

## Karyology of Conidiogenesis in Some Hyphomycetes

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One of the points repeatedly raised during the discussions at Kananaskis was the possibility that there might be karyological differences between the various methods of conidium ontogeny, with the logical corollary that we might be able to define certain of these processes in karyological as well as, or instead of, morphological terms.

With this possibility in mind, we have begun to retrace the steps of Kendrick and Cole, who carefully followed the sequence of morphological events involved in the conidium ontogeny of a number of hyphomycetes. We are examining these same fungi using a modification of the Giemsa nuclear staining procedure of Robinow (1961). This chapter reports on three fungi: *Scopulariopsis brevicaulis* [UW 236] (see Cole and Kendrick 1969a), *Gonatobotryum apiculatum* [UW 200] (see Kendrick, Cole and Bhatt 1968), and *Geotrichum candidum* [UW 203] (see Cole and Kendrick 1969b).

### MATERIALS AND METHODS

Each of the three organisms was grown in coverslip culture till the appropriate developmental stages were present (the times differed for each species, and were originally discovered by a comprehensive experiment in which many slides were inoculated concurrently and then fixed sequentially at half-hour intervals). The coverslip was then ripped off the agar block, immediately immersed in modified Helly solution (Robinow 1961), and fixed for 20 minutes. Two or three rinses in 70 per cent alcohol, then in water, were followed by hydrolysis in 1*N* hydrochloric acid for 10 minutes at 60°C. After three rinses in water, the preparation was stained in a Columbia jar for 10-30 minutes in a solution made up from 10 ml of Gurr's

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Giemsa buffer plus 18 drops of Gurr's Giemsa "R66" stock solution. The preparation was then rinsed and mounted in buffer for examination.

#### OBSERVATIONS

Our first two fungi, *Scopulariopsis* and *Gonatobotryum*, both form "blastic" conidia: there is marked enlargement of a recognizable conidium initial *before* the initial is delimited by a septum. In the simplest terms each conidium is derived from part of a cell. We hoped that the comparison between these two blastic forms and the thallic *Geotrichum* would be informative.

Zachariah and Metitiri (Chapter 8) have shown that the normal phialide of *Penicillium claviforme* contains a single nucleus. This divides once during the formation of each conidium, one of the daughter nuclei entering the conidium, the other remaining in the venter of the phialide where it will divide again each time a new conidium is formed. Thus the status quo is maintained: one nucleus in the phialide, one in each conidium of a basipetal succession. This confirms the general picture outlined by Pontefract (1956 Univ. of Western Ontario M.Sc. Thesis, unpub.).

The annellide superficially resembles the phialide. It has a deceptive air of possessing a definite length, and its contours frequently match those of phialides: an elongated, often somewhat widened body, and a narrower neck. Apparently genuine ontogenetic differences between phialides and annellides exist and are gradually being explored (see Chapters 7, 9, 19). The fixed endogenous meristem of the characteristic phialide and its disinclination to increase in length with conidium production (any percurrent or sympodial proliferation which may occur is not directly involved in conidium formation) clearly delineate it from the average annellide, with its repeated, short percurrent proliferations (annellations: one per conidium) and its consequent multiplicity of wall lamellae (Figure 9.1), though annellations are often difficult to see with the light microscope and the wall lamellae are revealed only by the transmission electron microscope.

As far as we know, the only previous karyological study of an annellidic fungus was carried out by Backus and Keitt (1940), who investigated conidial *Venturia inaequalis*. They reported that individual cells of this organism, whether vegetative hyphal cells, conidiogenous cells, or conidia, each contained only one nucleus. The nucleus in the conidiogenous cell apparently divided once for each conidium produced. This picture is essentially the same as that found in the phialide. *Scopulariopsis brevicaulis*, however, diverges markedly from this pattern.

Nuclear division in *S. brevicaulis* is of relatively short duration, and certain stages, such as anaphase, proceed very rapidly indeed. Although we have no absolute figures for *S. brevicaulis*, Robinow and Caten (1969) found that the entire process of nuclear division in vegetative hyphae of diploid *Aspergillus nidulans* lasted only 18 minutes, and that their stages II (late metaphase) and III (anaphase) were extremely ephemeral.

The photomicrographs of *S. brevicaulis* are all of 24-hour-old cultures. The vegetative hyphae give rise to short, lateral branches which are the conidiogenous cell

(annellide) initials (Figure 18.1 A). One or two nuclei migrate into each of these branches, and soon divide. In Figure 18.1 A, they are seen in the condition designated as stage II by Robinow and Caten (1969). Sometimes the young annellide is not delimited by a basal septum, and nuclei may continue to migrate into it quite freely. More usually, the annellide is cut off by a basal septum, nuclear migration ceases, and the narrowed apex soon blows out to form the first conidium initial. In B a nucleus (indicated by an arrow) is moving into the young conidium. In C, the nucleus in the conidium and those nuclei remaining in the conidiogenous cell are all dividing simultaneously (here seen in stage I, early metaphase, each nucleus a clump of irregularly disposed, arm-like chromatinic masses). D shows a young annellide with an almost completely differentiated first conidium; all four nuclei are in stage II of division. In E, the four dividing nuclei are all in telophase (stage IV), the eight daughter nuclei appearing at their most condensed, and the members of each pair still connected by a thin strand of chromatin (not to be confused with the spindle, which is not seen in Giemsa preparations.) In F, the first conidium has been delimited by a basal septum and contains two nuclei, probably the products of a division similar to that just described. However, if the conidium is not cut off by a septum, nuclei may continue to migrate into it. G shows a third nucleus (indicated by the arrow) entering the conidium, and H shows the likely product of this process after the basal septum has been laid down. Note that in F, G, and H several nuclei remain in the conidiogenous cell in every case. On occasion the migration may extend to the entrance of a fourth nucleus, as shown in Figure 18.2 A (arrow) before the septum apparently puts a stop to this movement. Migration is not the only way of arriving at a multinucleate conidium. Sometimes the number of nuclei is increased by division of those already inside the conidium (Figure 18.2 B).

After the delimitation of the first conidium, a very short percurrent proliferation of the conidiogenous cell takes place and the second conidium initial begins to blow out. As with the first, a nucleus migrates into it (Figure 18.2 C) and then divides (D, stage I, early metaphase). Note that although the nuclei in the conidiogenous cell divide synchronously with that in the second conidium, the nuclei in the first conidium are no longer affected by divisions below them. This may be a sign that all cytoplasmic connection between the first and second conidium has been lost. In E, the nuclei in the conidiogenous cell and in the young conidium (arrows) are in anaphase (entering stage III as described by Robinow and Caten).

Subsequently the third conidium initial forms. In Figure 18.2 F, one nucleus has already entered it and a second is actively migrating towards it. In G, a conidium has been delimited and a nucleus is moving up to take its place in the next conidium initial, which is just forming.

From this sequence of photomicrographs, it is clear that no single well-defined invariant pattern of nuclear division, migration, and population of conidia exists in this fungus.

Examination of large numbers of conidia and conidiogenous cells of *S. brevicaulis* has shown that there is indeed considerable variation in the nuclear number of both annellides and conidia. The histograms (Figure 18.3) show the frequency distribution of the different numbers of nuclei in 1,000 conidiogenous cells. The

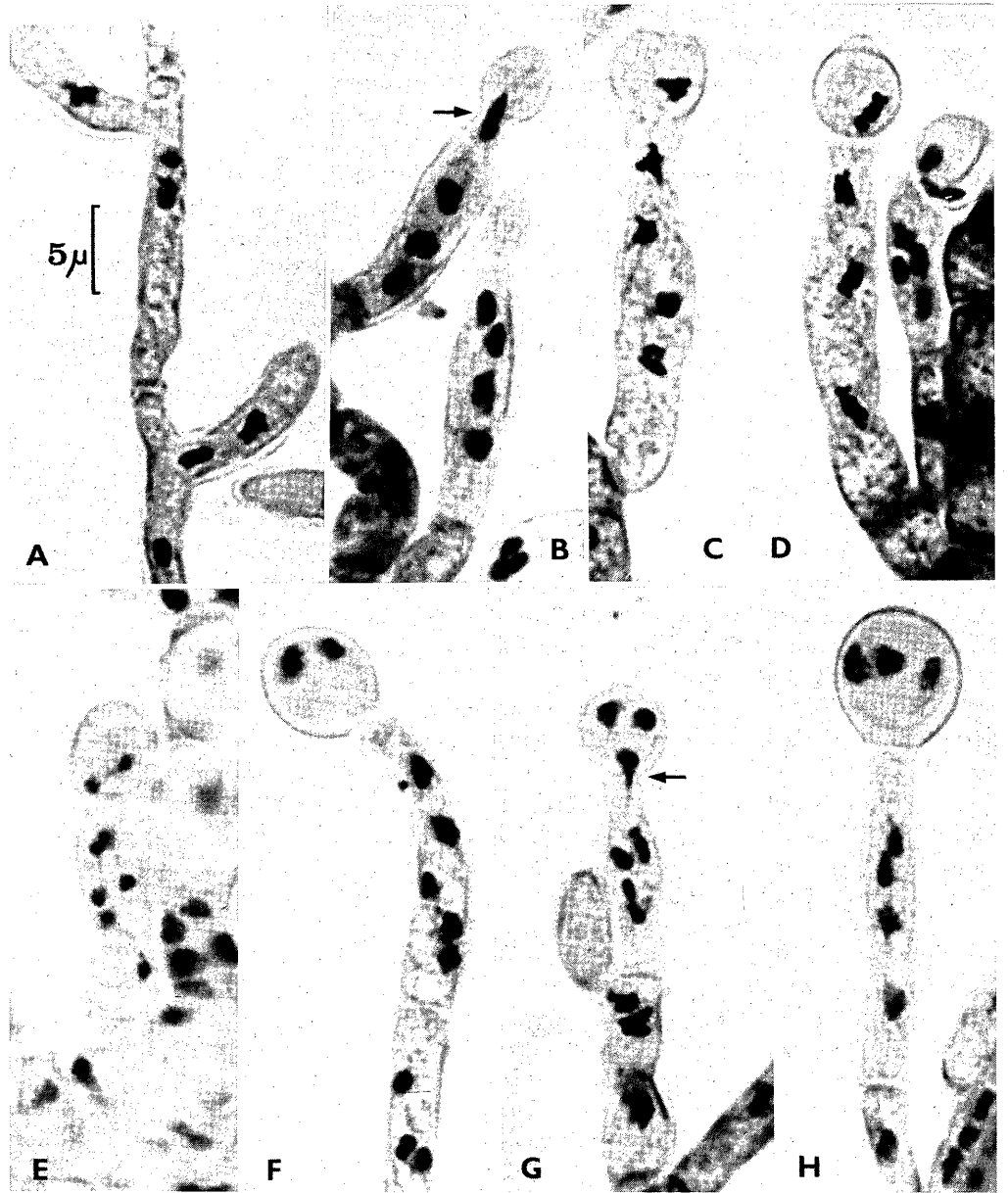


Figure 18.1 *Scopulariopsis brevicaulis*, karyology of conidiogenesis. Giemsa staining. For explanation, see text.

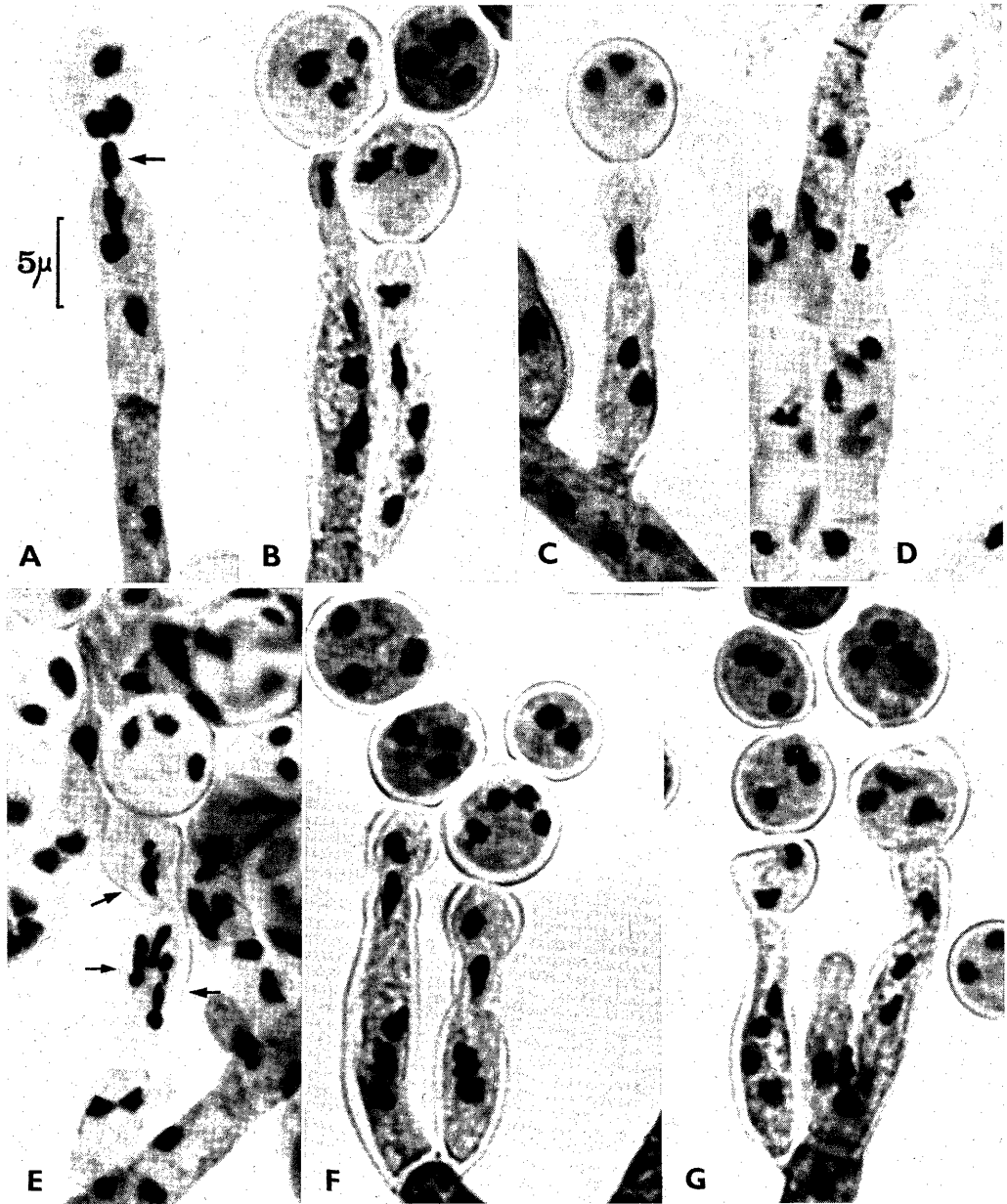


Figure 18.2 *Scopulariopsis brevicaulis*, karyology of conidiogenesis. Giemsa staining. For explanation, see text.

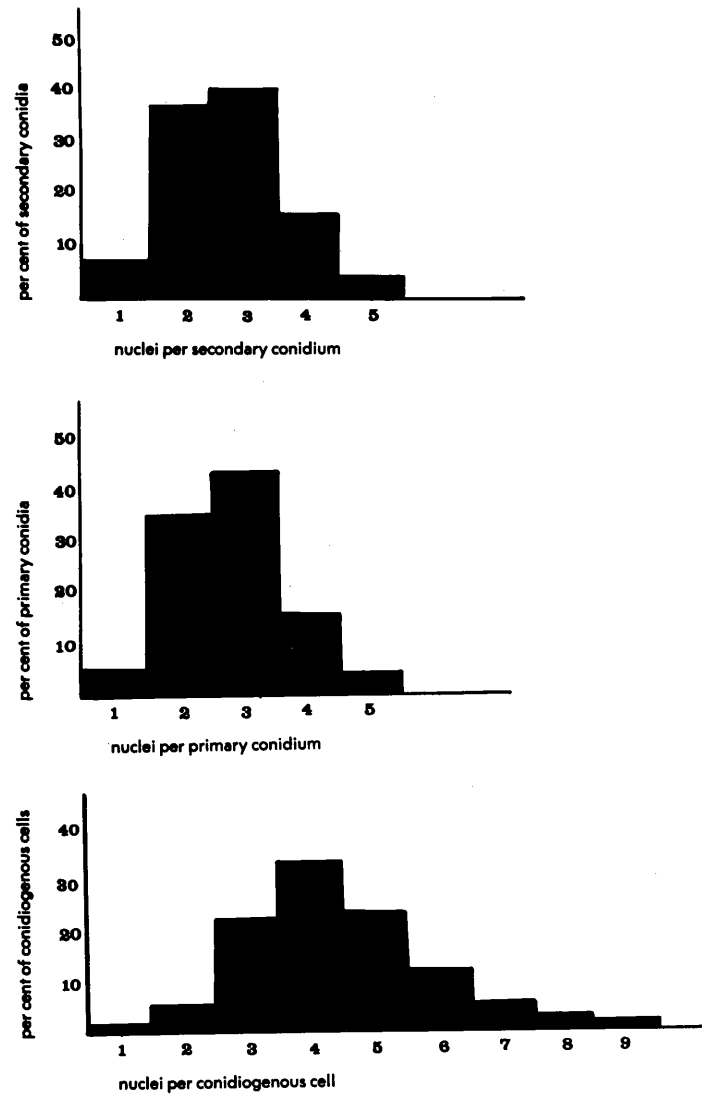


Figure 18.3 Histograms of nuclear number of conidiogenous cells and conidia in *Scopulariopsis brevicaulis*.

number of nuclei per annellide ranged from 1 to 9; 4 was the commonest number (33 per cent) but many cells possessed 3 (21.3 per cent) or 5 (22.9 per cent) nuclei, and a significant fraction contained 6 (10.8 per cent).

Comparison of 1,000 first and 1,000 subsequent conidia shows that there is little difference in nuclear number between them. The range in both cases is from 1 to 5 nuclei, and in both cases the 3-nucleate condition is commonest (42.2 and 40.1 per cent) with the 2-nucleate condition running it a close second (35.6 and 36.4 per cent). The only other nuclear complement present in a significant proportion of the conidia was 4 (15.1 and 15.8 per cent).

From this study we may conclude that the annellide of *S. brevicaulis* is significantly less regular in its karyological mechanisms than that of *Venturia inaequalis*, and than the few phialides whose karyology has been reported in the literature. Not only does nuclear number in both annellide and conidium vary considerably, but the origin of the nuclei also varies. It appears at first sight as if a 3-nucleate conidium may owe one, two, or all three of its nuclei to migration from the conidiogenous cell. But there is one generalization we feel fairly confident in making. In most cases when one nucleus in a conidiogenous cell or conidium goes into division, all nuclei in that cell or conidium will divide. Thus if there are two nuclei in a conidium, it is unlikely to become 3-nucleate except by the immigration of a third nucleus. We can draw up a tentative table of origins (see Table 18.1).

It seems, therefore, impossible to categorize the annellide of *S. brevicaulis* rigorously from the karyological point of view. We shall be interested to see the results obtained with the annellides of other organisms.

Our second example is a very different fungus. *Gonatobotryum apiculatum* produces "blastic" conidia, not from a phialide or annellide, but from an ampulla (a conidiogenous vesicle). It produces conidia, not singly in basipetal succession, but first in a synchronous cluster, then in asynchronously developing acropetal chains. After the primary conidia have been blown out, a conidiogenous locus moves into each, and a secondary conidium is produced from its distal end. The locus then moves into the second conidium, a third conidium is produced from its apex, and so on.

Clearly, we can expect to discover few parallels with the phialide or the annellide. The logistics of the basipetal and acropetal processes are very different. Once a phialide or annellide has elaborated and delimited a conidium, that conidium loses all cytoplasmic connection with the conidiogenous cell. The mature conidium is ready to be dispersed, and the conidiogenous cell is free to proceed with the formation of the next conidium.

In *Gonatobotryum*, all building material for each new secondary conidium must either pass through all previously formed conidia of the chain, or be derived from the previous conidium. If a chain of several conidia is formed, it is clear that food material must be coming from the parent hypha. This means that, if the chain is broken off by some accident, the conidiogenous locus will be cut off from its food supply and will cease to function. It is apparent to us that the acropetal chain is a less sophisticated mechanism than the basipetal chain.

Nobles (1935) reported that in the ampulliform conidiogenous cell of the ba-

Nuclear complement of conidium	Origin of nuclei
1	Migration
2	2 migrated, <i>or</i> one migrated and divided
3	1 migrated and divided and then a third migrated, <i>or</i> 3 migrated
4	1 migrated and divided, both daughter nuclei divided, <i>or</i> 2 migrated and divided, <i>or</i> 4 migrated

sidiomycete *Peniophora allescheri* one nucleus divides repeatedly to give 16 or more nuclei and then the nuclei "take up their positions around the inner surface of the distal half" of the ampulla. "At this stage the slender tapering sterigmata push out and a swelling, the developing conidium, appears on the end of each. While the spore is still small a nucleus migrates into it ..."

This neat and logical procedure, which is apparently also found in *Corticium effuscatum* (Nobles 1942), was more or less what we expected to find in *G. apiculatum*, though we knew that variations existed. Loveland, reported in Hughes (1953), found that in *Botrytis allii* one nucleus migrates into each conidium produced on an ampulla, but then divides once to produce binucleate conidia. She also observed that, in *Botrytis cinerea* and *Botrytis streptothrix*, several nuclei migrate into each conidium initial. Despite this foreknowledge, *G. apiculatum* managed to surprise us.

The conidiophores of *G. apiculatum* arise as lateral branches or apical extensions of vegetative hyphae. Conidiophore initials are wider, thicker-walled, and more darkly pigmented than the parent hyphae. At this stage the long apical cell of the conidiophore already contains many nuclei, sometimes more than 20. Some of these migrate in before the basal septum of the cell is laid down, others migrate through the septal pore (Figure 18.4 A, arrow), and others arise as products of nuclear division within the apical cell. Figure 18.4 B shows a young, extending conidiophore initial in which the nuclei appear to be at metaphase. In C, the nuclei are at telophase, the daughter nuclei very condensed. D shows the much larger interphase nuclei that are eventually reconstituted. In E, the apex of the cell, now densely populated with nuclei, has ceased elongation and has become slightly swollen. This slight ballooning is the ampulla, and its appearance heralds conidiogenesis, which soon begins with the precisely synchronous appearance of 17-25 tiny conical protuberances regularly spaced over the surface of the ampulla (F). Although in E and F nuclei crowd the ampulla, and might be assumed to be individually responsible for coordinating the development of adjacent denticles, and, subsequently, conidia, G shows that this is not necessarily the case. In this figure the formation of primary conidia is at an advanced stage, but the cluster of nuclei remains far below the ampulla (the nearest nucleus is 20 $\mu$  beneath it), yet conidium formation is proceeding normally.

Earlier observations (Kendrick, Cole, and Bhatt 1968) suggested that the apex of each denticle ruptures and that the primary conidium which blows out is clad in an extension of the inner wall of the ampulla. That would make these conidia en-

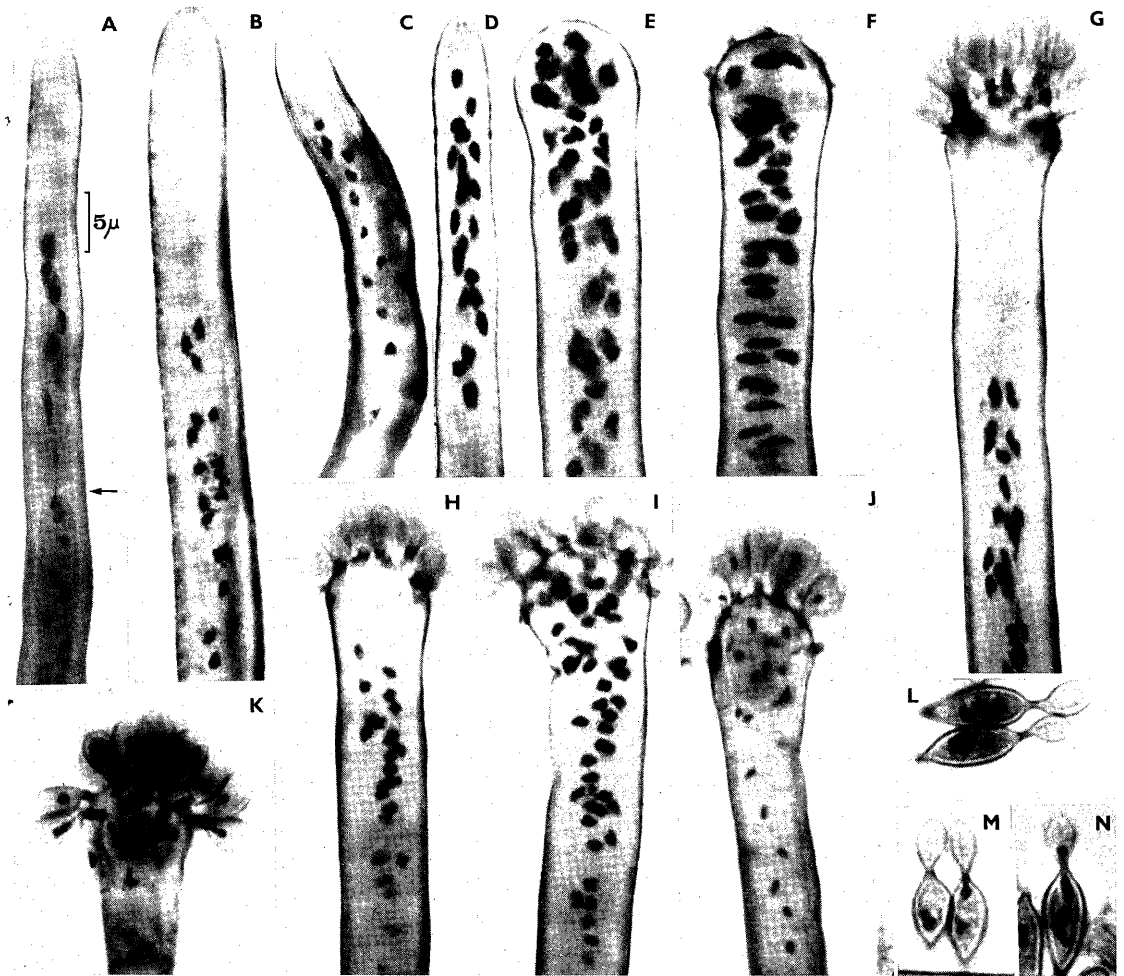


Figure 18.4 *Gonatobotryum apiculatum*, karyology of conidiogenesis. Giemsa staining. For explanation, see text.

teroblastic. Recent observations by Cole and Aldrich (see Figure 19.2 A, B) with the scanning electron microscope lend weight to this suggestion, but the matter must ultimately be settled by reference to the transmission electron microscope.

At this stage, if the nuclei of the apical cell of the conidiophore have not recently divided, they may do so. Figure 18.4 H and I show most nuclei at early metaphase, and J finds them in telophase and sees them migrating into the primary conidia. K clearly shows attenuated nuclei migrating into conidia. Nuclei generally move into the conidia only when the latter are almost full-size. Only one nucleus enters each primary conidium, and although nuclei do not populate all conidia simultaneously, all become nucleate in a relatively short time.

The secondary conidia are in many ways similar to the primary conidia. They form from denticles which develop at the distal end of the primary conidia, and they also are suspected of being enteroblastic. But they are not produced synchronously - the master control mechanism, whatever it is, has been fragmented - and their karyology seems to be less haphazard than that of the primary conidia. After the secondary conidium begins to blow out, the nucleus in the primary conidium divides. The upper primary conidium in Figure 18.4L is at telophase. In M, one of the daughter nuclei is just about to migrate into the secondary conidium, and N shows a nucleus which is in process of migrating into the young secondary conidium. We assume that, as an acropetal chain of conidia develops, each new apical conidium receives its nucleus as a result of a similar regular process of division and migration.

Conidia of *G. apiculatum* typically contain one nucleus. Conidia containing two nuclei are not uncommon, but are often about to give rise to a new apical conidium, or to initiate a branching of the conidial chain. Conidia with three or four nuclei are observed only occasionally.

The first two fungi discussed in this chapter produce their conidia blastically, *de novo*. Our third example, *Geotrichum candidum*, displays the thallic type of conidium ontogeny. Here, the main diagnostic characteristic is that if any enlargement of the recognizable conidium initial occurs, it occurs only after the initial has been delimited by a septum or septa. In the simplest terms, a thallic conidium is one derived from an entire cell, and its initial abutment with other conidia or the adjacent portion of the fertile hypha will always be the full width of that hypha.

The only previous cytological studies on thallic-arthric conidia of which we are aware were carried out by Brodie (1936), who described the formation of uninucleate conidia ("oidia") in *Collybia velutipes*. He reported that the single nucleus present in a hypha divided repeatedly, and that the hypha subsequently segmented basipetally. Again, as in the published work cited with reference to phialides, annelides, and ampullae, there is an over-all impression of neat and efficient organization: a state of affairs which seems to be much less in evidence in the fungi we have studied.

Cole and Kendrick (1969b) have already demonstrated unequivocally that in *Geotrichum* the process of hyphal segmentation and conversion into conidia does not follow a smooth basipetal sequence as had long been assumed, and we were prepared to discover less regularity in the karyological aspects of conidiogenesis than was reported by Brodie. We were not disappointed.



Figure 18.5 *Geotrichum candidum*, karyology of conidiogenesis. Giemsa staining. For explanation, see text.

The photomicrographs of *G. candidum* (Figure 18.5) are all of 12-hour-old cultures. The elongating hyphae are not septate for some distance behind their apex (Figure 18.5 A), and are populated with fairly regularly spaced nuclei which divide as required to maintain the appropriate spacing (arrowed nucleus in A is at late anaphase). In B, three septa, relatively widely spaced, are being laid down in an acropetal succession.

Each of these primary subdivisions of the hypha typically contains two nuclei (Figure 18.5 C). Subsequently, over a short period, each of the primary units becomes further subdivided by the deposition of a more or less median septum (Figure 18.5 D). Each unit now usually contains only a single nucleus. In many cases

the units are to undergo further subdivision, and the nucleus in each of these segments now divides (arrows in D and F). If the daughter nuclei migrate to opposite ends of the segment (D, E, F) the deposition of yet another median septum may be anticipated. If, however, they remain in relatively close proximity to one another, often at the equator of wider cells (E, F), it may be assumed that the ultimate unit size has been attained. This very soon becomes evident as the units, which may now be called thallic-arthric conidia, secede from one another, the thin outer wall which connected them rupturing easily as their end walls (formerly halves of double septa) become convex (F). The nuclear complement of *G. candidum* conidia is almost always either one or two, with uninucleate conidia predominating.

As we might have suspected, the karyological specialization found in the phialide and, to a lesser extent, the annellide is not present in the fertile hypha of *G. candidum*. In *Geotrichum* there does seem, however, to be some relationship between the size of the conidium and its nuclear complement. Figure 18.5F shows clearly that larger conidia tend to be 2-nucleate and smaller conidia, 1-nucleate. Each nucleus appears to have a definite sphere of action, and if it should be granted more than its allotted volume of cytoplasm, it apparently divides to restore the "volume per nucleus" to the appropriate level.

Clearly, with such a limited sampling as we have made, it is imprudent if not impossible to draw any general conclusion regarding the karyology of conidiogenesis in hyphomycetes, but we hope that, from work continuing in our laboratory and similar studies elsewhere, patterns may ultimately emerge which will allow us to formulate some useful generalizations. Any such source of insight into the labyrinths of hyphomycete ontogeny and systematics will be more than welcome.

#### ACKNOWLEDGMENT

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