ORGANIZATION OF THE UNWALLED ASCUS IN TWO SPECIES OF CERATOSTOMELLA

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INTRODUCTION

The ascus, characteristic of a major group of the fungi known as the Ascomycetes, is a familiar example of a type of reproductive body or special plant structure in which a number of independent cellular units are produced within the body of a walled parent cell. The unique process of free cell formation, by which ascospores are delimited within the parent cytoplasm, is commonly believed to distinguish the ascus from all similar plant structures. The investigations of Harper (14, 15) and his students, Sands (24), Schultz (25), and others on the cytology of the ascus have strongly influenced the views of present-day botanists in regard both to the nature of the ascus and to questions of homology of reproductive bodies in the lower plants. The work of these investigators, while in many respects comprehensive, does not represent adequately those species of fungi in which the asci are said to be "deliquescent."

Early disintegration or deliquescence of the ascus is a characteristic encountered in representatives of many genera of Ascomycetes, particularly those of the order Sphaeriales. The recent work of Mittmann (21) and of Andrus and Harter (1) on Ceratostomella fimbriata (Ell. and Hals.) Elliott has indicated that deliquescence of asci is not necessarily a result or a process of wall dissolution, since the ascus of C. fimbriata possesses no wall from the time of its origin. Deliquescence of the ascus seems to represent a disorganization of the peripheral region of a naked or plasmodial mass that has already reached a state of senescence. The same investigators observed the significant fact that the central spore-producing region of the ascus of C. fimbriata is vesiculate and sometimes distinctly walled, a condition not comparable to anything described in the more familiar type of ascus.

The early history of the unwalled cells that later become transformed into asci in Ceratostomella fimbriata further distinguishes this organism from the more familiar type of ascomycete. Although there is remarkably close agreement in the accounts given by Mittmann (21), and by Andrus and Harter (1) concerning the fragmentation of the ascogonium in C. fimbriata and the continuous divisions of the ascogenous cells, these writers have neglected to describe in sufficient detail the process of deliquescence.
detail the actual divisions of the isolated cells and the nuclear behavior in the ascus. The object of the present paper is to give a more complete picture of cell and nuclear behavior involved in the formation of asci of the C. fimbriata type and to compare these phenomena with those described in certain other fungi. This will be accomplished by describing additional observations on C. fimbriata and will be supplemented by a study of C. moniliformis Hedge.

MATERIAL AND METHODS

At the present time, in addition to species with deliquescent or unwalled asci, the genus Ceratostomella includes species in which the asci possess definite and persistent walls. Also included in this genus are species that possess an imperfect stage of the endoconidial type and various other very distinct types of conidial fructifications. So far as known all species with an endoconidial stage also possess asci of the deliquescent type. Recently Melin and Nannfeldt (20) have tentatively proposed to transfer to the genus Ophiostoma Sydow those species in which asci are deliquescent and to retain the genus Ceratostomella for species with persistent asci. Still more recently it has been proposed by Davidson (4) to segregate the endoconidial species into the genus Endoconidiophora Munch. Although there seems to be real justification for dividing the species of Ceratostomella among three different genera, the organisms used in the present investigation will be called by their more familiar names.

Ceratostomella fimbriata was isolated from sweetpotatoes, where it causes the disease known as black rot. Its taxonomic history has been reviewed in a previous publication (1). It has been the subject of morphological investigations by Elliott (6), Mittmann (21), and Andrus and Harter (1). C. moniliformis was isolated from lumber, but it does not seem to be an active agent in wood decay or blue stain. Both fixed and living material of the latter organism were supplied the writers by R. W. Davidson, of the Bureau of Plant Industry, United States Department of Agriculture. Additional fixations were made from the live cultures.

The two species included in this study are readily cultured on potato-dextrose agar, Thaxter agar, and malt agar media. Fixations were made from Thaxter agar cultures on the third day of growth in the case of Ceratostomella moniliformis, and on the sixth day with the less rapidly growing C. fimbriata. Material was embedded in commercial Parawax and sectioned 4μ to 6μ in thickness with a rotary microtome. Land's gum arabic-potassium bichromate method was used in attaching ribbons to the slide.

The following combinations of killing solution and stain were used with each of the species: Bouin's solution followed by the triple stain (safranin, gentian violet, and orange G); Bouin's solution followed by Heidenhain's haematoxylin; Flemming's solution followed by triple stain; and Flemming's solution followed by Heidenhain's haematoxylin. In addition, a small amount of material of Ceratostomella moniliformis was fixed in a formol-acetic solution 4 which was followed by the triple stain. The Flemming's solution was prepared after the stronger formula and then diluted with an equal volume of water. Some brilliant preparations were obtained from material fixed in this

*Prepared by adding 1.5 cc of glacial acetic acid and 8.5 cc of 40-percent formaldehyde to 90 cc of 50-percent alcohol.
solution and stained with the triple combination, yet they were in no way superior to the best results obtained when Bouin's fixative was used. Bouin's solution was used by Mittmann (21) in her work with *C. fimbriata* and by Andrus and Harter (1) in their investigation of the same species. Recently Raymond (23), in a comprehensive study of ascomycete cytology, recommended Bouin's in preference to solutions containing osmic acid. Although it had not previously been recommended for botanical work, recent experience demonstrates that Bouin's fixative can be used very successfully in the investigation of fungus morphology. Stages in nuclear division in *C. moniliformis* were observed more frequently in material fixed with the formol-acetic solution, yet for general purposes this solution seemed to be inferior to either Bouin's or Flemming's.

The various combinations of fixatives and stains were used to secure useful preparations and are not expected to indicate the relative merits of the several methods. The most generally useful preparations were those from material fixed in Bouin's solution and stained with haematoxylin. In some cases the haematoxylin alone proved satisfactory, yet various counter stains were tried (orange G, safranine, and erythrosin, or combinations of these three), and in a few instances good effects were obtained.

Recent use of Heidenhain's haematoxylin has resulted in bringing out numerous details of cell and nuclear behavior in *Ceratostomella fimbriata* that were lacking in previous publications on the morphology of the species. The earlier inability to observe certain stages in ascus formation apparently has not been due to the use of an inadequate fixative but has resulted from a failure to secure an adequate stain. It will be evident that a great many details of the development in each of the two species discussed herein are still lacking, yet to a considerable extent those available for one species may compensate for those lacking at a corresponding stage in the other. At the same time it will not be possible to overlook certain characteristic differences in cytological behavior.

**ORIGIN OF THE BINUCLEATE CONDITION IN CERATOSTOMELLA**

Early stages in the development of the fruiting body and the accompanying appearance of the binucleate condition in the ascogonium have been described previously in *Ceratostomella fimbriata* (1, 21). Corresponding stages in the development of *C. moniliformis* are similar to *C. fimbriata*. The earliest appearance of the fruiting body can be recognized in living cultures as a short hyphal outgrowth with a thin-walled recurved tip (fig. 1, A–H). After fixation and application of stain, the cells involved in the primary structure are seen to possess each a single nucleus (I). The terminal portion of the recurved hypha develops into a coil of several cells. The point a of figure 1 marks approximately the region in which the binucleate condition first appears and which later gives origin to the multitude of asci. Septa do not usually appear in the terminal portion of the coil until the latter has been enclosed by enveloping hyphae (J, K).

The manner in which the binucleate condition arises has not been definitely determined. There is evidence of an antheridium in both species, but no satisfactory evidence that the latter organ fuses with
the ascogonium. Both species are homothallic, since the fruiting bodies or perithecia are produced abundantly in cultures from single spores. Although there is the possibility of a true fertilization by fusion of antheridium and ascogonium, by "conidiation" (5), or by some other form of anastomosis involving nuclear migration, yet it seems probable that development of the fruiting body is apogamous.

The best available evidence indicates that the binucleate condition first appears as shown in figure 1, L. Apparently two successive nuclear divisions in the terminal portion of the ascogonium are followed by two cleavage planes that cut off a uninucleate terminal and basal cell and leave the subterminal cell with two nuclei. This is es-

**Figure 1.**—Development of perithecia in *Ceratostomella moniliformis*: A–H, Perithecial primordia from live material; a, approximate location of region in which binucleate condition first appears. I, Primordium from stained section. J–L, Young perithecia, showing origin of paired nuclei in the ascogonium. M, N, Young perithecia, showing the first divisions of the binucleate portion of the ascogonium. × 1500.
Organization of Unwalled Ascus of Ceratostomella

sentially a crosier device—an indirect type of cell cleavage originally described by Dangeard (3)—but there is no indication that the first and third cells fuse as in a true crosier. The crosier type of cell division, however, appears at a later stage, i.e., just previous to ascus formation.

Immediately after the first layers of enveloping hyphae are in place, the original wall of the ascogonium begins to disintegrate, although remnants of the wall can still be recognized at slightly more advanced stages. Following the complete dissolution of the wall, the cells of the ascogonium have the appearance of essentially naked protoplasts isolated in the small cavity of the perithecium. The unwalled condition persists throughout the period of cell proliferation and up to the time of ascospore maturation.

CELL DIVISIONS PREVIOUS TO ASCUS FORMATION

The first proliferation of the initial binucleate cell or fertile cell of the ascogonium in *Ceratostomella moniliformis* appears to follow a simple linear growth with divisions into binucleate daughter cells (fig. 1, M, N). The cells lose their linear relations but continue to divide independently, with the result that just preceding beak formation the perithecial cavity contains a multitude of binucleate cells that for the most part are isolated from one another and free from the wall of the perithecium. The early period of cell multiplication is distinguished to a slight degree from the later stage by the larger size of individual cells and nuclei and apparently by the absence of the crosier type of cell division. The corresponding stage of *C. fimбриата* has been described by Mittmann (21) and by Andrus and Harter (1). There is evidence in both species that uninucleate cells of the ascogonium undergo a limited and independent proliferation in the perithecial cavity. The presence of small scattered uninucleate cells with weak affinity for stain can be demonstrated in all early stages of perithecial development.

Approximately at the beginning of beak formation the perithecial cavity is seen to contain the first young asci and at that time also a change is noted in the appearance of the dividing cells. Individual cells frequently cling together in short chains or groups, and the nuclei are smaller though still quite distinct; but most characteristic of the later stage in development is the appearance of the crosier type of cell division. The various stages in this and other types of cell division in the two species are illustrated in figure 2, B to V, and figure 3, A to E1.

Earlier observers (1, 6, 21) believed that the formation of crosiers or ascus hooks did not occur in *Ceratostomella fimбриата*. It is evident from the illustrations here presented that structures which are at least the equivalent of crosiers do occur in *C. fimбриата* and in *C. moniliformis* as well. Some authors have attached considerable importance to the occurrence of the crosier type of cell cleavage at a point immediately preceding ascus formation, but the crosier is only one of several types of cell division that may be observed preceding ascus formation in *Ceratostomella*. These proliferative stages are described in full in the following paragraphs.

The cells concerned in crosier formation in *Ceratostomella* are considerably modified to conform to their detached and unwalled condition. The resemblance to hook formation in ascogenous hyphae of
discomycetous species is in some instances very remote; this being especially true where no protoplasmic connections between the individual parts of the crosier remain. Also, in many instances, owing to the shape of the parent cell, there is no clear differentiation of terminal and basal regions, since the two portions are abstricted together. In such cases the process approaches that of a simple division of binucleate cells (fig. 2, O), and in fact it is evident that both the crosier type of cell division and simple cell cleavage (figs. 2, A; 3, F) occur side by side even in later stages of perithecial development.

There are three reasonably distinct types of cell division in perithecia of Ceratostomella. In one type, following division of the paired nuclei, the parent cell is divided into three portions by two cleavage planes (figs. 2, E, L, P; 3, Z). The central portion retains two nuclei, and each of the two terminal cells possesses a single nucleus. The two uninucleate portions usually approach each other (fig. 3, K) and fuse (fig. 3, L). This differs in no important respect from the crosier device of discomycetous species. The two binucleate cells resulting from such a division may cling together, as in figure 3, E, or they may become separated. In either case each cell may continue to divide independently, or one or both may develop into an ascus (fig. 3, G).

In the second type of cell division the four-nucleate body (fig. 3, C), following division of the paired nuclei, is divided into three portions by three planes of cleavage. Here also there would seem to result a central portion with two nuclei and two uninucleate basal cells (U, W). But at the third point (V, a) cleavage does not seem to be completed, so that there actually results a pair of binucleate cells. Then follows a reunion of the two basal portions which apparently were never entirely separated. In the second type of division, also, the two binucleate fragments may continue to divide independently, or one or both may develop into an ascus.

In the third and simplest type of cell division, a single cleavage (figs. 2, A, G; 3, D, F) or two confluent planes of cleavage (fig. 3, A, C) divide the four-nucleate body into two binucleate portions, each of which may continue to divide independently.

The two uninucleate cells formed during crosier formation may remain separated, sometimes individually as an appendage of a fertile ascus (fig. 3, F). They may develop for a time independently, and eventually join the disorganized material in the center of the perithecial cavity. Frequently structures in Ceratostomella fimбриata that appear to be abortive asci (see fig. 7, M, R) may have originated from uninucleate portions of the crosier, while in C. moniliformis numerous

EXPLANATORY LEGEND FOR FIGURE 2

A, Direct cleavage of a binucleate ascogenous cell; B, Nuclear division in the crosier; the position of spindles indicates that a direct cell cleavage will follow; C, D, Nuclear divisions in the crosier, showing characteristic cell shape and divergent position of spindles; E, Approximation of uninucleate portions of a crosier; F, An appearance of budding that is probably equivalent to crosier formation; G, A unilateral form of direct cleavage; H, A four-nucleate stage in crosier formation; I, Two pairs of divergent spindles in an ascogenous cell of the crosier type; J, Nuclear division in the crosier; K, An early stage following nuclear division, with the original nucleoli still visible; L, M, Approximation of uninucleate portions of a crosier; N, O, The four-nucleate stage in crosier formation; P, An early indication of cleavage planes in a crosier; Q, S, Crosier formation and cell linkage; T, Change in cell shape previous to cell division; U, Fusion of uninucleate portions of a crosier; V, Linked components of a crosier in one of which nuclear division has again occurred; W, X, Nuclear fusion in the ascus; Y, Z, A–D, Linkage of young ascus; E, An abortive ascus or sterile body; F, Linkage of ascus; G, H, Nuclear fusion in the ascus; I, J, K, Stages preparatory to the first nuclear division in the ascus; K, L, Early stages in the first nuclear division in the ascus, showing the emergence of the nucleolus from the nuclear vesicle and emphasizing the vacuolation of the peripheral region of the ascus that accompanies increase in cell size; Q, A first-division figure showing four discrete chromatin bodies and the persistent nucleolus; R, A later stage indicating the movement of two chromosomes or a single bilobed chromatin body to each pole; All X 3000.
Figure 2.—Cell and nuclear division in Ceratostomella moniliformis. (For explanatory legend see opposite page.)
FIGURE 3.—Cell and nuclear division in Ceratostomella fimbriata. (For explanatory legend see opposite page.)
fragile, inflated, and apparently uninucleate cells that precede the fertile asci in the center of the cavity (figs. 2, E₁; 4, L; 6, E) may have had a similar origin. The weak haploid cells sometimes display a series of nuclear changes that seem to correspond to those that take place in a fertile ascus.

It may be necessary to distinguish two types of haploid cells in the perithecial cavity, namely, those resulting from chance irregularities in the division of binucleate ascogenous cells and those that have developed independently from uninucleate portions of the original ascogonium. The former could be regarded as haploid asci, while the latter are perhaps homologous with paraphyses of discomycetous fungi.

There is ample evidence that the uninucleate components of the crosier sometimes fuse to form a second binucleate cell (figs. 2, U; 3, X) which undergoes further division. It appears that the originally uninucleate portion of the crosier usually, but not invariably, develops directly into an ascus, whereas the portion resulting from fusion of the uninucleate proximal cells continues to divide. Evidence for this is based largely on the shape and position of cells during and after crosier formation.

Change in cell shape plays an important role during the process of cell division. The changes in conformation are made conspicuous by the circumstance that a wall, commonly present on ascogenous hyphae of discomycetous species, is lacking on ascogenous cells of Ceratostomella. The cells undergoing rapid multiplication within the perithecium correspond conceivably to the protoplasmic contents of septate hyphae after the hyphal walls have been dissolved away, and the slender filaments that frequently provide a connection between individual cells (figs. 2, F₁; 7, A) perhaps correspond to the plasmodesma that extend through the perforations in hyphal cross walls.

Change in cell shape will account for the fact that a cell of the kind shown in figure 2, D, will later appear as in figure 2, N and T, without any appreciable increase in cell size. By change in cell shape two distal portions of a crosier are brought together and fused. Also, the appearance of budding shown in figure 2, F and M, may be produced by change in shape of the cell and not necessarily by a true growth process. It is not intended to imply that the cells undergo amoeboid movements. The movements that produce the changes in shape probably are made slowly, and the fact that such movements actually take place is only inferred from the appearance of the cells in the fixed and stained material.

EXPLANATORY LEGEND FOR FIGURE 3

A, An ascogenous cell immediately following cell division, showing the reappearance of nucleoli and "centrosomes"; B, Reorganized nuclei in young crosier, showing the usual position of a satellite or centrosome at the margin of each nuclear vesicle; C, A typical crosier following conjugate nuclear division; D, A stage in unilateral cell cleavage; E, Cell linkage following an apparently unilateral cleavage; F, A stage in simple and direct cell division; G, Cell linkage. The parallel position of spindles may indicate that a direct cell cleavage will follow; H, A stage in cell division that may have been direct or indirect; I, Spindle formation in the crosier; J, A stage in one type of direct cell division; K, A crosier at the four-nucleate stage; L, Fusion of uninucleate portions of a crosier; M–O, Crosiers with paired nuclei prepared for division; P–S, Spindle formation in the crosier; T–Y, Stages in crosier formation; V, a, Point of incomplete cleavage resulting in pair of binucleate cells; Z, A₁–C₀; Stages in cell division brought about by two confluent planes of cleavage; D₅, E₅, Cell cleavage accompanied by an irregular distribution of nuclei; F₅, A young ascus with an appended uninucleate cell fragment; G–J, Nuclear structure previous to the first division in the ascus. All X 3000.
Figure 4.—Nuclear division and ascus formation in *Ceratostomella moniliformis*. (For explanatory legend see opposite page.)
Characteristic bodies that appear to behave like centrosomes are present in prefusion nuclei of both *Ceratostomella moniliformis* and *C. fimbriata*. They are more conveniently observed in the latter species. The single chromatic granule situated conspicuously on the periphery of each nucleus (fig. 3, B, M) appears to become attenuated and bilobed just preceding nuclear division (E, N, O). The spindle-shaped structure at first traverses the nuclear vesicle, but later each end of the spindle extends beyond the limits of the vesicle. Nuclear division seems to take place along the slender axis. The nucleolus during this time is situated usually midway of the spindle (I, S) and remains visible until division is complete. Following nuclear division the old nucleolus dwindles in size and commonly disappears before cell cleavage. At the same time a new nucleolus, together with a satellite or centrosome, appears in the reorganized nucleus (A).

**NUCLEAR STRUCTURE AND NUCLEAR FUSION**

**CERATOSTOMELLA MONILIFORMIS**

In *Ceratostomella moniliformis* the apparent absence of a nuclear membrane is a notable characteristic of prefusion nuclei. The nuclei commonly have the appearance indicated in figure 2, G, H, with the limits of the nuclear vesicle quite indistinct. At the time of nuclear fusion the two vesicles become confluent, so that at a certain stage two nuclei appear to be floating in a single hyalosphere (W). The outline of the hyalosphere becomes more distinct at this time, although a well-defined nuclear wall rarely is seen. A mingling of the two separate spheres of nucleoplasm (X, G) marks the second stage in nuclear fusion, and a fusion of the two nucleoli (H, I) indicates completion of this stage in ascus formation.

While the content of prefusion nuclei appears to be homogeneous, with affinity principally for plasma stains, the fusion nucleus possesses an elaborate network to which chromatin stains adhere (fig. 2, I). The comparatively large and frequently lens-shaped nucleolus of the young ascus (A) commonly assumes a position between the central body of nucleoplasm and the margin of the nuclear vesicle. In this condition the whole structure, including the cell body, increases in size. Just previous to or during the first nuclear division in the ascus characteristic large vacuoles appear peripherally in the cytoplasm (M1–R1).

**EXPLANATORY LEGEND FOR FIGURE 4**

A–H. First nuclear-division stages that in most cases show evidence of a persistent nuclear vesicle; I, stage in spindle formation, with chromatic granules or inclusions marking the periphery of the original nuclear vesicle; J, an advanced spindle with evidence of a persistent membrane at each pole; K, reorganization of daughter nuclei following the first division, and showing an early appearance of cleavage at points originally occupied by the definitive nuclear membrane; L, nuclear division in a probably haploid ascus; M, beginning of the second nuclear division in the ascus; N, spindles during the second nuclear division traverse the contents of an inner vesicle which at this stage becomes conspicuously separated from the outer body of the ascus; O–Q, second-division stages after formal-acetic fixation; R, linkage of asci, with an appended uninucleate fragment; S, contraction of the interior region of the ascus at the second nuclear division; T, U, the four-nucleate stage in ascus formation; V, linkage of asci in a manner to indicate that cell cleavage was incomplete; W, the third nuclear division in the ascus; X, the eight-nucleate stage in ascus formation; Y, Z, emergence of the eight nuclei on the surface of the spore-producing mass suspended in the ascus vesicle; A1, outgrowth of spore rudiments from the surface of the vesicle contents; B–D1, continued growth of spore rudiments into the outer cytoplasmic body of the ascus, with indication that the spores extend from a common base; E1–F1, the four-nucleate stage showing the vesiculate structure of the ascus interior; G1, an abortive ascus, or one from which the spore-producing contents have been displaced; H1–J1, ascus during the third nuclear division, showing a frequent appearance of chromatic contents of the nuclei. All X 3000.
CERATOSTOMELLA FIMBRIATA

Ceratostomella fimbriata is similar to C. moniliformis in the structure of prefusion nuclei (fig. 3) and in the various stages of nuclear fusion. The structure of the fusion nucleus, however, deserves careful consideration because of the important role that the nuclear vesicle is believed to play during later stages of ascus formation.

The fusion nucleus possesses a network of nodose filaments that gather into thick strands just preceding nuclear division (figs. 3, G, I; 5, B) and form an increasingly compact plate of chromatic substance across the nuclear vesicle (fig. 5, C, D). The nucleolus becomes spherical instead of lens-shaped and withdraws from the nuclear vesicle. Unlike the nucleolus in Ceratostomella moniliformis, which persists throughout ascus formation, that in C. fimbriata disappears during the first nuclear division (fig. 5, J, K). Local concretions or thickenings sometimes observed at the periphery of the nucleus (fig. 5, A) may presage the later transformation of the nuclear membrane into an endogenous ascus wall, yet this is improbable since in many cases the original nuclear vesicle is distinguishable only by a faint outline during the first and second nuclear divisions.

ORGANIZATION OF THE ASCUS

FIRST NUCLEAR DIVISION

Ceratostomella Moniliformis

In Ceratostomella moniliformis the beginning of the first division is indicated by the aggregation of chromatin threads in a crude plate across the face of the nuclear vesicle (fig. 2, J). The threads condense into an irregularly lobed or sometimes spherical mass of chromatin (D, K, M, N) that breaks into two portions situated midway on a simple rod-shaped axis (L, O, P). Later there are four reasonably distinct bodies distributed along the axis of the spindle (Q), two of which move to each pole. Often a single bilobed chromatin body appears to be drawn to each pole (R). The amount of chromatin substance passing to the opposite poles frequently appears unequal (fig. 4, A, B), but the resultant daughter nuclei as well as the spindles of the second division appear equal. The advanced spindle is a dumbbell-shaped structure (fig. 4, E, H–J) with a simple axis that rarely seems to consist of two filaments (fig. 4, I).

The number as well as the rather characteristic shape of the chromatin bodies distributed on the spindle has been observed repeatedly, and there seems little reason to doubt that these bodies correspond to the chromosomes described in other species of fungi. At the same time,

EXPLANATORY LEGEND FOR FIGURE 5

A. An ascus with local thickenings at the periphery of the definitive nucleus; B. Withdrawal of the nucleolus from the nuclear vesicle at the first stage in nuclear division; C. Gathering of the chromatic contents of the nuclear preparatory to division; D. Separation of chromatin bodies at metaphase; E–J, Metaphase figures showing chromosomes attached at various angles to the axis of the spindle; K, L. Four-lobed spindles at metaphase. The nucleolus is disintegrating at the margin of each nuclear vesicle; M. A typical figure at late anaphase, showing fusion of two chromosomes into a single chromatin mass at each pole; N. A typical figure at late anaphase, showing two pairs of chromosomes, each pair consisting of a simple and a compound chromatin body; O. A first-division figure at anaphase, with the old nucleolus still visible at the margin of the nuclear vesicle; P. Reorganized daughter nuclei following the first division in the ascus, showing their structure and arrangement in the vesicle; Q–T. The second nuclear division in the ascus; U, V. Eight-nucleate ascus, showing the spore-producing region enclosed by a definite wall. All X 3000.
FIGURE 5.—Nuclear division and ascus formation in Ceratostomella fimbriata. (For explanatory legend see opposite page.)
time it is conceivable that discrete chromatin bodies in fungi may not be equivalent to chromosomes of higher plants. The latter viewpoint was expressed by some of the earlier workers in the field of fungus cytology (10), but the more recent workers have come to regard such a reservation as unnecessary.

The outline of the original nuclear vesicle usually remains visible during the first division in the ascus, although there seems to be no definite nuclear wall. Chromatic granules that appear in material killed with osmic acid sometimes indicate the margin of the vesicle (fig. 4, D).

The behavior of the nucleolus during the nuclear divisions in the ascus is a conspicuous and perhaps an important characteristic of the species. Just preceding the first spindle formation, the nucleolus withdraws to one side of the nuclear vesicle (fig. 2, D, K) and remains there during the nuclear divisions and up to the time of ascospore formation (figs. 2, L-R; 4, A-N, S-V; 6, A, F, H). It is surrounded by a faint or sometimes distinct halo, which, however, often appears to be continuous with the outline of the nuclear vesicle (figs. 2, O; 4, M, T). The persistence of the nucleolus in its relation to the vesicle (fig. 4, T-V) comprises part of the evidence upon which it is concluded that the ascospores are formed within the nuclear vesicle. In Ceratostomella moniliformis the vesicle appears to be without a wall, whereas in C. fimбриata a definite endogenous wall is frequently observed just previous to ascospore formation.

The reorganized daughter nuclei following the first division in the ascus are similar in size and general appearance to prefusion nuclei. An eccentric highly chromatic body, as indicated in figure 4, K, does not seem to be the equivalent of the nucleolus in the prefusion stage. Rarely at this time a break occurs in the cytoplasm of the ascus at a position corresponding to the margin of the original vesicle of the fusion nucleus. More often this cleavage of the cytoplasm does not occur until the second nuclear division is nearly completed.

CERATOSTOMELLA FIMBRIATA

In Ceratostomella fimбриata the first-division figure is enclosed by an elliptical hyalosphere within the limits of the original nuclear vesicle, which in many instances is indicated only by the regular contour of a central mass of denser protoplasm (fig. 5, N). There is little evidence that a centrosome is present during nuclear division in the ascus. While two denser points of granulation can be observed occasionally during early stages of division, the two poles of the advanced spindle (E-I) seem to merge into the cytoplasm at the margin of the hyalosphere.

During the first nuclear division in the ascus the spindle first appears as a continuous two-lobed mass of chromatin that later separates into four distinct bodies attached at various angles to a single filament or axis (fig. 5, F, I, K). In some figures five chromatic granules appear to be distributed along the axis (E, G); in other instances six may be seen (H, N). It will be observed, however (H, N), that the four bodies nearest the midpoint on the spindle appear as two dumbbell-shaped structures, each pair probably representing a single "chromosome." This arrangement of chromosomes, if such they may be called, seems to be characteristic. Two chromosomes, one oval or comma-shaped
and the other irregularly lobed, move toward each pole \((L)\). They appear as a single mass of chromatin after they reach their respective poles \((M, O)\).

Since the writers have found no evidence of a double nuclear fusion in *Ceratostomella fimbriata* or in *C. moniliformis*, they have no reason to suppose, as Gwynne-Vaughan and Williamson do in the case of *Pyronema confluens* (11) and *Lachnea scutellata* (12), that the first nuclear division in the ascus is a reduction division. The stages in division of prefusion nuclei are too few and too obscure to provide an adequate estimate of chromosomes in haploid nuclei. In those stages observed a single chromatin body appeared to move to each pole of the spindle (fig. 3, \(O, I\)). On the basis of rather meager evidence it appears that there are two unequal chromosomes in diploid nuclei of *C. fimbriata* and that haploid nuclei probably contain a single chromosome. Presumably, if reduction occurs in the ascus, one half of the ascospores and the mycelial growth to which they give origin would contain nuclei possessing a dumbbell-shaped chromosome and the other half would contain nuclei possessing a globular or comma-shaped chromosome. It would be possible, of course, to interpret the bilobed chromatin body as representing two separate chromosomes. In such a case it would be necessary to conclude that the diploid number is three, and that some of the haploid cells contain nuclei with an extra chromosome. In either case the inference would exist that there are two kinds of haploid cells in *C. fimbriata*, differing in the amount of chromatin in their nuclei if not in the number of chromosomes.

As suggested elsewhere, it is probable that no reduction occurs in the ascus of this species and that the chromosome complement of each ascospore is equal. Reduction may occur at the time of ascospore germination or at some later time. Obviously the situation needs to be clarified much further before it can be said that nuclei in *Ceratostomella fimbriata* are not differentiated in respect to sex, and that apparent inequalities in the distribution of chromatin among nuclei may not be in some way related to their behavior.

**SECOND NUCLEAR DIVISION**

*Ceratostomella Moniliformis*

In *Ceratostomella moniliformis* the second division in the ascus resembles in many respects the division of prefusion nuclei. Polar bodies that may correspond to centrosomes are observable in some instances (fig. 4, \(M\)). Late metaphase stages of this division are particularly obscure, and the observer is disposed to rely upon data from *C. fimbriata* (see below), in which it is shown that no reduction in number of chromatin bodies occurs during the second division. Figure 4, \(O\) and \(Q\), however, shows a difference in the amount of chromatin and in the general appearance of the chromatin mass passing to each pole. The bodies shown probably do not correspond to individual chromosomes.

At an early stage the spindles appear to follow the periphery of the inner vesicle (fig. 4, \(P\)). Later they appear to traverse the entire breadth of the vesicle \((S)\), which by this time has become conspicuously separated from the outer region of the ascus. The curious isolation of the inner vesicle from the outer body of the ascus \((N, R-Z, A_1-G_1)\) is perhaps a characteristic of *Ceratostomella moniliformis*. Mittmann
observed a similar phenomenon at a certain stage during ascus formation in *C. fimbriata*; she was uncertain whether or not a wall was present about the vesicle. The present writers have observed the cleavage of the ascus cytoplasm only infrequently in the latter species, while a definite wall is frequently seen in the position corresponding to the periphery of the vesicle in *C. moniliformis*.

No wall appears to surround the spore-producing vesicle in *Ceratostomella moniliformis*. The contracted appearance of the vesicle contents, however, suggests the occurrence of plasmolysis, which in itself may suggest the presence of a membrane. If the phenomenon is actually a result of plasmolysis it nevertheless demonstrates that a material difference, morphological and perhaps physiological, exists between the inner spore-producing mass and the outer much-vacuolated cytoplasm. It seems, however, that the phenomenon, rather than being due to plasmolysis, is similar in nature to the cleavage of the cytoplasm during the formation of ascospores. This is further indicated by the fact that in *C. fimbriata* the inner zone does infrequently develop into a single giant ascospore. (See fig. 7, L.)

It is important to note that the contents of the ascus vesicle are not entirely isolated from the outer body of the ascus. The inner mass remains continuous with the external region of the ascus usually at the point where the old nucleolus emerged from the nuclear vesicle (figs. 2, O; 4, T), and is also connected by slender strands at various points on the periphery corresponding, perhaps, to those observed in the definitive nucleus (fig. 6, B). The nucleolus remains permanently in its position at the main attachment of the vesicle contents, and this fact is regarded as evidence that the ascus vesicle (figs. 4, S–Z, A; 6, F) actually corresponds to the nuclear vesicle (fig. 2, A, J), which has undergone a continuous increase in diameter. The same conclusion was previously reached with respect to *Ceratostomella fimbriata* (1), where the ascus vesicle can be shown to possess a definite wall at certain stages.

At the close of the second division in the ascus the daughter nuclei again assume an appearance resembling prefusion nuclei (fig. 4, T, U). This stage seems to persist somewhat longer than did the corresponding one following the first division. In figure 4, T to V, are shown characteristic views of the ascus vesicle at the four-nucleate stage.

*Ceratostomella Fimbriata*

In *Ceratostomella fimbriata* the second nuclear division in the ascus is similar to the first, there being no appreciable reduction in the size of spindles or in the amount of chromatin. Figures at this stage are less numerous, and details in the distribution of chromatin are not easily observed. It is evident, however, that no reduction in chromosome number occurs at the second division. Each spindle appears as a four- to six-lobed body (fig. 5, Q, R), with a two- or three-lobed mass of chromatin passing to either pole (T). These resemble in shape and size the chromatin masses most often observed in the first division.

Following the second nuclear division the endogenous ascus wall begins to appear in some of the asci in the position formerly indicated by the outline of the inner mass of denser protoplasm (fig. 5, S). While not visible in every instance, it is in many cases distinct and is undoubtedly a true endogenous wall surrounding the inner spore-producing area of the ascus.
THIRD NUCLEAR DIVISION

CERATOSTOMELLA MONILIFORMIS

In Ceratostomella moniliformis, owing partly to the reduced size of the various structures, the third division in the ascus is difficult to study. Figure 4, I₁, J₁, shows a stage frequently observed. Metaphase figures are obscure; consequently the division appears to involve only a single chromatin body. The recently reorganized nuclei (fig. 4, X) again resemble those previous to fusion. At a more advanced stage deeply stained bodies, usually crescent-shaped and often bilobed, may represent the reappearance of individual chromosomes at the beginning of ascospore formation (figs. 4, H₁; 6, A, B).

FIGURE 6.—Morphology of the ascus in Ceratostomella moniliformis. A, An eight-nucleate ascus, with evidence of a membrane surrounding the central mass and with the original nucleolus still visible; B, linkage between asci (note the curious shape maintained by chromatic nuclear contents at one stage in spore formation; C, an advanced ascus, showing the origin of membranous plates that persist between pairs of ascospores; D, an exceptional appearance of ascus cleavage during spore formation; E, an abortive ascus, or one from which the spore-producing contents of the vesicle have been removed; F-H, typical views of immature asci showing the attachment of the rudimentary spores to a common cytoplasmic base and the formation of spore sheaths by the invagination of the outer region of the ascus; I-K, typical mature asci, showing the arrangement of spores in pairs and the absence of a peripheral ascus wall; L, an oblique view of a pair of ascospores held together by membranous plates; M, a single mature ascospore with membranous attachment; N, an ascospore, with a second spore sheath from which the contents have been displaced or in which a spore has failed to develop; O, a pair of ascospores held together by membranous plates; P, Q, groups of four spores held together by membranous attachments and remnants of cytoplasm. All X 3000.
In *Ceratostomella fimbriata* there is a slight reduction in the size of spindles and in the amount of chromatin in third-division figures (fig. 7, C, D). A reduction in number of chromosomes presumably might occur at this stage, yet that is not a necessary conclusion, as is pointed out elsewhere. A brief period of reorganization of daughter nuclei follows the third division. The nuclei frequently have the appearance shown in figure 7, E, H, where a single deeply stained body in each nucleus resembles in size and shape the chromatin bodies observed at certain stages during the previous nuclear divisions.

A break in the cytoplasm at the limits of the inner cell mass (fig. 7, E–G), first described by Mittmann (21), seems to be exceptional in this species, although it is a characteristic feature of ascus formation in *Ceratostomella moniliformis* (fig. 4). The occasional occurrence of the break does, however, indicate the vesicular nature of the inner spore-producing mass and is additional evidence of the presence of a membrane surrounding the denser region of the ascus, even though a definite wall is not always to be seen.

**SPORE FORMATION AND ORGANIZATION OF MATURE ASCUS**

*Ceratostomella Moniliformis*

In *Ceratostomella moniliformis*, at the first stage in the delimitation of ascospores, the eight nuclei are distributed near the surface of the ball of protoplasm suspended within the ascus vesicle. The chromatic contents of each nucleus appear as a lens-shaped cap with its convex face producing a small wartlike protuberance on the surface of the suspended sphere (fig. 4, Y, Z). The lens-shaped bodies have somewhat the appearance of polar caps or cones that are observed during spore formation in more familiar Ascomycetes. Polar rays diverging from the apical beak, such as were believed by Harper (15) to function in the formation of ascospore walls, seem to be entirely absent in this and related species.

The protuberances on the surface of the sphere of protoplasm suspended in the vesicle are young or rudimentary ascospores. By their continued growth they encroach upon the limits of the vesicle, causing it to become angular in shape (figs. 4, A₁; 6, F) and eventually deeply lobed. Each spore rudiment forms a niche in the surrounding wall of protoplasm (fig. 6, G, H). Numerous indications lead to the conclusion that the eight spores, early in the process of delimitation, radiate from a common center or base (figs. 4, B₁–D₁; 6, F–H). This attachment base appears to be a point at which the vesicle contents are continuous with the outer body of the ascus.

As a result of its continued growth each young spore becomes enclosed by a sheath of cytoplasm that originally constituted a portion of the external ascus (fig. 6, B, F–I). Although the external spore walls appear to be produced by a transformation of the cytoplasm which formerly composed the peripheral region of the ascus, a rudimentary wall may already have existed as a membrane lining the inner face of the epiplasm. The separate origin of the spore wall and the surface membrane of the spore cytoplasm would be somewhat contrary to the observations of Faull (7) on *Hydnobolites* and other genera, where the two membranes appeared to originate at
the same point and time. The separation of the spore-producing mass from the epiplasm (as the term is used by Faull) in *Ceratostomella* occurs at approximately the four-nucleate stage, and it may be supposed, of course, that the surface membrane of the inner mass and the membrane that presumably lines the vesicle cavity have a common origin. The two membranes later would appear to become transformed respectively into the surface membrane of the individual spores and into the spore walls.

During advanced stages in spore formation the external portion of the ascus appears to be an inert mass of protoplasm with its outer margin often fading imperceptibly into the unorganized debris of the perithecial cavity. The young ascospores appear to grow into their encasement, yet it is possible that the spore wall is, in part, a result of active growth processes within the cytoplasm. There is no true ascus wall in *Ceratostomella moniliformis*. Close study of the disorganization of the external ascus reveals no stage at which there is an outer membrane of any more definite nature than that which surrounds the numerous vacuoles within the protoplasmic body. The group of mature spores simply fall apart (fig. 6, I–K), and remnants of the disorganized cytoplasm remain attached to them after they are discharged from the perithegium.

A characteristic membranous attachment (fig. 6, M) that occurs on the ascospores originates from the interspore layers of cytoplasm and seems to be composed of the same substance as the spore walls. The membranous disk-shaped structures are frequently responsible for the spores clinging together in pairs or sometimes in groups of four (fig. 6, L–Q).

**Ceratostomella Fimbriata**

In the initial stage of ascospore formation in *Ceratostomella fimbriata* the eight nuclei emerge near the periphery of the inner cell mass (fig. 7, G), and each spore rudiment grows outward and becomes separately enclosed by the surrounding cytoplasm (H–J). The young spores extend radially from one or more points of attachment.

The behavior of the endogenous wall during the period of spore formation is variable. As pointed out in a previous publication (1), a wall is not always present in the ascus, in which case the spores mature in a group held together only by remnants of cytoplasm (fig. 7, O, Q). In other instances a wall is clearly visible following the four-nucleate stage (figs. 5, U; 7, B), and portions of it are still to be seen after the spores are completely formed (fig. 7, K). It appears, therefore, that ascus formation in *Ceratostomella fimbriata* may follow two courses. Where no endogenous wall encloses the spore-producing mass, the cleavage of the young spores may extend into the cytoplasm of the outer ascus (fig. 7, I), in which case the spore wall would be principally cytoplasmic in origin. But where an endogenous wall is present, spore cleavage must occur entirely within the vesicle (figs. 5, V; 7, K), with the vesicle wall being partially used up in the formation of spore walls. The occasional early separation of the central spore-producing region of the ascus from the epiplasm, which is evidenced by a conspicuous cleavage space (fig. 7, E–G), suggests that the processes of spore delimitation and membrane formation are fundamentally similar to those described under *C. moniliformis*. 
Problems concerning the nature and origin of the spore-producing vesicle and of the vesicle wall in *Ceratostomella* cannot immediately be solved, since there is no precedent for such a structure in any of the Ascomycetes. The opinion has already been expressed that the vesicle wall has its origin from the membrane of the fusion nucleus. Such a view is supported by considerable evidence derived from the study of *C. moniliformis* and is discussed elsewhere. Further information regarding the nature and origin of the endogenous wall in *C. fimbriata* may be had from certain abortive structures that are sometimes observed in the same species. Figure 7, *M*, shows an abortive ascus that appears to contain a single uninucleate spore; figure 7, *R*, seems to represent a similar abortive ascus. Infrequently, as late as the eight-nucleate stage, the central spore-producing mass may become enclosed by a wall of such an indurated appearance that the vesicle seems to be a single giant spore (fig. 7, *L*). It is suggested, therefore, that the delimitation of the vesicle in *Ceratostomella* may be comparable in some respects to the delimitation of a single ascospore.

Mature ascospores of *Ceratostomella fimbriata* possess membranous attachments similar to those of *C. moniliformis*. The attachments originate in a manner similar to the spore walls, namely, from the intervacular layers of the cytoplasm (fig. 7, *N, P, Q*). Spore walls, as represented in figure 7, *O*, for example, at one stage appear as ropy strands or layers of cytoplasm (fig. 7, *P*) and the spore attachments, similar to those shown in figure 6, *M*, are constructed of the same material. These cytoplasmic layers in their turn must have originated from the cytoplasm that formerly enclosed the rudimentary spore cavity (fig. 7, *I*) or from the lining of the vesicle. If spore walls are formed from the cytoplasm of the external ascus, as appears to be the case, and not from the surface layer of the spore rudiments, as is commonly understood to occur in other Ascomycetes, it may be necessary to suppose that ascospore walls in *Ceratostomella* are not entirely equivalent to or homologous with ascospore walls in the more familiar genera. The apparent spore wall in *Ceratostomella* may be a special sheath that encloses both the spore and its true wall; the latter, in these species, may be limited to a surface membrane.

**DISCUSSION**

Any investigation in the field of cytology that purports to describe something new or something at variance with what is already known of cell or nuclear behavior is apt to meet the familiar query concerning proper fixation. In answer to any possible criticism of this nature it should be said that the several methods used are similar to

**EXPLANATORY LEGEND FOR FIGURE 7**

A. Cell linkage previous to ascus formation.  
B. A four-nucleate ascus with endogenous wall.  
C, D. Ascii during the third nuclear division.  
E–G. Ascii with the spore-producing interior contracted away from the vesicle wall.  
H. An immature ascus, showing the chromatic contents of each spore nucleus organized into distinct bodies.  
I, J. Stages in ascospore formation, showing the radial disposition of the young spores.  
K. A nearly mature ascus with a portion of the endogenous vesicle wall still identifiable.  
L. An abortive ascus with the interior developed into a single giant spore.  
M. An abnormal or abortive ascus with a central region that might develop into a single giant spore.  
N. A group of ascospores held together by membranous attachments and disorganized cytoplasm of the parent cell.  
O. A well-developed ascus indicating the absence of any peripheral wall.  
P. A pair of ascospores held together by membranous remnants of cytoplasm.  
Q. A fully developed ascus; four of the eight ascospores frequently stain more deeply than the others.  
R. An abnormal or abortive ascus in which a nucleus has persisted at the margin of the inner cell mass.  
All X 3000.
Fig. 7.—Morphology of the ascus in *Ceratostomella fimbriata*. (For explanatory legend, see opposite page.)
those used successfully by other investigators in the same or related fields and that many of the exceptional phenomena observed in *Ceratostomella*, such as the unwalled condition of the asci and the vesiculate condition of the spore-producing region of the ascus, are clearly discernible in preparations made by the acetocarmine method, where the fruiting bodies are dissected directly in the mounting fluid.

An attempt has been made to describe in some detail the origin and development of an ascus of the so-called deliquescent type. In the course of the discussion considerable emphasis has been placed on the fact that the deliquescent asci of *Ceratostomella* possess no external wall at any stage of their development. The situation at first seems to be unparalleled among other genera of Ascomycetes, but it is possible that those who have had occasion to observe the deliquescent type of ascus have neglected to distinguish between a cell wall, such as occurs on the more familiar type of ascus, and a plasma membrane, which occurs in addition to the external wall. The ascus wall, as it is understood in the case of discomycetous species, for example, is clearly homologous with the hyphal wall present on the filaments on which the ascii are characteristically borne. The plasma membrane, on the other hand, is equivalent to the surface layer of cytoplasm such as occurs on any essentially naked or plasmodial mass. The ascus of *Ceratostomella* is of the latter kind. The point at which the external wall is discarded or dissolved away and the point from which the cells of the ascogonium begin to multiply as naked protoplasts can be detected in an early stage of perithecium formation.

Stages in the multiplication of the naked cells previous to ascus formation in *Ceratostomella* have not been adequately described in previous work on the development of the perithecial stage. Crosier cell divisions are of an unusual type and may lead to a better interpretation of the function and nature of the curious device frequently referred to as the "ascus hook." Cell divisions may follow a single cleavage plane, two separate planes of cleavage, two confluent planes of cleavage, or three confluent planes. In particular instances the appearance of a compact group of cells may suggest that simultaneous divisions have occurred in three dimensions. Division of a four-nucleate body into two binucleate portions may follow one of several different courses. The appearance of a crosier may actually be followed by a simple division into two binucleate cells.

All previous investigators, with the exception of Varitchak (26), who believed that ascus crosiers occur in *Ceratostomella piceae* Münch, have failed to observe this type of cell division in the genus. Elliott (6), Mittmann (21), and Andrus and Harter (1), all previously overlooked their occurrence in *C. fimбриata*, and the last-named authors were led to question Varitchak's statement concerning *C. piceae* partly owing to the absence of figures showing nuclear division in the supposed crosier. The present observations show that the external form of an ascus hook is not sufficient evidence that the later cell division will be of the crosier type. The position of spindles during the conjugate nuclear division seems to provide the most reliable information as to whether cell division will be direct or indirect. The shape of the cell and the divergent position of spindles, shown in

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8 The so-called "smear" technique frequently employed by cytogeneticists.
figure 2, J, are very characteristic, especially in *C. moniliformis*. It seems necessary to suppose that conditions of polarity within the cell determine both the shape of the cell and the position of spindles at the time of nuclear division but that the two conditions may not always be correlated.

The vesiculate structure of the spore-producing region of the ascus in *Ceratostomella moniliformis* and *C. fimbriata* seems to be quite without parallel in other fungi. Mittmann (21) first described the break in the cytoplasm bounding the inner spore-producing region in *C. fimbriata*. She was uncertain whether or not a membrane or wall was present. Andrus and Harter (1) did not observe the cleavage of the cytoplasm in *C. fimbriata*, but frequently observed the presence of an endogenous wall surrounding the spore-producing area. They believed that the position of the wall corresponded to the periphery of the original (definitive) nuclear vesicle and concluded that the nuclear membrane, if such had existed, had been transformed into the endogenous ascus wall. In the present investigation the break in the cytoplasm of the ascus is found to occur in some asci and the existence of an endogenous wall is more clearly demonstrated. In *C. moniliformis*, on the other hand, the break in the cytoplasm surrounding the spore-producing region is highly characteristic and there appears to be no endogenous wall, except such as might be suggested by the appearance of plasmolysis (fig. 4, S–Z).

The remarkable structure of the spore-producing area of the ascus in *Ceratostomella moniliformis* and *C. fimbriata* does not seem to be merely an exaggeration of the cytoplasmic condensation so frequently observed in more conventional Ascomycetes. To be sure the vacuolization of the outer region of the ascus and the denser, more homogeneous appearance of the inner region correspond to the condition in other species, but in *C. moniliformis* and *C. fimbriata* nuclear divisions and spore formation seem to take place within a true vesicle that is variously separated from the outer region of the ascus by a true wall, by a conspicuous cleavage of the cytoplasm, or by an abrupt and regular contour.

The fact that the cleavage of the ascus cytoplasm in *Ceratostomella moniliformis* sometimes occurs as early as the first nuclear division is considered to be especially strong evidence that the margin of the spore-producing vesicle actually corresponds to the margin of the original fusion nucleus, since the first spindle, at least, is intranuclear. The position of the nucleolus in relation to the nuclear vesicle during the first division and in relation to the ascus vesicle at a later stage is further evidence that the original nuclear vesicle is continuous with or is transformed into the spore-producing vesicle. These evidences would seem to be very conclusive were it not for the fact that there is ordinarily a brief period following the first division during which the outline of the vesicle is very faint or, in particular asci, not at all apparent. It may be difficult to determine whether the nuclear membrane actually becomes transformed into the membrane of the spore-producing vesicle, but such a view seems to be a logical one.

The occurrence of successive nuclear divisions within an original nuclear vesicle is not without precedent, for it has been described frequently in other species of fungi and in algae. The regeneration of an entire cellular unit from a nucleus or from chromatic portions of a nucleus also is not without precedent if the opinions of Komocki
in regard to the erythrocytes of the salamander *Batrachoseps attenuatus* Esch. are accepted. The formation of an endogenous wall or walled inclusion within a cytoplasmic body certainly is not without precedent. Nevertheless, the statement concerning the "transformation of a nuclear membrane into an ascus wall" is astonishing and much further study is necessary before it can be accepted fully. Doubts such as were expressed by Gwynne-Vaughan and Williamson (13) regarding earlier work on *Ceratostomella fimбриata* (1) are not surprising.

The question of the nature of the spore-producing vesicle in ascii of *Ceratostomella fimбриata* and *C. moniliformis*, and perhaps other members of the genus, is quite as important as that concerning the origin of the vesicle membrane. It has already been indicated that the spore-producing region of the cytoplasm in ascii of more familiar genera often appears condensed in contrast to the vacuolated peripheral or sometimes basal region of the ascus, but the denser portion does not appear to be vesiculate. The vesiculate condition appears to be lacking also in *C. piceae* (26), which belongs to the group of non-endoconidial species. Since the outer region of the ascus in *Ceratostomella* is a naked and sometimes shapeless mass, the view that the vesicle membrane represents or substitutes for an ascus wall would seem to be an obvious one. As a matter of fact, some ascii appear to have no wall either inside or out, the intervacular layers of cytoplasm representing the nearest approach to a membranous structure.

The interpretation now suggested is that the spore-producing vesicle corresponds to a single giant ascospore. In *Ceratostomella fimбриata* the "giant spore" first appears as a definitely walled structure; in *C. moniliformis* (fig. 4, K, N) it is formed by a process of cytoplasmic cleavage that appears to be very similar to the cleavage by which ascospores are formed in more familiar genera of the Ascomycetes. The eight-nucleate "spore" seems sometimes to persist as a single abortive ascospore (fig. 7, L), but characteristically the vesiculate body either gives rise to eight protuberances (fig. 4, Z) that at first remain attached to a common base (figs. 4, B, D; 6, F; 7, I) but later appear as eight separate ascospores (figs. 6, B; 7, O) or becomes separated into eight spores, apparently by the usual processes of ascospore delimitation.

Whether or not the above interpretation of the ascus vesicle in *Ceratostomella* is correct, it is still evident that the process of ascospore formation differs from that commonly understood to occur in other Ascomycetes. Although the cleavage of the cytoplasm about the spores may in some instances be comparable to that observed in other species, the manner of outgrowth of spores, i.e., their whorled arrangement and their attachment to a common base, does not seem to conform to any previous description of ascospore formation, and the clearly vesiculate condition of the spore-producing region is of course unique.

The absence of spindle fibers and polar rays, such as were believed by Harper (15), Schultz (25), and others to function actively in the process of spore-wall formation, is further evidence that the process of ascospore delimitation in *Ceratostomella* may differ fundamentally from that commonly observed to occur. If it may be conceived that the differentiation of the spore-producing vesicle in *Ceratostomella* corresponds to the delimitation of a single ascospore, then the process
would seem to conform more nearly with the process of ascospore formation described by Faull (7), especially in regard to membrane and spore-wall formation.

The question of where the reduction division occurs may turn out to be an especially significant one in respect to the genus Ceratostomella. Buisman (2) observed that single-conidium cultures of C. fagi Loos fall into two sexual groups and that ascospores seem to be bisexual. Single-ascospore cultures produced perithecia, but cultures from single conidia did not. Mating of single-conidium cultures resulted in the formation of perithecia. Buisman suggested that sex segregation may occur at the time of ascospore germination. A difference in the behavior of cultures derived from ascospores and from conidia had been observed previously by McCallum (19), Münch (22), and Loos (18) in several species of Ceratostomella. Leach (17) also has observed recently a difference in the ability of C. ips Rumbold to produce perithecia in cultures of conidium and ascospore origin, but neither type of reproductive cell appeared to be wholly bisexual or wholly unisexual. These curious facts suggest that a segregation occurs at the time of ascospore germination or at some time during the period of mycelial growth or conidium formation. It is probable, although perhaps it is not a necessary conclusion, that sex segregation occurs at the time of, or is accompanied by, reduction in the number of chromosomes.

Ceratostomella moniliformis and C. fimbriata appear to be homothallic. Single-ascospore cultures of both species produce perithecia. Out of 32 single-conidium cultures of C. fimbriata, only 4 failed to produce perithecia. At present it is unknown whether the four cultures represent sex segregates or some other type of heritable sterility, such, for example, as frequently appear as sectors in plate colonies of the same species. The homothallic condition of the two species seems to support the earlier observation that there is no reduction in number of chromosomes in the ascus, and that the two chromosomes observed actually represent the diploid number. On the other hand it may be a mistake to assume that a single-ascospore culture is diploid merely because it is able to produce perithecia.

The view of Gwynne-Vaughan and her students that a double reduction occurs in the ascus is made necessary by their belief that a double nuclear fusion occurs in the ontogeny of certain species. The occurrence of nuclear fusion in the ascogonium, however, has been to a considerable extent discredited, especially by the recent work of Raymond (23), who observed that fusions of the type described by Fraser (8) and Fraser and Brooks (9) in Lachnea stereo-rea and by Gwynne-Vaughan and Williamson (11) in Pyronema confluentus occur also in wholly vegetative cells.

Conclusions in regard to sex segregation and chromosome reduction in Ceratostomella should be deferred for the present. It is conceivable that reduction as well as sex segregation might occur at various points in the life cycle. Many fundamental facts regarding cytological behavior and sex segregation in fungi still remain to be discovered. Certainly it cannot be said that knowledge of the life history and cytology of C. moniliformis and C. fimbriata is in any way complete. It may yet be possible to determine whether nuclei at many different stages are haploid or diploid, and such information would be of great value in interpreting life histories.
SUMMARY

Stages in the multiplication of unwalled cells previous to ascus formation in *Ceratostomella moniliformis* and *C. fimbriata* include the crosier type of cell division as well as several other types of direct and indirect cleavage. The position of spindles at the time of nuclear division is believed to provide the most reliable indication as to whether cell division will be direct or indirect. Cell divisions are accompanied by significant changes in cell shape. A structure resembling a centrosome in behavior is present as a satellite of nucleoli during the prefusion stage.

Nuclear fusion in the ascus is followed by three successive nuclear divisions, all of which are alike except in the size of the various structures. The axis of the spindle is a simple rod without evidence of polar bodies or astral rays. Two and later four chromatin bodies appear on the axis of the spindle, and two bodies move to each pole. The chromosome number in each species is interpreted to be 2 (diploid). No reduction occurs in the ascus.

Following the nuclear divisions the spore-producing region of the ascus is differentiated as a single distinct vesicle. The vesiculate condition in *Ceratostomella moniliformis* is made evident by the presence of a cleavage space surrounding the spore-producing region, while in *C. fimbriata* a definite endogenous wall frequently encloses the vesicle. Data are presented which tend to show that the membrane of the spore-producing vesicle is continuous with or derived from the membrane of the fusion nucleus.

The eight ascospores in *Ceratostomella moniliformis* originate as protuberances on the surface of the sphere of protoplasm contained in the spore-producing vesicle. By continued growth each spore becomes enclosed by a wall of cytoplasm that originally composed a part of the external ascus. The process of spore formation in *C. fimbriata* is frequently modified to an extent imposed by the presence of a wall enclosing the spore-producing region of the ascus. In both species the immature spores appear to be attached to a common base which is usually lateral to the longer dimension of the ascus.

The mature ascus consists of a compact group of spores to which are attached remnants of inert cytoplasm. Deliquescence of the ascus involves a disorganization of the peripheral layer of cytoplasm but does not appear to involve any process of wall dissolution in *Ceratostomella moniliformis* and *C. fimbriata*, since the asci possess no external wall. Mature ascospores possess disk-shaped membranous attachments that appear to originate in a manner similar to spore walls and often hold the spores together in pairs or in groups of four.

LITERATURE CITED

(1) ANDRUS, C. F., and HARTER, L. L.

(2) BUISMAN, C.
1933. UEBER DIE BIOLOGIE UND DEN PARASITISMUS DER GATTUNG CERATOSTOMELLA SACC. Phytopath. Ztschr. 6: [429]–439, illus.

(3) DANGEARD, P.
1894. LA REPRODUCTION SEXUELLE DES ASCOMYCÈTES. Botaniste 4: [21]–58, illus.
(4) Davidson, R. W.

(5) Dodge, B. O., and Swift, M. E.

(6) Elliott, J. A.

(7) Faull, J. H.

(8) Fraser, H. C. I.

(9) —— and Brooks, W. E. St. J.

(10) Griggs, R. F.

(11) Gwynne-Vaughan, H. C. I., and Williamson, H. S.

(12) —— and Williamson, H. S.

(13) —— and Williamson, H. S.

(14) Harper, R. A.

(15) ——

(16) Komocki, W.

(17) Leach, J. G.

(18) Loos, W.
1932. Über eine Buchenholzbewohnende Ceratostomella, Cerato-

(19) McCallum, B. D.

(20) Melin, E., and Nannfeldt, J. A.

(21) Mittmann, G.
1932. Kulturversuche mit Einsporstämmen und zytologische unter-

(22) Münch, E.

(23) Raymond, J. R.
(24) Sands, M. C.
1907. **Nuclear Structure and Spore Formation in Microsphaera Alni.**
Wis. Acad. Sci., Arts, and Letters, Trans. 15: [733]-752, illus.

(25) Schultz, E. S.
1927. **Nuclear Division and Spore-Formation in the Ascus of Peziza Domiciliana.**

(26) Varitchak, B.
1931. **Contribution à l'Étude du Développement des Ascomycètes.**