Somatic Nuclear Division in *Tilletia* Species Pathogenic on Wheat

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ABSTRACT

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Somatic nuclear division was studied in secondary sporidia and hyphae of *Tilletia caries*, *T. foetida*, and *T. controversa* by means of HCl-Giemsa and H_3PO_4-acetic orcein staining techniques. The morphology of division figures was determined by observing individual nuclei from different perspectives in cells suspended in glycerine. The chromatin in resting nuclei was granular to filamentous; one or more dark-staining bodies were observed near the periphery of the nucleus. During sporidia formation, most of the parent cell protoplast migrated into the developing sporidium concomitant with basipetal septation of the parent cell. In the bud cell, the nucleus contracted into an intensely staining spherical mass. The contracted nucleus was transversed by a linear, unstained area (the spindle) that usually was positioned eccentrically and oriented parallel to the long axis of the bud cell. Chromatin separating from the contracted nucleus was in the shape of a cylinder surrounding the unstained spindle. In side view, the chromatin appeared as two elongating parallel lines stretching out from the contracted configuration. During chromatin migration, most of the chromatin aggregated on opposing sides of the spindle forming two parallel lines which separated transversely as the chromatin aggregated at the poles. The spindle transversed the daughter nuclei giving them a bisected appearance in side view. In polar view, chromatin at the ends of division figures and the daughter nuclei were doughnut-shaped, with the unstained spindle occupying the hole in the doughnut. After division, one daughter nucleus returned to the parent cell and usually remained contracted; the other migrated to the distal end of the bud cell and entered an interphase-like condition. Septation occurred between the parent and daughter sporidium and then the parent sporidium usually senesced. The same division process was observed in monokaryotic and dikaryotic sporidia and hyphae of the three species studied. Chromosomes could not be counted, but our observations cast serious doubt on previous reports that $n = 2$ in these *Tilletia* spp.

Additional key words: bunt, cytology, smut, *Triticum aestivum*.

The genus *Tilletia* (the bunt fungi) includes some of the most destructive pathogens of cereal crops. Because they are of long-standing economic importance, the bunt fungi have been intensively investigated. Early studies of *Tilletia* spp., particularly those parasitizing wheat, established fundamental concepts that are part of the foundations of mycology and plant pathology. Continuing research on the bunt fungi has elucidated many aspects of their biology including life-histories, physiology, genetics, host-parasite relationships, and methods of control (9).

Despite numerous investigations of nuclear cycles in *Tilletia* spp. (9), there is little information on the details of nuclear division. Both Kharbush (15) and Wang (24) presented drawings of nuclear divisions in *T. caries* (DC.) Tul. (= *T. tritici*) as well as other smut species and described essentially classical mitosis involving a haploid chromosome number of two. These and other reports that $n = 2$ in the smut fungi (3,4,14,17) have been questioned (2,7,16,22). Moreover, recent cytological and genetical work with *Ustilago violacea* (6,7,18–20) has shown that somatic nuclear division in this species differs from classical higher eucaryotic mitosis in several respects and that previous reports that $n = 2$ are untenable.

Our knowledge of nuclear cytology of fungi has advanced greatly in recent years with the development of modern techniques of light and electron microscopy. Thus, it seems timely to reexamine nuclear phenomena in this important group of plant pathogens. Results of a study using light microscopy are reported here.

MATERIALS AND METHODS

Stocks and cultures. Teliospores of *T. caries* (race T-5) and *T. foetida* (Wallr.) Liro (race L-16) were collected from wheat inoculated and grown in field nurseries at Logan, Utah. Teliospores of *T. controversa* Kühn were collected from commercial wheat fields near the Blue Creek Experiment Station, Box Elder County, Utah.

Teliospores were germinated on soil extract (75 g of soil extracted with 500 ml of boiling deionized water per liter) agar (SEA) under continuous light. Those of *T. caries* and *T. foetida* were incubated at 15–17 C for 5–7 days; those of *T. controversa* were incubated at 5 C for 35–40 days.

Monokaryotic (haploid) cultures were obtained by isolating single primary sporidia from germinating teliospores onto potato-sucrose agar (PSA) with a micromanipulator. Each isolate was transferred to a PSA slant and incubated at 17 C for 2–3 wk. Monosporidial isolates varied in their propensity to produce secondary sporidia. Cultures of *T. caries* and *T. foetida* were predominantly sporidial, whereas those of *T. controversa* were predominantly mycelial.

Isolates that produced secondary sporidia most abundantly were selected for this study. Subcultures were grown at 17 C on PSA slants, or in shake culture in potato-sucrose broth, or Trione's chemically defined medium (23). In shake culture, filiform secondary sporidia (morphologically similar to primary sporidia) were produced in abundance. To prepare cultures for staining, a few drops of sporidial suspension were transferred onto a 1- to 1.5-mm-thick layer of PSA (1.5% agar) and incubated for 3–10 days at 5 C or 17 C. Under those conditions the filiform sporidia produced typical sickle-shaped secondary sporidia directly or hyphae from which sickle-shaped secondary sporidia were produced subsequently.

Nuclear division in the dikaryotic phase of *T. foetida* was examined in secondary sporidia that were produced following teliospore germination. For staining purposes, teliospores were germinated on a 1- to 1.5-mm-thick layer of SEA (1.5% agar).

Fixation. When petri-dish cultures were producing secondary sporidia abundantly, 4 × 5-mm agar blocks were removed and inverted onto microscope cover slips. When the HCl-Giemsa stain-

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ing technique was used, cover slips were first coated with Mayer's albumen adhesive; no adhesive was required with the H_3PO_4-acetic orcein staining procedure. The preparations were fixed by immersion of the agar-bearing cover slips in absolute ethanol-glacial acetic acid solution (3:1, v/v) for 10–20 min. After fixation, cover slips were rinsed in 95, 75, and 50% ethanol for 10–15 sec each and then rinsed in deionized water. The agar blocks still adhering to the cover slips were dried at room temperature or in an oven at 40 C. Drying insured adhesion of the cells to the cover slip and apparently did not distort the nuclei.

HCl-Giemsa hydrolysis and staining. Fixed and dried preparations were hydrolyzed in 5 N HCl at room temperature for 60 min, rinsed in three changes of deionized water for 1 min each, and briefly rinsed in 0.15 M phosphate buffer, pH 7.2. During rinsing the agar blocks floated away, leaving most of the cells attached to the cover slip. The cover slips were immersed in a mixture of four to eight drops of Giemsa stock solution (25) and 8 ml 0.15 M phosphate buffer, pH 7.2, for 15–30 min, rinsed briefly in deionized water, drained, and air-dried for 15 min. Drying at this step apparently set the stain in the nuclei rendering it less soluble during destaining. Preparations were destained in the buffer solution for 15–30 sec, rinsed briefly in deionized water, drained, and mounted in immersion oil on microscope slides. Slides prepared in this manner remained suitable for observation for up to 18 mo.

Phosphoric acid-acetic orcein hydrolysis and staining. Fixed and dried preparations were hydrolyzed in 40% H_3PO_4 at room temperature for 20–40 min (depending on the thickness of the agar), rinsed vigorously in three changes of deionized water, and drained. The H_3PO_4 dissolved the agar and all traces were removed by the rinses, leaving the fungal cells firmly attached to the cover slip. Cover slips were mounted in a drop of acetic orcein stain (2% orcein in 60% acetic acid) on microscope slides and, after the excess stain had evaporated, ringed with nail polish. Such preparations were usable for 1–2 days.

Stained cell suspensions. Cells prepared for viewing from different angles were stained with acetic orcein. After fixation and hydrolysis, a few drops of stain were placed on the cover slip which was then placed in a covered petri dish to prevent the stain from evaporating. After 15–30 min, the cover slip was drained of excess stain, sequentially rinsed in 50% acetic acid, 10% acetic acid, and deionized water for 15 sec each, and then drained. A drop of glycerine was carefully spread over the surface of the cover slip. After 20–30 min, the cells were loosened from the cover slip and suspended in the glycerine by gently brushing the surface with a fine sable brush.

To allow movement of the cells between the microscope slide and the cover slip, two parallel lines of fingernail polish were brushed on the surface of a microscope slide about the same distance apart as the width of the cover slip. When the nail polish was semi-dry, the cover slip with the cells was mounted on the slide so as to provide a uniform space between the slide and cover slip. The cover slip was ringed with nail polish.

When a cell in the desired stage of division was located with the microscope, a micromanipulator equipped with a glass needle was used to apply pressure on the cover slip. This caused the cell to change position and rotate in the glycerine. Cells could be manipulated into different positions with some precision enabling observation and photography of the same figure from different perspectives.

Photography. Preparations were photographed with a 35-mm camera mounted on a Leitz Orthoplan microscope equipped with an ×100 apochromatic objective (N.A. 1.32) and a green filter. Kodak Panatomic-X and Kodak High Contrast Copy films were used and were developed in Kodak Microdol-X. An extended development time of 12 min at 24 C was used with the Kodak High Contrast Copy film.

RESULTS

Similar results were obtained with both acetic orcein and Giemsa staining procedures. However, Giemsa tended to overstain the division figures which made details more difficult to observe. Consequently, acetic orcein was used in most of the study. Morphology of division figures was the same for the three species investigated; therefore, they were used interchangeably to describe and illustrate nuclear division and related phenomena.

Sporidial development and nuclear numbers. Development of secondary sporidia in the *Tilletia* spp. that were examined was similar to that described recently for *U. violacea* (7) and *Sorosporium consanguineum* (8). During sporidial budding, the cytoplasm and nucleus of the parent cell migrated into the developing bud. However, in *Tilletia*, the increase in volume of cytoplasm corresponding with bud development was small; instead, septations were formed progressively behind the migrating cytoplasm in the parent cell until the parent cell was nearly devoid of cytoplasm, except for a small amount in the end nearest the bud (Fig. 1–8).

The nucleus divided inside the bud cell, after which one daughter nucleus migrated back into the remaining cytoplasm in the parent cell (Fig. 9,10). A septum formed in the isthmus between the parent and bud cell and the newly formed sporidium was ejected forcibly. The parent cell (Fig. 4, bottom center) then usually senesced but occasionally produced a hypha. Whenever secondary sporidia were produced, whether on hyphae, on fused or unfused primary sporidia, or on other secondary sporidia, nuclear division invariably occurred inside the developing sporidia.

The number of nuclei in secondary sporidia was variable. Sporidia from monokaryotic cultures (monosporidial lines) usually contained a single nucleus but rarely contained two or three nuclei. In the dikaryophase, secondary sporidia usually contained two or three nuclei; but sporidia also were observed with as few as one and as many as 16 resting nuclei (Fig. 40).

Nuclear division. Resting nuclei in secondary sporidia were located near the middle of the cell, with the chromatin varying in appearance from granular (Fig. 1) to filamentous. Resting nuclei often consisted mostly of chromatin strands and appeared much like prophase nuclei in higher plants (Fig. 11,12). Usually one or more spherical or disk-shaped bodies were observed near the periphery of the nucleus (Fig. 1–4,10,13,29).

After the nucleus migrated into the developing bud, the chromatin (and apparently the entire nucleus) contracted into a compact, deeply staining mass that was usually positioned in the lower third of the bud cell nearest the parent cell (Fig. 14). In side view the contracted nucleus appeared to be evenly (Fig. 15) or unevenly (Fig. 16) bisected by a linear, unstained area oriented in a longitudinal direction in relation to the long axis of the bud cell. This unstained area, usually positioned eccentrically through the nucleus, is believed to represent an achromatic spindle. Polar views of nuclei at this stage showed that the spindle passed completely through the nucleus (Fig. 31B) rather than separating it into two portions as it appeared in side view (Fig. 31A). The image of a bisected nucleus in side view was created by the limited depth of field of the microscope which gives an "optical section" when focused at a median plane in the nucleus. It is uncertain whether the chromatin contracted around the spindle or whether the spindle was formed as the nucleus contracted. This stage was relatively long-lasting and is thought to be analogous to metaphase, although metaphase plates and chromosomes were not observed.

As mitosis progressed, the spindle gradually elongated taking the surrounding chromatin with it. During this process the chromatin appeared in side view as two elongating parallel lines extending out from the metaphase configuration (Fig. 17–20). The ends of the figure appeared bisected in side view. This stage may represent the beginning of anaphase and is called "anaphase" throughout this paper.

In cells suspended in glycerine that were rotated on their longitudinal axes during observation, the bisected appearance of the ends of early anaphase figures and the appearance of two parallel chromatin lines running the length of the figures were maintained throughout rotation (Fig. 30A–F). This revealed that the chromatin, as it pulled away in opposite directions from the metaphase configuration, was in the shape of a cylinder surrounding the achromatic spindle.

In polar views, the chromatin at the ends of anaphase figures was doughnut-shaped (Fig. 32B,D) in contrast to the bisected appearance of the ends in side view (Fig. 32A). The unstained spindle

represented the hole in the doughnut. The midportion of anaphase figures (the remaining metaphase chromatin) appeared in polar view as a spherical mass with the unstained spindle usually positioned eccentrically (Fig. 32C). In some polar views of anaphase figures the cylinder of chromatin could be followed from end to end. As the chromatin migrated along the spindle in opposite directions toward the poles (Fig. 21–24), the amount at the midportion of anaphase figures gradually decreased, whereas at the ends it gradually increased.

During mid- to late anaphase, as the spindle elongated, the chromatin (or at least most of it) gradually aggregated on opposing sides of the cylinder and formed two parallel lines on opposite sides of the spindle (Fig. 22,33A,34A). When the same cells (suspended in glycerine) were observed at an angle perpendicular to the first view, only a single line of chromatin was seen lying between the chromatin at the poles (Fig. 33B,34B). In polar views, the ends of the same division figures were oval, like an elongated doughnut (Fig. 34C), rather than round as seen at an earlier stage of anaphase (Fig. 32B). The length of the oval corresponded to the width of the incipient daughter nucleus as seen in side view. The development of two chromatin lines from a cylinder was not apparent in all dividing nuclei and the parallel lines were farther apart in some dividing nuclei than in others.

Occasionally, the ends of anaphase figures were at different dis-

Fig. 1–10. Budding and nuclear division in secondary sporidia originating from monosporidial cultures of *Tilletia foetida* (Fig. 1,2,3,6,7,9,10) and *T. controversa* (Fig. 4,5,8) stained with acetic orcein. Scale bar represents 20 μm. **1**, Resting nucleus in a secondary sporidium. Note prominent dark-stained body near the periphery of the nucleus. **2,3**, Nucleus migrating into the bud cell. **4–7**, Contraction and division of the nucleus in the bud cell. Most of the protoplast has migrated into the developing bud and septa have formed in the parent cell. **8**, Division completed; note bisected appearance of daughter nuclei. **9**, Migration of one daughter nucleus back into the parent cell. **10**, One relaxed nucleus in the bud cell and one nucleus still contracted in the parent cell. Only a small amount of cytoplasm remains in the end of the parent cell.

tances from the original metaphase site suggesting that one group of chromatids had migrated away from its sister chromatids, rather than each group migrating equal distances from the metaphase site. This left one developing daughter nucleus with most of its chromatin strung out along the spindle and the other with most of its chromatin already aggregated into a daughter nucleus (Fig. 21, 33A). Occasionally, distinct chromatin bodies were observed in mid- to late anaphase figures that may have represented chromosomes or aggregates of chromosomes (Fig. 24,42).

By late anaphase-early telophase most of the chromatin had migrated to the poles resulting in a transverse separation of the two parallel chromatin lines (Fig. 23). Before separation was complete, one or two thin, stained lines, often extremely attenuated, were observed between the daughter nuclei (Fig. 25,26).

In side view telophase nuclei usually appeared to be bisected (Fig. 27). This was thought to result from the presence of the unstained spindle through the daughter nucleus near the center, as was seen in polar views (Fig. 35).

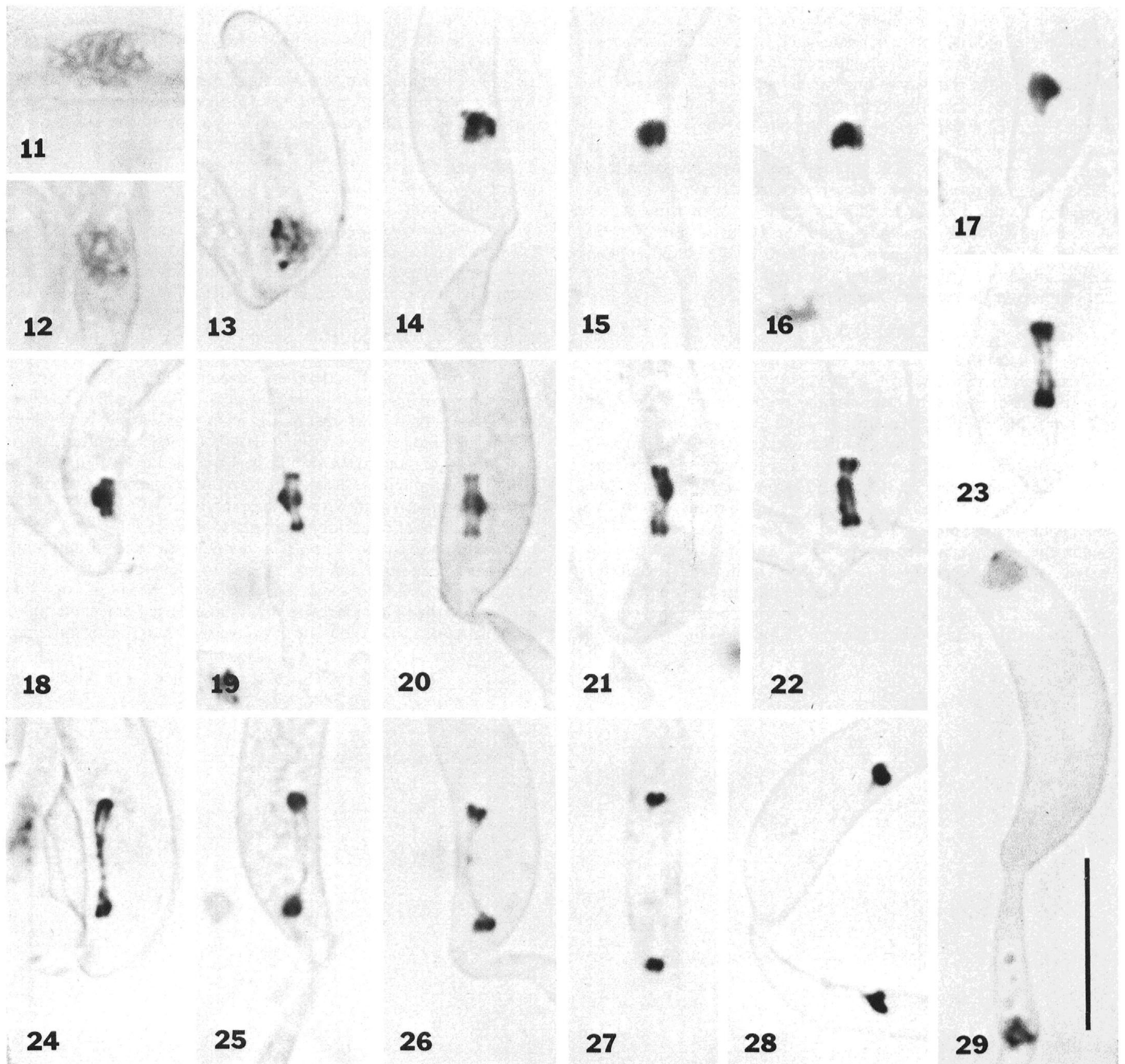

Fig. 11–29. Nuclear division in budding monokaryotic secondary sporidia of *Tilletia foetida* (Fig. 11,13,18–24,26,28,29), and *T. controversa* (Fig. 12,14,15,16,17,25,27) stained with acetic orcein. Scale bar represents 10 μm. **11,12,** Resting nuclei consisting of filamentous chromatin. **13,** Nucleus after migration into the bud cell; note the two prominent, dark-stained bodies. **14,** Nucleus contracting in bud cell. **15,16,** Contracted (metaphase) nuclei, each with an unstained linear area (the spindle). In Fig. 16 the spindle is positioned eccentrically (see Fig. 31-A,B). **17–20,** Early stages of chromatin separation (anaphase). The elongating spindle is surrounded by a cylinder of chromatin appearing in optical cross-section as two parallel lines in Fig. 19,20 (see Fig. 30-A–F). **21–24,** Chromatin migrating along the spindle. The chromatin has aggregated on opposite sides of the spindle in Fig. 22. Note distinct chromatin bodies (chromosomes?) in Fig. 24. **25,26,** Late anaphase-early telophase nuclei with attenuated chromatin between the developing daughter nuclei. The dark area on the attenuated chromatin in Fig. 26 is the remaining portion of the metaphase chromatin. **27,** Telophase; note the bisected appearance of the daughter nuclei (see Fig. 35). **28,** Migration of one daughter nucleus into the parent cell and the other to the distal end of the bud cell. **29,** Daughter nucleus in bud cell in an interphase-like stage with diffuse chromatin and prominent dark body; daughter nucleus in parent cell still somewhat contracted.

When division was completed, the two daughter nuclei moved away from the division site in opposite directions (Fig. 28). One nucleus returned to the remaining cytoplasm in the parent cell and usually remained in a contracted condition. The other nucleus migrated to the distal end of the bud cell and entered an interphase-like condition characterized by diffuse, evenly stained chromatin and a small, dark-stained body located near the periphery of the nucleus (Fig. 29).

In multinucleate sporidia, nuclear division occurred more or less synchronously, each nucleus dividing in the manner described above. One daughter nucleus of each resulting pair returned to the parent cell and the other remained in the bud cell (Fig. 36–39).

In secondary sporidia of the filiform type (Fig. 41), migration of the protoplast into the bud cell, nuclear division, and senescence of the parent cell took the same course as in typical sickle-shaped secondary sporidia. The same nuclear division stages observed in sporidia also were observed in hyphae (Fig. 42).

Estimation of the duration of division phases. For estimation of the relative time periods involved in different phases of nuclear division, the frequencies of metaphase, anaphase, and telophase figures were determined in monokaryotic secondary sporidia of *T. foetida*. The number of nuclei in each of the three division phases was recorded from 12 slides having numerous division figures. Of 527 division figures, 59% were in metaphase, 19% in anaphase, and 22% in telophase. This suggested that metaphase is the longest lasting phase in the division sequence.

DISCUSSION

Although details of somatic mitosis in *Tilletia* spp. are lacking in the literature, there are several early reports of meiotic and subsequent nuclear divisions in the spore and promycelium of *T. tritici* (= *T. caries*) (5,15,24). These authors each described nuclear divisions in the spore and promycelium as typical mitosis. Kharbush (15) also stated that nuclear divisions in secondary sporidia were mitotic. Kharbush (15) and Wang (24) both reported that the diploid nucleus contained four chromosomes that fused into two bivalents at meiotic metaphase. At anaphase of reduction division two chromosomes separated to each pole. Subsequent divisions in the spore and promycelium were described and drawn as occurring in the same manner. Essentially the same nuclear division process and a haploid chromosome number of two has been reported for numerous other smut species (3,4,14,17,24).

Despite the almost unanimous conclusion that $n = 2$ in the smut fungi, there remains considerable doubt whether division figures have been interpreted correctly (7,22) and whether chromosomes in the smut fungi have been observed at all (2,16). These doubts seem justified in the light of genetic studies with *U. violacea* (6) which on the basis of mapping by mitotic haploidization have shown a haploid chromosome number of at least 10 to 12 in this species. Although chromosomes could not be counted in our study, the division figures observed would be difficult to interpret on the basis of two chromosomes.

The division figures illustrated in the smut fungi in previous studies (3,4,7,14,24) resemble those observed in this study. Basically, a bisected-appearing metaphase structure divides transversely giving rise to bisected-appearing chromatin at the poles. Reports that $n = 2$ in the smut fungi may be due to misinterpretation of bisected appearing chromatin at metaphase and telophase as two chromosomes. Day and Jones (7) likewise explained previous reports that $n = 2$ in *Ustilago* species as a misinterpretation of the bilobed or "two-track" stage of mitosis in *U. violacea*.

Mitosis comparable to that described in this study has been observed in many different fungi, including *Aspergillus nidulans* (21), *Fusarium oxysporum* (1), *Tricothecium roseum* and *Cladobotryum variospermum* (12), *Puccinia* species (10,11), and *Uromyces phaseoli* var. *vigne* (13). These fungi do not exhibit a typical metaphase plate. Instead, the chromosomes at metaphase seemingly are arranged at various points around an elongated, intranuclear spindle. Development of this metaphase-equivalent stage was not observed in detail and is poorly understood.

Likewise, nothing resembling a typical metaphase plate was observed in *Tilletia* spp. Rather, development of the metaphase stage was observed as a gradual contraction of the nucleus resulting in an intensely stained chromatin mass transversed by a short, unstained spindle. No additional details could be resolved. Chromosome attachment to the spindle was assumed to be completed by this stage inasmuch as chromatin migration to the poles (anaphase) proceeded directly and without interruption.

Usually the chromatin in anaphase figures of *Tilletia* spp. appeared to migrate to the poles as a whole; discrete chromosomal units were not seen. This may be due to a combination of small chromosome size and close association of chromatids as they migrated to the poles. Occasionally, in anaphase figures, clumps of chromatin were observed that may have represented chromosomes,

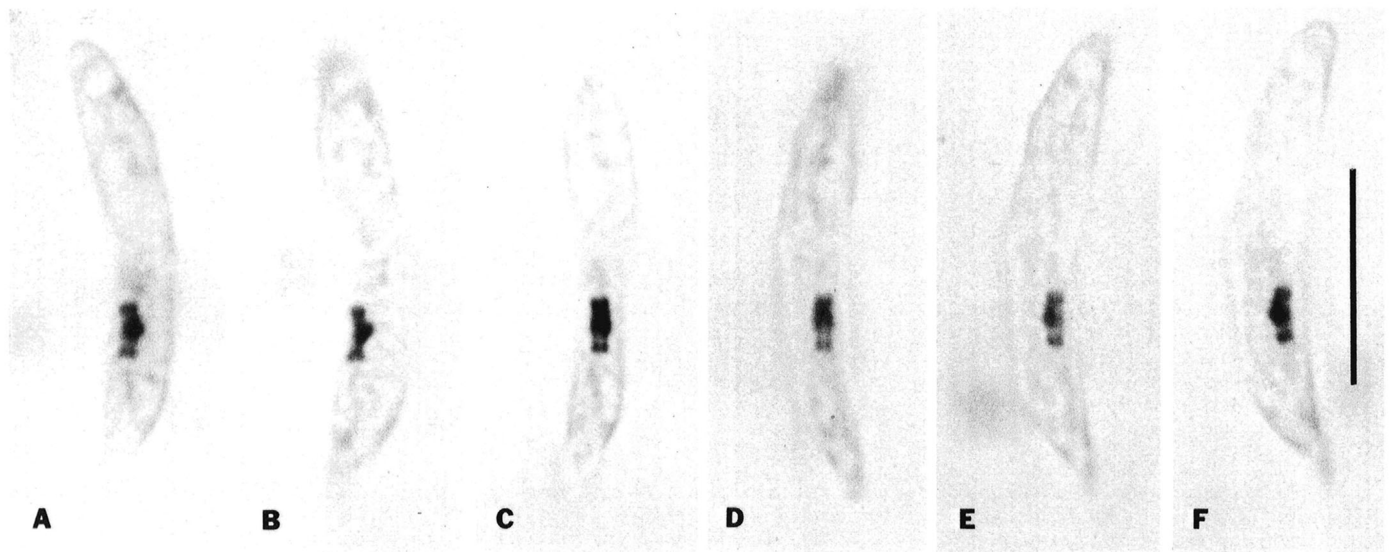

Fig. 30. A secondary sporidium of *Tilletia controversa* stained with acetic orcein and suspended in glycerine. The same anaphase figure photographed as the sporidium was rotated clockwise 180 degrees on its longitudinal axis. The appearance of two parallel lines of chromatin and the bisected appearance of the chromatin at the ends of the figure is maintained throughout the rotation. This shows that the chromatin constitutes a cylinder which surrounds the unstained, eccentrically-positioned, spindle. Scale bar represents 10 μm. **A,** The midportion of the figure (the remaining metaphase chromatin) is above and slightly to the right of the chromatin cylinder. **B,C,D,** As the sporidium is rotated, the midportion of the figure is positioned directly beneath the chromatin cylinder giving the figure a symmetrical appearance. **E,F,** With further rotation, the midportion appears below and to the left of the chromatin cylinder.

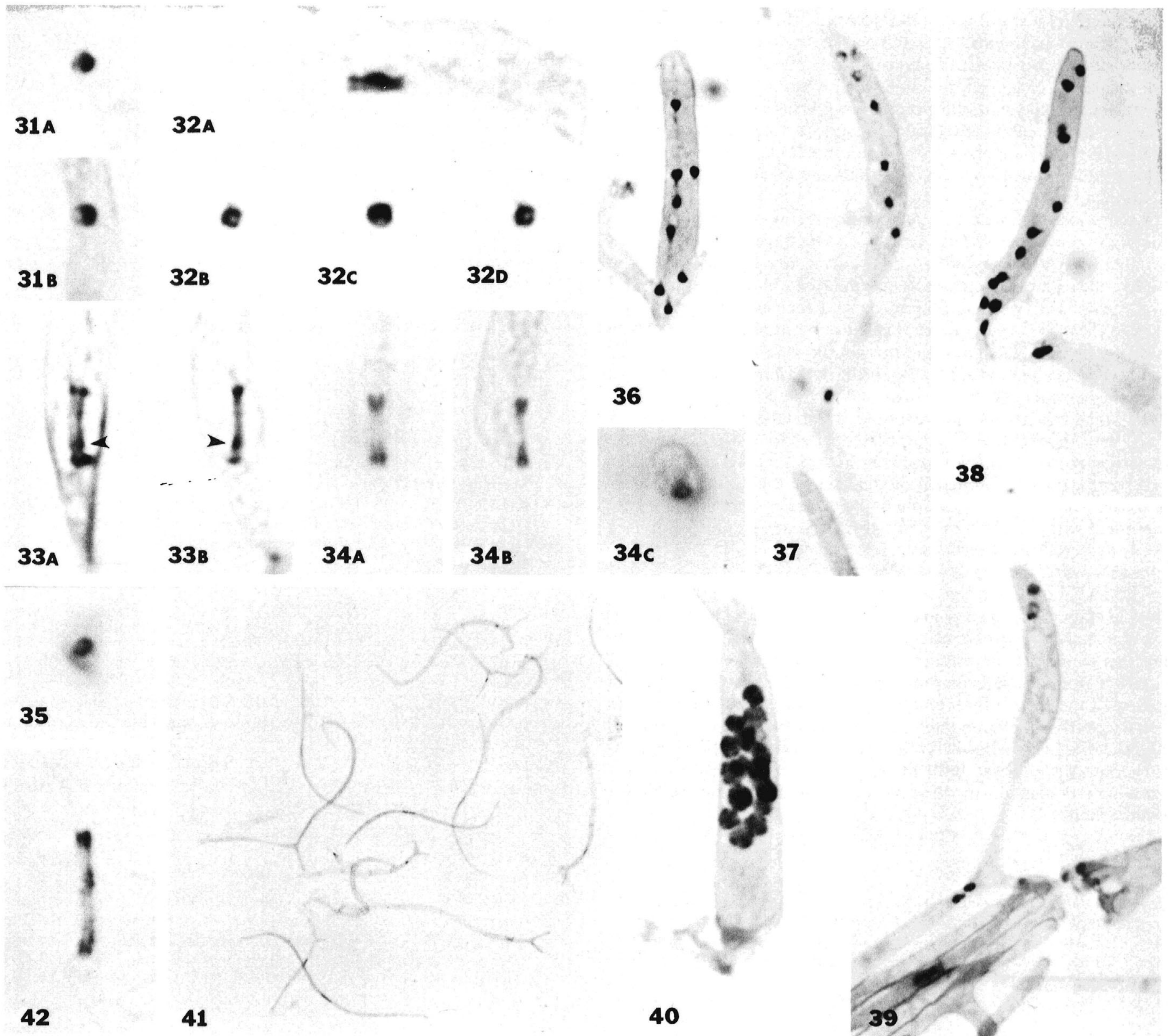

Fig. 31–42. (Fig. 31–35) Secondary sporidia of *Tilletia controversa* stained with acetic orcein and suspended in glycerine. (Fig. 31-A,B) Side and polar views of the same nucleus at metaphase (×2,960). **31-A,** Side view showing an unstained area (the spindle) eccentrically transversing the nucleus. **31-B,** Polar view showing the spindle as a round unstained area. (Fig. 32-A–D) Side and polar views of the same anaphase figure (×3,560). **32-A,** Side view. The right end of the figure is slightly out of focus. **32-B,** Polar view of the left end of the division figure showing doughnut-shaped chromatin surrounding the unstained spindle. **32-C,** Polar view of the midportion of the division figure. The spindle is positioned eccentrically. **32-D,** Polar view of the right end of the division figure showing essentially the same structure as in B. (Fig. 33-A,B) Different side views of the same anaphase figure. The dark area in the figures (arrows) is the remaining metaphase chromatin (×2,960). **33-A,** At this stage most of the chromatin is arranged on opposite sides of the spindle. The ends of the sporidium are pointing upward. **33-B,** Side view after rotating the sporidium on its longitudinal axis 90 degrees clockwise from 33-A. The two parallel chromatin lines now appear as a single line. The extreme lower end of the division figure is below the plane of focus but still appeared bisected in this view. (Fig. 34-A–C) Side and polar views of the same anaphase figure at a later stage than in Fig. 33-A, B (×2,810). **34-A,** Side view. Most of the chromatin has migrated to the poles but a small amount is on opposite sides of the unstained spindle. The ends of the sporidium are pointing upward. **34-B,** Side view after rotating the sporidium on its longitudinal axis 90 degrees clockwise from A. A single line of chromatin lies between the ends of the figure, which appear slightly bisected. **34-C,** Polar view of the upper end of the figure. The incipient daughter nucleus is seen as an oval mass of chromatin with an unstained area (the spindle) near the center. The length of the oval corresponds to the width of the incipient daughter nucleus as seen in A. **35,** Polar view of a telophase nucleus similar to one shown in side view in Fig. 27 (×2,960). (Fig. 36–40). Multinucleate secondary sporidia originating from dikaryons of *T. foetida* stained with Giemsa (Fig. 36–39, ×1,650; 40, ×2,250). **36,** Early telophase nuclei resulting from the synchronous division of four nuclei. **37,** A stage after the division of four nuclei before the migration of half the daughter nuclei back to the parent cell. Three of the four nuclei in the distal end of the sporidium have begun to enter interphase; one nucleus has migrated into the parent cell. **38,** A secondary sporidium with 18 telophase nuclei (not all in focus) resulting from the synchronous division of nine nuclei. Typically, one of each pair of daughter nuclei returns to the parent cell. **39,** Completed nuclear division in a secondary sporidium formed on a fused pair of primary sporidia (H-body). Two daughter nuclei have migrated back to the H-body. **40,** Fourteen nuclei in a secondary sporidium. **41,** Filiform monokaryotic secondary sporidia of *T. controversa* grown in shake culture and stained with Giemsa (×350). **42,** An anaphase figure in monokaryotic hypha of *T. foetida* stained with acetic orcein (×2,960).

although this was not proven.

The stage at which chromatids separate is unknown. The general morphology of division figures suggested that chromatids separate asynchronously as they migrated away from the contracted metaphase configuration. It is possible, however, that chromatid separation did not occur until after the chromatin became arranged on either side of the spindle. The mechanism or reason for the apparent formation of two parallel lines of chromatin from a chromatin cylinder at some variable point during anaphase is obscure. A similar phenomenon ("two-track" stage) has been observed in late metaphase-early anaphase in *A. nidulans* (21), late anaphase-early telophase in *Puccinia* sp. (11) and in a metaphase-like stage in *U. violacea* (7).

Except for the linear spindle and the lack of a metaphase plate, the division cycle in *Tilletia* spp. appears to have the same characteristics of division stages that occur during conventional mitosis in higher eucaryotes. Therefore, the terms "metaphase", "anaphase", and "telophase" were used here where it seemed appropriate. We realize, however, that such terms must be used with reservation until further knowledge of the division process is obtained.

The significance or function of the dark-staining bodies observed near the periphery of the nucleus in nondividing nuclei is unknown. We assume that at least one represented a spindle-pole body. In any case, they appeared to be incorporated with the rest of the nucleus as it contracted into the deeply stained metaphase configuration and were not seen again until the nucleus entered interphase after division. Although neither spindle-pole bodies nor nucleoli were identified with certainty in our light microscope studies, both have been observed in interphase nuclei using electron microscopy (authors *unpublished*).

The technique for observing the same cell in different perspectives in stained cell suspensions was extremely useful to distinguish the true morphology of division figures from optical artifacts. Without this technique (or without extensive examination of serial sections) it would have been difficult to demonstrate the cylindrical nature of the chromatin in mid-anaphase figures and to distinguish this stage from the later stage when most of the chromatin formed two parallel lines. Although this technique was useful for observation, photography was difficult because the cells usually did not remain motionless during the relatively long exposures required for micrographs.

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