MITOSIS IN THE YEAST *LIPOMYCES LIPOFER*

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ABSTRACT

A study of mitosis in *Lipomyces* has been carried out because preliminary observations by Ganesan and Roberts, 1959 (9), had indicated that the nucleus of this yeast might be unusually favourable for morphological observations. This impression has proved correct. The chromosomes of *Lipomyces* are visible as separate, countable bodies for the greater part of mitosis. The pattern of mitosis differs from the common one in that in *Lipomyces* the proper distribution of sister chromosomes is accomplished without the help of a spindle apparatus. At the end of prophase sister chromosomes are found in pairs which align themselves parallel to one another to form a palisade or stack whose long axis coincides with the axis of the impending division. At anaphase-telophase the stack of paired chromosomes fuses into a seemingly homogeneous cord which divides by constriction.

INTRODUCTION

The mode of division of the nucleus of baker’s yeast (*Saccharomyces*) has been studied for many years but remains difficult to understand despite improvements in staining techniques and the advent of electron microscopy. Chromosomes have been convincingly demonstrated at meiosis (21) but few workers claim to have been able to follow the movements of the chromosomes in dividing nuclei of cells multiplying by budding. In fact, there is good evidence that the yeast nucleus divides directly and that in the course of this process its chromosomes do not normally become visible in the light microscope as separate, countable individuals (10, 24, 25, 26). The poor visibility of the nucleus in living yeast examined by ordinary microscopy (the excellent photographs of budding cells by Ingram (16) are a good example) has also played a part in the persistence of a contentious literature on yeast caryology. It is therefore of considerable interest that a sharply defined ordinary-looking nucleus has recently been described and photographed in another member of the Saccharomycetaceae, namely in *Lipomyces lipofer* (9), and that in squashed preparations of budding cells of this yeast chromosome-like bodies, some of them in pairs, have been seen arranged in what “appeared to be disturbed metaphase plates.” It seemed important to try to work out the course of mitosis in a yeast with such favourable nuclei. A study of the behaviour of the nucleus in growing and dividing cells of *Lipomyces* was therefore undertaken and is described in the present paper. An account of the nucleus of baker’s yeast, which has been studied at the same time, will follow.

The work of Ganesan and Roberts (9), which forms the starting point of the present study, was done on the nuclei of “giant cells” which regularly arise in considerable numbers in cultures of *Lipomyces* (29). The observations to be described in the present paper have mainly been made on the nuclei of cells of normal size. Their nuclei behaved in the same way as the nuclei of giant cells but were easier to study and photograph because they were usually less obscured by stain taken up by the cytoplasm.

MATERIALS AND METHODS

The Organism

A culture of *Lipomyces* designated CBS 944 was obtained from Dr. N. J. W. Kreger-van Rij at Delft, who, with J. Lodder, has given the first definition of this genus of the yeasts (19, 29). The organism was

* To Professor Irene Manton., F. R. S.
grown and maintained on a medium composed of 2 per cent glucose, 0.5 per cent yeast extract (Difco), and 1.5 per cent agar. Growth was slow to start but abundant, and continued for a long time. Stock cultures on slants of this medium were kept at 4°C, and were plated out and transferred to fresh slants every two or three months.

**Microscopy of Living Cells**

Cells from growing cultures were examined by phase contrast microscopy in 16 per cent gelatine containing 1 per cent glucose and 1 per cent yeast extract. This way of looking at living microorganisms is derived from the work of Barer *et al.* (6) and in the hands of Mason and Powelson (21) has already provided much useful information on the behaviour of the nuclei of living bacteria. The high refractivity of the gelatin medium reduces the brightness of the halos with which the phase contrast microscope surrounds yeasts that are examined in watery media. It also increases the contrast between the nucleus and the cytoplasm.

**Fixation**

Several fixatives were tried. The most instructive preparations were obtained after fixation with the fluid of Helly; with the sodium sulfate of the original formula omitted it is a solution of 5 per cent mercuric chloride and 3 per cent potassium dichromate in water to which 5 per cent formalin is added before use. Helly fixation was chosen for two reasons: it has been found very satisfactory by Heim (11-15) in studies of the nuclei of a wide range of fungi, and it was already known to the present writer (from work to be published in another paper) that Helly is an excellent preservative of the size, shape, and texture of the nucleus of baker’s yeast. Osmium tetroxide vapour and acetic acid-alcohol were also tried but were less helpful because nuclei fixed by either of these reagents were less easily resolved into chromosomes than were Helly-fixed nuclei.

To make preparations of *Lipomyces*, a loopful of the slimy growth from a 2- to 5-day-old plate culture was placed on a No. 1 coverslip. A second slip, its corners turned through 45 degrees relative to the first one, was placed on top. The drop of yeast was allowed to spread and the two glass slips were then rapidly pulled apart in opposite directions. The two instantly drying films of yeast cells so obtained were minimised in the fixative. Ten minutes later the fixed films were rinsed several times with 70 per cent alcohol and were then transferred to small jars of the mixture recommended by Newcomer (27) for the long-term preservation of tissues to be stained for nuclei by the Feulgen technique. These jars were kept in a domestic refrigerator.

It is a shortcoming of this procedure that it allows the cells to dry more or less before they are fixed. Preservation is indeed often poor in the thinly spread “comet’s tail” of a film, where drying has been most rapid. Preservation is best in the thicker regions around the point of origin of the film. Brief exposure of the yeasts to formalin vapour before spreading them on coverslips has given encouraging results, and the possibilities of some such pretreatment before proper fixation deserve to be further explored.

**Staining**

**A. HCl Giemsa Preparations:** The nuclei were studied mainly in Giemsa preparations that had been treated with N/1 HCl at 60°C. before staining. The usefulness of this kind of preparation in nuclear studies of yeasts and filamentous fungi is now well established:

To increase the contrast between chromatin and cytoplasm, fixed cells were usually first extracted for 1½ hours with 1 per cent NaCl at 60°C as suggested (with a slight difference in time, concentration, and temperature) by Ganesan (8). They were then treated for 10 minutes with N/1 HCl at 60°C, rinsed with tap water, and stained for several hours in Columbia staining jars (supplied by A. H. Thomas and Co., Philadelphia) containing 16 drops of Gurr’s Giemsa R66 (George T. Gurr Ltd., London, S.W. 6, England) dissolved in 10 to 12 ml. of Gurr’s Giemsa buffer at pH 6.9. A few preparations were stained directly with Giemsa solution.

Stained films were differentiated by moving them about repeatedly for 10 to 12 seconds at a time in a Petri dish with 40 ml. of distilled water to which one wire loop, 2 mm. in diameter, of acetic acid had been added. The acidulated water had a pH of 4.2. The extraction of excess stain was controlled with a water immersion lens. When the nuclei stood out clearly the preparation was mounted over a drop of buffer containing 2 or 3 drops of Giemsa per 10 ml. Excess buffer was removed by touching strips of bibulous paper to the edge of the coverslip. A sheet of such paper was then placed firmly over the preparation and while the first and second fingers of one hand held the coverslip in place through the paper, heavy pressure was several times brought to bear on it with the thumb of the other hand. The preparation was then sealed with wax, and either examined immediately or first stored for several days in the refrigerator at 4-6°C. Some films were sealed and examined without having been squashed.

**B. Feulgen Test:** Helly-fixed films were hydrolysed for 10 minutes, rinsed with tap water, and placed for 3½ hours in Schiff reagent prepared with Diamant Fuchsir (Chroma GMBH, Stuttgart, Germany). They were then rinsed quickly with 10 changes of SO2 water (tap water 90 ml., N/1 HCl 5 ml., 10 per cent sodium metabisulfite 5 ml.), followed by 20
FIGURE 1

Schematic drawing illustrating the main steps in the proposed sequence of mitosis in *Lipomyces*. It has been assumed that *Lipomyces* is normally haploid and has six chromosomes, but, as explained in the text, this may not be right. In the absence of precise information it has been further assumed that all the chromosomes are more or less of similar shape and size, though this again is unlikely to be correct.

A. Resting nucleus with six single chromosomes and a nucleolus.
B. Beginning of prophase. The chromosomes have divided and are long and thin.
C. Late prophase. The chromosomes have contracted. Arrows point at pairs of sister chromosomes. The remaining ones may be regarded either as having not yet joined in pairs or as having been forced apart by squashing.
D. Palisade or stack of paired sister chromosomes. The second and third pair from the top have fused. The arrows indicate that the two genomes that are now aligned opposite each other have somehow to be separated and moved in opposite directions. How this is achieved is not known. There is no spindle.
E. Anaphase. The stack of separately visible pairs has fused into a seemingly homogeneous cord. At its side is the gradually disappearing nucleolus.
F. Telophase. During anaphase the two genomes have moved to opposite ends of the cord, which now divides transversely into daughter nuclei.
G. The new nuclei emerge from their dense telophase condition and can again be resolved into chromosomes. A new nucleolus has been formed.

minutes in running tap water, and were examined mounted in water or in aceto carmine (23, 30).

**Microscopy**

Stained preparations were examined and photographed through a Zeiss microscope, 1935 model, equipped with an achromatic condenser of N.A. 1.4, apochromatic oil immersion lens X 90, N.A. 1.3, and X 15 compensating eyepiece. Light from a tungsten ribbon lamp was adjusted to give Koehler illumination. A Baird Associates interference filter was used with maximum transmission at 5460 A. Photographs were taken on Kodak Panatomic film at an initial
magnification of 1800 and enlarged to ×3600 in printing.

RESULTS

A. Observations on Living Cells (Figs. 2 and 3)
The cytoplasm of *Lipomyces* contained numerous watery vacuoles of different sizes, filamentous mitochondria, shiny droplets of lipid, and small dense granules whose nature has not been determined. The nucleus stood out clearly as a sphere of low density containing an excentric, dense, round nucleolus. In slide cultures prepared for phase contrast microscopy cells of *Lipomyces* remained alive for many hours in good condition, but during the time they were kept under observation their nuclei were not seen to divide.

B. Observations on Stained Preparations
The "chromatic bodies" of *Lipomyces*, first seen by Ganesan and Roberts (9) and suspected by them of being chromosomes, were found in the nuclei of all cells of this yeast. Their positive Feulgen reaction combined with their affinity for the Giemsa stain, their orderly behaviour, and their occurrence in regular numbers leave no doubt that they are indeed chromosomes. A large number of nuclei were studied until further preparations no longer provided types of constellations of chromosomes that had not been seen before. The best-resolved photographs were then arranged in what the writer believes to represent a sequence of steps in the duplication of the chromosomes and their distribution to daughter nuclei. These photographs have provided the illustrations for this paper and contain the main evidence for the account of mitosis that follows. The principal features of the proposed sequence are schematically illustrated in Fig. 1.

The cultures used in this work resemble those which Roberts and Ganesan (29) have raised

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**Explanation of Figures 2 to 57**

All photographs are of *Lipomyces*. All except Figs. 2 and 3 are from Giemsa preparations. All except Figs. 2, 3, 6, and 56 are from squashed preparations.

The magnification everywhere is close to 3600 times.

**Figures 2 and 3**
Living cells. In each cell can be seen a pale nucleus with dense nucleolus and vacuoles both large and small. In Fig. 3 there is a shiny droplet of lipid next to the nucleus.

**Figure 4**
Young nuclei recently emerged from division in mother cell and fully grown bud. Four to six chromosomes and a nucleolus can be made out in the upper nucleus.

**Figure 5**
Young nucleus in single cell as yet without bud. Four chromosomes are visible above the nucleolus.

**Figure 6**
Budding cell with large nucleus in which four or five chromosomes can be seen. From a preparation that had not been squashed. Comparable to Fig. 3 and, probably, Fig. 10, which is from a squashed preparation.

**Figures 7 to 14**
Budding cells with nuclei in prophase. Three single chromosomes and one or two (newly formed?) pairs can be seen in the nucleus of Fig. 7. Fig. 8 illustrates a similar stage. The chromosomes have duplicated themselves in Figs. 9 to 14. Six pairs of delicate chromosomes surround the nucleolus in Fig. 9; four, perhaps five pairs in Fig. 10. Ten to twelve tangled strands, some running parallel, can be counted in Figs. 11 and 13; six pairs, already somewhat contracted, in Fig. 14.
from single presumably haploid ascospores. It is therefore assumed that the present writer's cultures too are composed of haploid cells.

In the full grown bud or detached single cell the nucleus is small and compact. It seems to contain four to six slender chromosomes and a nucleolus (Figs. 1, A; 4, 5, and 49). Mitosis begins with a long prophase during which the chromosomes duplicate themselves in the intact and expanding nucleus (Figs. 1, B; 6 to 14 and 57). Towards the end of prophase the chromosomes contract into minute shapes (Figs. 15 to 17) and are later found in pairs (Figs. 18 to 28 and 56b; Fig. 1C). An early stage of these changes is shown in Fig. 24, a late one in Fig. 28. The origin of these pairs is uncertain. Are they formed by independently moving isolated sister chromosomes? It is not easy to see why sister chromosomes once formed and cleaved apart should later form a fresh association. It is easier to imagine that sister chromosomes are able to come together at this stage because they have never completely lost contact with each other. The wide scattering of many separate chromosomes in Figs. 15 to 24 seems to argue against this view, but it must be remembered that these are figures of nuclei that have been much flattened. Energetic squashing may have forced chromosomes apart that would have appeared joined in the intact nucleus (Figs. 56a, 56b); joined, perhaps, so it has been suggested to the writer, by short lengths of non-staining material. This attractive hypothesis would make the mechanism of the events that followed easier to understand than other schemes, but electron microscopy is required to either prove or disprove it.

Several factors combine to make counting of the chromosomes in the photographs difficult: imperfect resolution, overlapping, insufficient depth of focus, tricks of perspective, and the fact that all chromosomes in one nucleus apparently do not undergo duplication, contraction, and the pulling together into pairs at precisely the same time. Where these shortcomings are least apparent, as in Figs. 9, 15 to 17, and 19, ten to twelve chromosomes can be counted. The higher number, six chromosomes in the resting nucleus and six pairs of sister chromosomes at late prophase, has been tentatively accepted as normal for this strain of Lipomyces. The various difficulties mentioned above probably account for most instances where smaller numbers are found. For details see legends to the figures.

The pairs of chromosomes at first lie around at random but later somehow arrange themselves parallel to one another and form a stack or palisade. The cytoplasm around the stacks is stained as in the rest of the cell and no evidence has been seen that a spindle apparatus is involved in these movements. This is not likely to be due to either the fixative or the stain employed. Helly's solution is a good cytoplasmic fixative and spindles are clearly visible in the HCl-Giemsa-stained preparations of meiotic nuclei of the perfect stage of Helminthosporium recently described by Knox-Davies and Dickson (17). Half-way stages of the process of alignment are illustrated in Figs. 29 to 35 and in Fig. 11 of Ganesan and Roberts (9), finished stacks in Figs. 1, D, and 36 to 43. Counts

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**FIGURES 15 TO 17**
Contraction of chromosomes in late prophase. Ten may be counted in Fig. 15, eleven or twelve in Fig. 16, ten or twelve in Fig. 17.

**FIGURES 18 TO 28**
Stages in the gradual reduction of the number of chromosomes through pairing. In the figures not separately commented on, the chromosomes overlap so much that reliable counts cannot be made. Fig. 18 may be interpreted in two ways. On the face of it there are three large chromosomes, four smaller ones, and two very small ones, making a total of nine. It is also possible to regard the three largest objects as pairs of sister chromosomes with the remaining six still unpaired, giving a total of twelve. Six complexes (pairs?), one including the nucleolus, are seen in Fig. 19. A constellation suggestive of the beginning of a process of pairing is shown in Fig. 24. Five pairs and two single dots can be counted in Fig. 27; five pairs also, if the two angular dots are regarded as doublets, in the very similar Fig. 28.
of the pairs of chromosomes in the stacks are likely to be inaccurate for the reasons given above. The numbers most commonly found are four and five. It is suggested that the stacks are composed of pairs of sister chromosomes, and they are regarded as marking the metaphase of mitosis.

At anaphase (Figs. 1, E, and 44 to 46) the chromosomes, surprisingly, come still closer together. They now cease to be separately visible and appear to become fused into a single, solid cord. At telophase this divides into two drop-shaped solidly chromatinic masses which move away from each other but remain connected for a while by a thin strand (Figs. 1, F; 47, 48, and 55). A cell whose nucleus has reached telophase has invariably grown a bud. Into this, one of the two dense, angular sister nuclei now migrates. It soon unfolds into a small sphere complete with nucleolus and filamentous chromosomes (Figs. 1, G; 49, and 4, which completes the circle).

The presence of a nuclear envelope can be inferred from the rounded, compact arrangements of chromosomes in early prophase, especially in Fig. 4, and is obvious in electron micrographs of sections of Lipomyces made by Dr. R. G. E. Murray (personal communication). The nuclear envelope remains intact through most and perhaps all of mitosis. This is obvious from the smoothly rounded shape of the nuclei in all stages of division in preparations that have not been squashed. There is a suggestion that the nuclear membrane breaks down before the stacks are formed. This is hinted at in Fig. 1, C but awaits confirmation by electron microscopy.

During the greater part of mitosis the nucleolus, as already noted by Ganesan and Roberts (9), remains in contact with one or two of the chromosomes, as seen e.g. in Figs. 18, 31, 32, and 40. It is later cast out into the cytoplasm and disappears from view at the end of telophase.

Every culture of Lipomyces harbours a considerable number of cells that are very much larger than their fellows. As Roberts and Ganesan (29) have already pointed out, the large or “giant” cells may have several nuclei of ordinary size or one or two unusually large ones. A few giant cells with large nuclei are shown in Figs. 50 to 55. The chromosomes in the large nuclei are larger (longer) but apparently not more numerous than those in ordinary nuclei, and the two classes of nuclei occupy the same volume at telophase (Fig. 55).

C. Feulgen Reaction
The chromosomes are Feulgen-positive, most strongly so at metaphase and telophase. The

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**FIGURES 29 TO 35**
Prometaphase. Clusters of condensed and paired sister chromosomes, each pair having the appearance of a short rodlet or dumbbell, are beginning to align themselves for inclusion in the palisade or stack of metaphase. The nucleolus, a soft-contoured round body, less deeply stained than the chromosomes, can be seen among them or free in the cytoplasm in all the figures. In Fig. 29 it has been drawn out into a crescent. Four or five pairs can be counted in Figs. 31 to 34. Note the similarity between these four figures. In all of them a single small body of chromatin is clinging to the nucleolus. It is no longer found there from anaphase onwards.

**FIGURES 36 TO 43**
Metaphase. Four or five pairs of chromosomes are aligned more or less parallel to one another in metaphase stacks. The alignment is most perfect in Figs. 36, 38, 39, 41, and 42.

**FIGURES 44 TO 46**
Anaphase. The several pairs of chromosomes of which a stack is composed now fuse into a continuous cord.

**FIGURES 47 TO 49**
Telophase and beginning emergence of daughter nuclei.
nucleolus, conspicuous in hydrolysed Giemsa-stained preparations, is entirely Feulgen-negative and is invisible in Feulgen-aceto carmine slides.

D. Direct Staining

In preparations stained directly with Giemsa the contrast between nuclei and cytoplasm was poor, and only a few such slides were examined. Prophase chromosomes appeared bright red and were more delicate than in preparations stained after hydrolysis. Metaphase chromosomes were of a darker purple or bluish red. Variation of the pH of the stain might have produced better results, but the course of mitosis, the main subject of the present paper, was so much more easily followed in hydrolysed preparations that experiments with direct staining were not continued.

DISCUSSION

The sequence of steps in the form of mitosis that is claimed for Lipomyces is unusual and lacks the support which precedent lends to schemes of mitosis advanced for newly described species of higher organisms. A summary of the logic behind the proposed sequence might be useful at this point. Neither the beginning nor the end of the sequence is fixed arbitrarily. It is reasonable to regard the chromatinic masses in Figs. 55, 47, and 48 as illustrating the end of a process of division which provided a nucleus for the bud that has grown on the mother yeast. It is also obvious that nuclei emerging from mitosis (Figs. 4, 5, and 49) are small and contain few chromosomes. Constellations of approximately twice that number of chromosomes (Figs. 9, 10, 14, 16, and 17) have presumably arisen through the division of the chromosomes of the original set and have therefore been referred to as prophaeses. The question now arises, by what means the duplicated chromosomes are first sorted into two sister sets and then separated. It is a far step from prophase to the division stage of Figs. 46 to 48. An intermediate phase is required and is provided by the palisades or stacks of Figs. 36 to 42. Long, narrow, and densely chromatinic, the stacks merge smoothly enough into the equally dense cords and drop-like final division stages; their link with prophase is less immediately obvious but consists in their being composed of a small number of chromosome-like subunits. Since that number is approximately half that of the number of units in the scattered prophase constellations, chromosomes must have either dropped out or joined with others when the stacks were assembled. The fact that the stacks (through the cords) give rise to two nuclei forces us to accept the pulling together of sister chromosomes as the event that precedes the construction of the stacks. Evidence for such a process is, I believe, to be found in Figs. 17 to 28. The gradual transformation of a random cluster of pairs of sister chromosomes into the tidy array of the finished palisade is illustrated in Figs. 29 to 35.

The behaviour of the nucleolus corroborates this story. Its position at a considerable distance...
from the chromosomes in Figs. 29, 35, 36, and 41 to 43 makes it obvious that these photographs illustrate aggregates that are formed after the prophase constellations and do not represent side views of them, as one might be tempted to think by analogy with more conventional forms of mitosis.

The close palisade of chromosomes at metaphase and the even tighter cord of telophase may perhaps be regarded as devices for the rapid and accurate separation of two sets of chromosomes without the help of a spindle apparatus. Because they are already aligned opposite each other in the stack, the two genomes can presumably be gathered more swiftly than if at metaphase their members were still scattered at random. The relative scarcity of the cord stage in preparations of Lipomyces indicates that this phase is indeed passed through very quickly. In this connection it is interesting to note that in Euglena, where a spindle is also lacking but where the chromosomes do not contract but remain relatively far apart at metaphase, mitosis occupies several hours (Leedale, 16). It is not known how the two genomes which together form a stack slide past each other, as they must, at anaphase-telophase (Fig. 1, D). A possible and plausible device would be first to transform the two genomes that confront each other in the metaphase stack into two parallel chains and then to develop some form of repulsion between them. Confirmation of this idea must be left to the electron microscope.3

In a note published while this paper was being written, Dowding and Weijer (7) state that in somatic nuclei of Neurospora crassa the chromosomes at all stages of mitosis are visible as separate bodies through most of mitosis. The peculiar behaviour of Lipomyces chromosomes which makes newly formed sister sets move together rather than apart at the end of metaphase is probably also not restricted to this yeast. The bouquets of contracted "chromosomal filaments" which mark the halfway stage of mitosis in Neurospora and Gelasinospora, according to Bakerspigel (3, 4), are perhaps the result of similar manoeuvres. The example of Lipomyces further encourages a fresh look at other fungal nuclei which late in their division assume the shape of a densely chromatinic cord (or two of these parallel and close together). Such cords which later divide by elongation and constriction have been described in Blastomyces and Schizopyllum (1, 5).

It would not be unreasonable to expect the nuclei of baker's yeast to resemble those of Lipomyces. In reality they are different. The nuclei of budding yeast cells, fixed and stained by the methods used in the present work, have at all times a fine-grained structure and, at the level accessible to the light microscope, do not divide by the form of mitosis found in Lipomyces. In comparison the nuclei of this fungus by Bakerspigel (3) and Somers et al. (31), who also differ among themselves on many points of interpretation.

### Figures 56a, 56b, and 57

These figures are intended to give an impression of the general character of Lipomyces preparations that cannot be obtained from the preceding small figures of isolated cells. They also serve to illustrate the difference between intact nuclei and squashed ones.

#### Figures 56a and 56b

Two different levels of focus of the same group of cells of Lipomyces in a preparation that had not been flattened. In the centre of both pictures is a cell with a nucleus in prophase. Arrows point to cells with nuclei at that stage of mitosis where the chromosomes shorten and pairs begin to emerge. These nuclei are comparable to the squashed ones in Figs. 25 to 26.

#### Figure 57

Cells with nuclei in prophase. This preparation had been squashed.
mon with McClary et al. (22). I have seen countable chromosomes in baker's yeast only at meiosis. This work will be described in a separate paper.

The author is indebted to Mrs. Elizabeth Berry for several useful suggestions.

BIBLIOGRAPHY