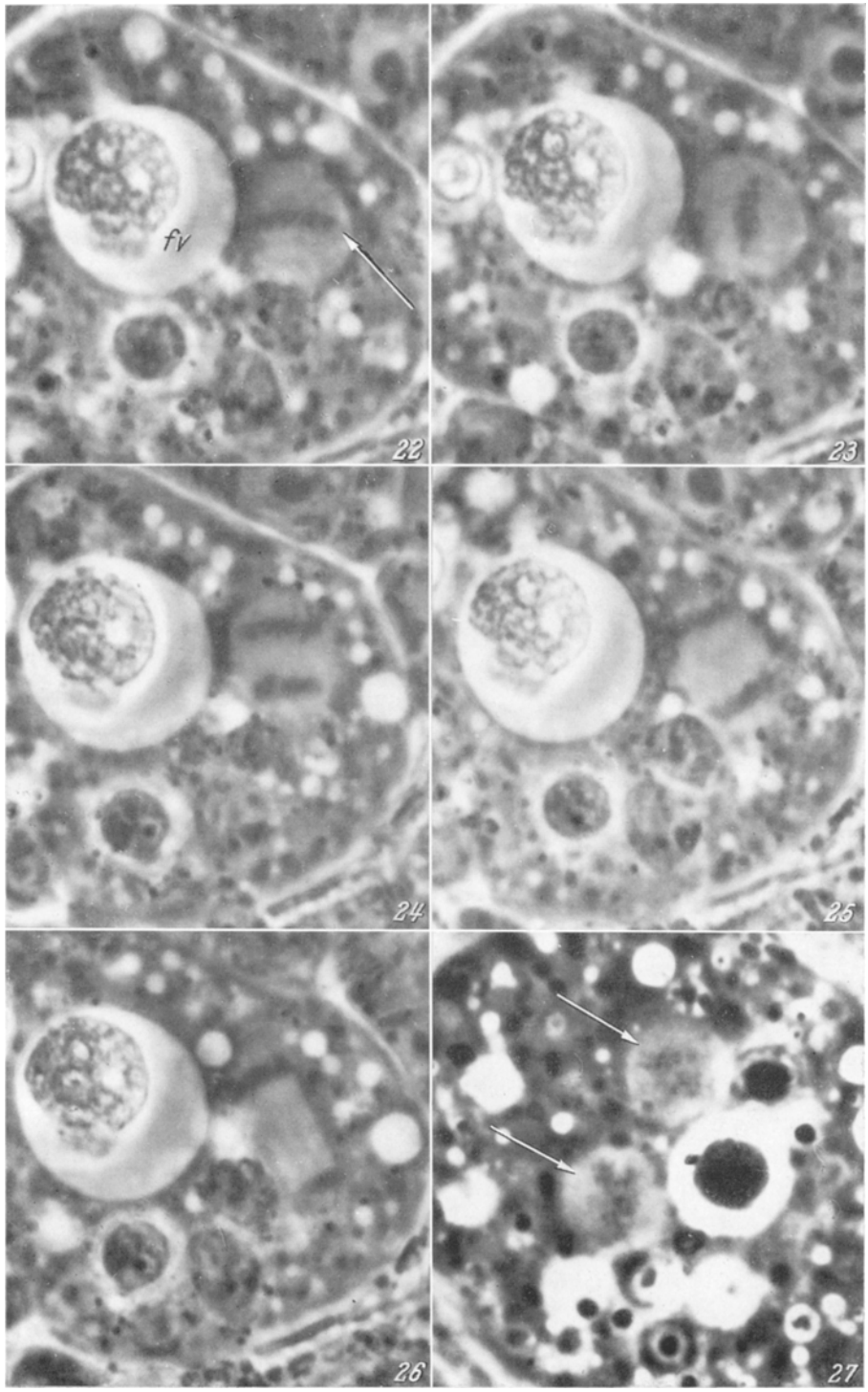

Plate III. Figs. 22–27. *Didymium iridis*

4. Discussion

The evidence presented here confirms the reports cited in the introduction that the Myxomycetes possess two patterns of nuclear membrane behavior during mitosis in the vegetative phases. As mentioned above, this difference has been used to differentiate between haploid and diploid mitosis, on the assumption that since plasmodia have one kind of membrane behavior and are believed to be derived from a sexual union of haploid cells, the mitotic behavior is a reflection of the diploid nature of the plasmodial nuclei. In many of the previous studies on amoebae and plasmodia of Myxomycetes there has been considerable doubt as to the origin of the plasmodial phase and, therefore, of actual difference in ploidy between the amoebal and plasmodial phases (cf. ALEXOPOULOS 1966). The zygotes and plasmodia of *D. iridis* used here were known, by continuous observation, to have been derived from the plasmogamy and karyogamy of amoebae and amoebal nuclei and to have followed the same pattern and timing as described earlier (Ross 1967 c) and were consequently known to be of ploidy different from the amoebae. This in itself would seem to indicate that the difference in nuclear membrane behavior is indeed a function of the ploidy of the cells involved. That this concept is not wholly tenable is shown by the mitotic behavior in the heteroploid *B. curtisii* and is indicated by recent work of N. KERR on a mutant form of *D. nigripes*. The *B. curtisii* amoebae possessed a considerable range of chromosome numbers in steps indicating autopolyploidy (Ross 1966). Amoebae of all sizes and degrees of ploidy were seen in mitosis and in every case the nuclear membrane disappeared, indicating that it is not the ploidy of the cell alone that determines the behavior of the nuclear membrane. N. KERR (1967) in a study of a mutant, apparently asexual, form of *D. nigripes*, reported that a single cell could undergo mitosis with the loss of the nuclear membrane, followed by cytokinesis, and that the daughter cells, without any apparent cell or nuclear fusion, could undergo mitosis with the retention of the nuclear membrane, without cytokinesis following, and thus develop directly into a plasmodium with the characteristic nuclear membrane behavior. This change in nuclear membrane behavior occurred without any apparent change in the ploidy of the cells involved.

Other evidence indicates further that the nuclear membrane behavior is not a function of multi-nuclearity of the cells. The loss of the nuclear membranes during mitosis in multi-nucleate amoebae of *B. curtisii* and an earlier report

Fig. 28. Same bi-nucleate plasmodium as Fig. 27, 4 minutes later, metaphase. ×1,800

Fig. 29. Same, 2 minutes later, anaphase beginning. ×1,800

Fig. 30. Same, 45 seconds later, late anaphase, nuclear membranes visible as black lines (arrows). ×1,800

Fig. 31. Same, 10 minutes later, 4 daughter nuclei visible (arrows). ×1,800

Fig. 32. Multi-nucleate plasmodium, most nuclei in metaphase (*m*), some still in prophase (*p*). ×1,000

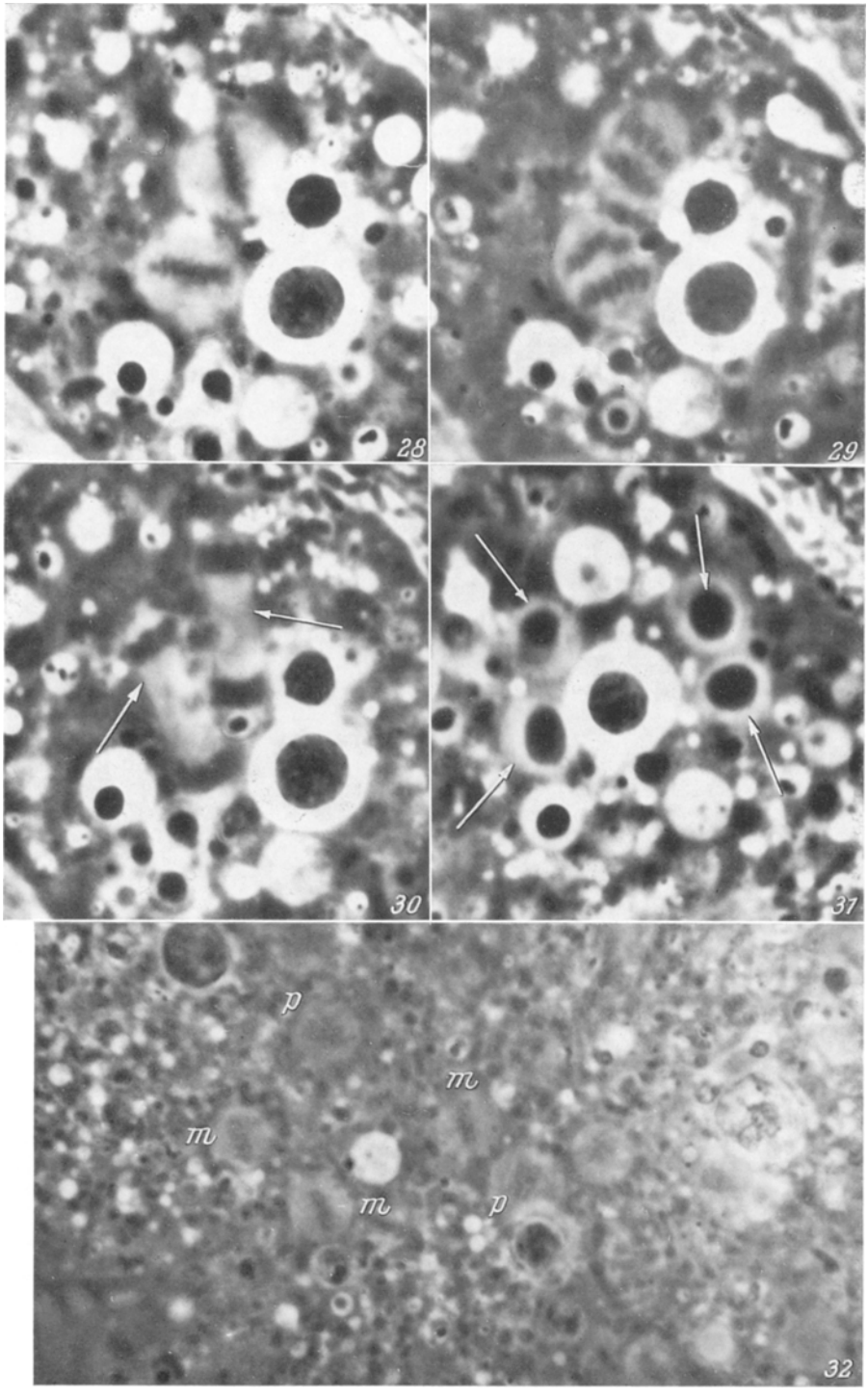

Plate IV. Figs. 28–32. *Didymium iridis*

(Ross 1967 a) on nuclear behavior in abnormally bi-nucleate amoebae of *D. iridis* shows that the mere presence in one cell of more than one nucleus is not sufficient to cause the retention of the nuclear membrane. Conversely, the fact that uni-nucleate zygotes do retain the membrane during mitosis shows that membrane disappearance is not a result of there being but one nucleus in the cell.

It is evident that the change in nuclear membrane behavior cannot be directly or simply related to a change in ploidy nor to a change in nuclear number as a result of coalescence or lack of cytokinesis. The nuclear membrane difference is but one of the numerous differences that exist between the normally uni-cellular uni-nucleate amoeboid phase and the multi-nucleate acellular plasmodial phase. This transformation from one cell type to another may not be necessarily mediated by sexuality even though sexuality is known to or suspected to occur at the time and location of the transformation in nearly every case examined with possible exception of the system described by N. Kerr (1967) (cf. Alexopoulos 1966). Evidence has been presented elsewhere (Ross 1967 a, b, c) suggesting that the trigger mechanisms responsible for the initiation of this transformation are independent of nuclear behavior and may reside in changes in and interactions between the cell plasma membranes. The difference in behavior of the nuclear membranes may merely reflect an overall difference in the cell membrane structure and physiology between the amoeboid and plasmodial phases. The occurrence of a sexual union at the time of this transformation could therefore be a more or less uniform result of such membrane changes, rather than a cause.

In most plants and animals the nuclear membrane breaks down during mitosis (cf. Mazia 1964) and only in some protozoa (Elliot 1963, Mazia 1964), in vegetative hyphae of fungi (Robinow and Bakerspigel 1965, Motta 1967), in some algae (Godwood 1966) and in Myxomycete plasmodia is the nuclear membrane known to or suspected to remain intact during most of the mitotic cycle. It may be significant that these organisms have all been regarded at one time or another as "acellular". Disregarding the applicability or validity of such a term, the existence of such a common nuclear membrane behavior pattern in these organisms does imply that such behavior may be of some functional importance to organisms whose structure and functions do not fit strictly into the classical concepts invoked by the cell theory. The possession of a persistent nuclear membrane in these "acellular" and predominately coenocytic organisms suggests that such behavior is necessary for the maintenance of that kind of form.

Fig. 33. Same multi-nucleate plasmodium as Fig. 32, 1 minute later. All nuclei in metaphase, some viewed obliquely (*om*) and some from the side (*sm*)

Fig. 34. Same, 30 seconds later, metaphase plates rotating (compare *om* and *sm* with those of Fig. 33)

Fig. 35. Same, 3 minutes later. Anaphase (*a*) beginning in bottom half, rest of nuclei in metaphase (*m*) (arrows indicate separating groups of anaphase chromosomes)

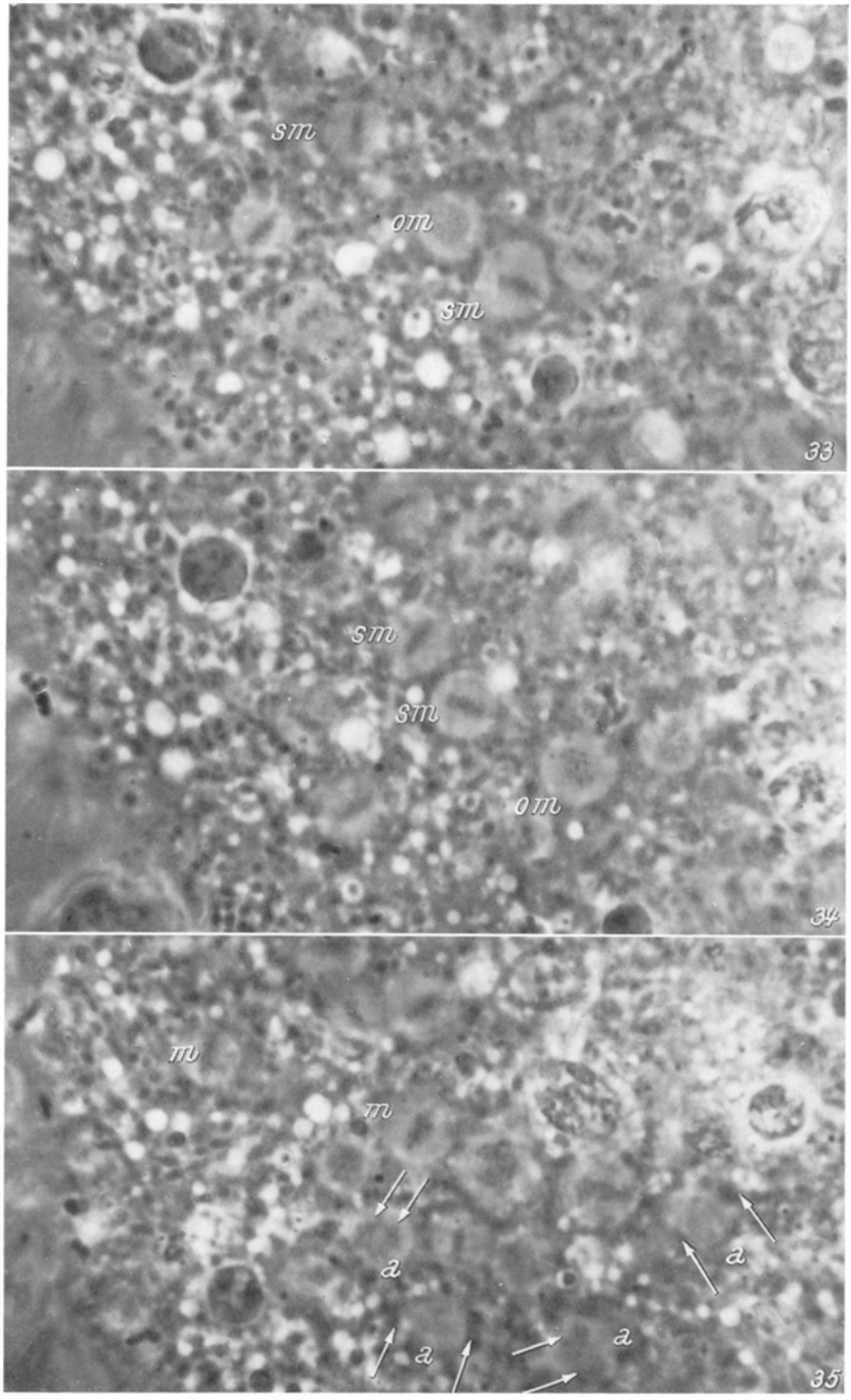

Plate V. Figs. 33–35. *Didymium iridis.* All ×1,000

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