PHYLOGENY AND ULTRASTRUCTURE IN
EOCRONTARTIUM MUSCICOLA: MEIOSIS AND BASIDIAL DEVELOPMENT

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ABSTRACT

Eocrontartium muscicola (Fr.) Fitz., a heterobasidiomycetous moss parasite, has been hypothesized to occupy a pivotal taxonomic position between the Uredinales and the simple-septate auricularioid phytoparasites occurring on archeaic hosts. Sporulating fruitbodies were fixed in the field for transmission electron microscopy, and epifluorescence microscopy was used to preselect representative nuclei in precise meiotic stages with which secondary characters could be correlated. Ultrastructural features of nuclear division, spindle pole body (SPB), septa and patterns of basidial development were analyzed in E. muscicola and compared to findings in the Uredinales, Auriculaires sensu stricto, and other simple-septate Heterobasidiomycetes. The septal pore apparatus in E. muscicola is similar to that found in the Uredinales, differing significantly from the Auriculaires sensu stricto. However, ultrastructural details of meiosis, such as the SPB and its relation to the nuclear envelope during division, the presence of endoplasmic reticulum caps at the spindle poles, nucleolus behavior and wall breakage phenomena associated with vegetative branch initiation serve to distinguish E. muscicola from the Uredinales. The host-parasite interface and the phylogenetic antiquity of the mosses parasitized are discussed in relation to rust evolution.

Key Words: Eocrontartium, Uredinales, ultrastructure, nuclear division, phylogeny.

Eocrontartium muscicola (Fr.) Fitz., Auriculaires sensu lato, is a simple-septate heterobasidiomycete found parasitizing numerous temperate moss genera (Boehm and McLaughlin, 1988). It has figured prominently in the phylogenies of lower basidiomycetes, especially with regard to the origin of the Uredinales (Hennem and Burtt, 1980; Hiratsuka and Sato, 1982; Khan and Kimbrough, 1980, 1982; Lepik, 1955; Oberwinkler, 1982). The presence of hyaline thin-walled probasidia (Stanley, 1940) germinating without dormancy to yield rust-like metabasidia, the simple septum with the characteristic pulley-wheel pore occlusion, seemingly transitional between the Uredinales and the Septobasidiales (Khan and Kimbrough, 1982), germination by repetition and the presumed phylogenetic antiquity of the host group, suggest that E. muscicola occupies a pivotal taxonomic position between the primitive Uredinales and the simple-septate auricularioid phytoparasites.

The present study was initiated to clarify the relationship of E. muscicola to the Uredinales, Auriculaires sensu stricto, and the simple-septate ballistosporic auricularioid taxa. Specifically, E. muscicola was analyzed to determine whether it can be accommodated in the Uredinales, as proposed by Jülich (1982) and Hennem and Burttica (1980). Ultrastructural features of nuclear division and the associated spindle pole body (SPB) seem to be highly conserved, suggesting that they are sufficiently stable to be useful for judging natural relationships at the higher taxonomic levels (Heath, 1978, 1980b, 1986; Kubai, 1978). SPB morphology has been characterized for some members of the Uredinales (Heath and Heath, 1976; O'Donnell and McLaughlin, 1981a-c), Auriculaires sensu stricto (McLaughlin, 1981), and for two genera of the simple-septate auricularioid Heterobasidiomycetes (Bourret and McLaughlin, 1986; McLaughlin, 1987). Ultrastructural features of the SPB and its relation to the nuclear envelope during meiosis, nucleolar behavior, septal fine structure, wall breakage phenomena, and patterns of basidial development have been analyzed for E. muscicola and suggest that E. muscicola and allied organisms are distinct from the Uredinales.

MATERIALS AND METHODS

Infected moss gametophytes of Haplocladium microphyllum (Hedw.) Broth., Pylaisiella poly-
antha (Hedw.) Grout and Climacium dendroides (Hedw.) Web. and Mohr, bearing sporulating basidioecars of Ere. muscicola, were collected near Dam Lake, Aitkin Co., and from Cedar Creek Natural History Area, Anoka Co., Minnesota. Vouchers are deposited in MIN (DJM #333, 334, 339, 340; EWB #045, 046).

Fungal fruitbodies were excised from the moss host and chemically fixed in the field with 2% glutaraldehyde in a 0.1 M sodium cacodylate buffer, pH 7.2, 20 C for 8 h. Fixed basidioecars were then macerated in the laboratory and teased into fine hyphal fragments bearing developing probasidia and metabasidia on a microscope slide in the primary fixative. Acetone-cleaned, No. 2 coverslips (Corning Glass Works, Corning, New Jersey) were sprayed four times with Crown dry film lubricant (Crown Industrial Products Co., Hebron, Illinois), wiped free of excess lubricant between coatings and coated with poly-L-lysine (P1524, Sigma Chemical Co., St. Louis, Missouri). The hyphal fragments were transferred to the poly-L-lysine-coated coverslip, briefly dipped in 0.5% water agar at 40 C and transferred to Columbia staining jars (A. H. Thomas Co., Philadelphia, Pennsylvania) containing the primary fixative. Coverslips were prepared for epifluorescence microscopy by inverting them, cell side down, onto depression microscope slides containing a 0.1 mg/ml solution of mithramycin (Sigma Chemical Co., St. Louis, Missouri) with 15 mM MgCl₂·6H₂O in 0.1 M sodium cacodylate buffer (Bourett and McLaughlin, 1986; Heath, 1980b). Coverslips were sealed to the depression slides with parafilm to retard excess evaporation.

Probasidial nuclei in pachytene of prophase I and metabasidial nuclei in later divisional phases were selected with a Zeiss Photomicroscope III using a 100 W mercury lamp, appropriate filters (BP 450–490 excitor filter, LP 520 barrier filter, and FT 510 dichroic mirror), and a Zeiss Planapochromat 40×/1.0 n.a. oil immersion objective. Selected nuclei were marked on the coverslip using a diamond scribe objective. Low magnification phase contrast (for mapping purposes) and higher magnification epifluorescence micrographs were taken with Kodak Tri-X Pan film. Only basidia whose longitudinal axes were parallel to the plane of the coverslip and near the surface of the agar film were selected.

Following nuclear selection, the coverslips were returned to the Columbia staining jars and postfixed with 1% OsO₄ in a 0.1 M sodium cacodylate buffer for 4 h at room temperature, rinsed with distilled water and en bloc stained overnight in 1% uranyl acetate in the dark. Dehydration was via a graded acetone series and the cells were infiltrated with Quetol (Abad et al., 1988; Fujita et al., 1977). The coverslips were flat embedded, in a modification of the technique of O'Donnell and McLaughlin (1981a), by inverting them onto teflon-coated sheets of mylar and placing a lead weight on them to insure a flat, thin mount. The resin was polymerized for 18 h at 74 C. The coverslips were easily removed from the mylar by carefully peeling the sheets at a 45 degree angle. Selected nuclei were retrieved from the resin wafer by relocating the scribe mark on the coverslip, and resubmerging the resin. Hyphae of interest were excised from the coverslip and mounted cell-side up on blank resin blocks with a cyanoacrylate glue (Super Drop, Ormsteen Chemicals, Seabrook, New Hampshire). The block face was then trimmed to a trapezoid of 0.1 mm² whose upper and lower edges were coated with molten tackwax to insure the adhesion of serial sections. Complete serial sections of each preselected nucleus were obtained using a Reichert-Jung Ultracut E microtome, harvested from the water surface using 2 × 1 mm single-slot grids and placed onto carbon-coated formvar film supported by a perforated aluminum platform (Rowley and Moran, 1975). Sections were stained in 5% uranyl acetate followed by 0.2% lead citrate and examined with a Hitachi H-600 transmission electron microscope operated at 75 kV.

Efforts to grow Ere. muscicola in axenic culture were attempted by suspending sporulating fungal fruitbodies over 2% water agar, PDA (Difco Laboratories, Detroit, Michigan) and MYP media (Bandoni and Johri, 1972). An even spore deposit was obtained by turning the Petri dish lid every 20 min and incubating at 25 C with alternating periods of light and dark.

RESULTS

Eocronartium muscicola fruits in the field for a short time in early summer. Attempts to culture it were unsuccessful, necessitating the use of field-collected material. The limited supply of basidioecars has restricted the number of stages that could be analyzed ultrastructurally and the preparative methods that could be used. Fungal fruitbodies were processed for epifluorescence microscopy so that nuclei could be preselected at precise meiotic stages prior to serial sectioning.
Epifluorescence microscopy of mithramycin-stained nuclei provided useful secondary characters, such as metabasidial length, which could be correlated with specific divisional phases. Metabasidia in metaphase I and II measured 55 μm and 75 μm, respectively. Metabasidial length permitted the use of phase contrast microscopy to select embedded metabasidia in known meiotic phases.

The meiotic cycle in *E. muscicola* is illustrated in Figs. 1–5. Karyogamy through late pachytene–early diplotene of meiotic prophase I occurs in the probasidium (Figs. 1, 10–13) while subsequent phases of meiosis are completed in the metabasidium (Figs. 2–5, 16, 18). Throughout metabasidial maturation and consequent sporulation the probasidium remains attached to the metabasidium as a vacuolated compartment (white arrowheads, Figs. 2–6). The probasidium in *E. muscicola* is a globose, thin-walled apical cell measuring 22 μm in length in which two nuclei fuse to initiate the meiotic cycle (Figs. 1, 10, 11). FIGURES 7–9 represent post-meiotic developmental stages and will be discussed later.

Following karyogamy the probasidium germinates to form the metabasidium (Figs. 1, 2, 11, 12, 13). This involves the rupture of the thin outer wall of the probasidium at its apex where a new inner wall is laid down (Fig. 11, insert). The fusion nucleus, however, does not immediately migrate into the developing metabasidium, but delays in the probasidium as it completes the assembly and disassembly of synaptonemal complexes characteristic of zygotene (Fig. 11), pachytene (Fig. 12) and diplotene (Fig. 13) of meiotic prophase I. Each end of the paired homologues forming the synaptonemal complex, is attached to the nuclear envelope except for the nucleolar organizing region which is immersed in the nucleolus (not illustrated). Recombination nodules, indicative of chiasmata formation, and chromosome-nucleus envelope attachment sites can be discerned (Fig. 14). The synaptonemal complex is composed of two lateral elements, each side of which supports the chromatin of the two sister chromatids, separated by a ladder-like central element (Fig. 15).

Separation of the homologues initiates diplotene and occurs well after metabasidial emergence, concomitant with nuclear migration from the probasidium (Fig. 13) into the metabasidium (Figs. 2, 16). Condensed chromatin is discernible but all traces of synaptonemal complexes are gone (Fig. 16). Typically, the migrating late prophase nucleus is polarized into a leading chromatin-containing (karyokinetic) region and a trailing nucleolus-containing (nucleolar) region (Fig. 16), although the nucleolus does not always assume a distal position (Fig. 2).

Eight prophase SPBs of *E. muscicola* were analyzed during the zygotene–pachytene transition in the probasidium, prior to the exiting of the fusion nucleus. The SPB was situated upon the nuclear envelope, often in a lateral orientation with respect to the long axis of the probasidium, and consisted of a pair of layered disks connected by an osmiophilic middle piece (Fig. 17). The middle piece measured approximately 110 × 40

Figs. 1–11. Developing probasidia and metabasidia of *Eocronartium muscicola*. 1–5. Epifluorescence micrographs of mithramycin-stained nuclei illustrating the meiotic cycle. 1. Germinating globose probasidium with developing metabasidium (arrowheads). Fusion nucleus (arrow) is in meiotic prophase I. The probasidium remains attached throughout metabasidial maturation (white arrowheads in Figs. 2–5), ×1550. 2. Late prophase I nucleus (arrow) migrating into the developing metabasidium. Note evacuating probasidium (arrowhead), ×950. 3. Metaphase I nucleus (arrow) showing chromatin aggregated along the spindle, ×650. 4. Metaphase II nuclei (arrows) in singly-septate metabasidium, ×1100. 5. Non-sequential sterigmal initiation in mature transversely-septate auricularid metabasidium. Note nuclei migrating into the developing sterigmata, ×650. 6. Phase contrast micrograph illustrating non-sequential sterigmal initiation, supernumerary metabasidial compartments and the vacuolated thin-walled attached metabasidium (arrowhead), ×850. 7. Micrograph using oblique illumination showing a sporulating metabasidium containing seven compartments, not all of which initiate sterigmata, ×650. 8, 9. Phase and epifluorescence micrographs of the same basidiospore prior to its discharge. Note adventitious septum delimiting spiculum from the vacuolated metabasidial compartment (arrowhead). One of the mitotic nuclei remains in the basidiospore while the other descends into the spiculum (arrows), ×1600. 10, 11. Transmission electron micrographs (TEM) of developing probasidia in *E. muscicola*. 10. Prekaryogamy probasidium with two compatible nuclei (N) subtended by a developing vegetative branch (arrow), ×6500. 11. Postkaryogamy probasidium containing the fusion nucleus (N) in zygotene of meiotic prophase I. Note the initiation of synaptonemal complexes (arrowheads) and germinating apex (arrows), ×5500. Insert: Close-up of same probasidial apex illustrating breakage of the thin outer wall (arrowheads) during metabasidial emergence, ×22,500.
nm (length × width). Surrounding the cytoplasmic side of the prophase SPB was a ribosome-free zone in which could be detected cytoplasmic microtubules (Fig. 17). Heterochromatin was seen to associate with the SPB on the inner nuclear envelope in all prophase nuclei analyzed (Fig. 17). An intranuclear element (McLaughlin, 1981) was not observed subtending the prophase SPB but was present in interphase (Fig. 24). The two SPB disks presumably separate during late prophase (prometaphase) to initiate the metaphase spindle; however, this stage was not observed.

Metaphase I is initiated as the fusion nucleus approaches the midregion of the metabasidium (Figs. 3, 18). At mid-metaphase there is a well developed central spindle with condensed chromatin arranged along its equatorial periphery (Figs. 18, 19). The mean pole-to-pole measurement, based on five metaphase nuclei examined in medial section, was 14 μm. Late metaphase-early anaphase I is characterized by the poleward movement of separated homologues. It is during late metaphase through telophase that the SPB disks achieve their largest diameter, measuring approximately 110 nm. Stratification of the disks is also most pronounced at this time (Figs. 20, 21). Three distinct layers can be discerned at late telophase I (Fig. 21), although additional layers may be present. A distinct perforated cap of endoplasmic reticulum (ER) was observed from mid-metaphase I through anaphase and telophase I (Figs. 20, 21). The ER cap encloses the layered SPB at each pole and extends downward along the flanks of the fenestrated nuclear envelope (Fig. 20). A clear separation exists between the ER cap, the layered SPB disk and the nuclear gap in which the latter resides (Figs. 20, 21). Astral microtubules emanate from the cytoplasmic side of the metaphase-telophase I SPB, passing through perforations in the ER cap (Fig. 21). During late telophase I a dissolution of the ER cap was observed along with a greater population of astral microtubules as the chromatin aggregated at the poles (Figs. 21). Metaphase II appears to be entered semisimultaneously by the two sibling nuclei as they reach the midregion of the two metabasidial compartments (Fig. 4). Following meiosis and septal maturation the characteristic transversely separte auricularioid metabolasidum can be resolved (Figs. 5, 6, 7, 22).

Sterigmal initiation in E. muscicola was nonsequential, and basidial compartments showed no centripetal or basipetal pattern of vacuolation (Figs. 5, 6, 7, 22). In some instances, basidial compartments were completely vacuolated, having discharged their basidiospores, prior to the initiation of sterigmata in neighboring compartments. Metabolasidum containing more than four compartments were routinely observed (Figs. 6, 7). The development of sterigmata in E. muscicola involves the rupture of the outer metabasidial wall (Figs. 22, 23). Each metabasidial compartment behaves in an independent fashion during sterigma initiation due to the occluded and walled-over septal pore plug (Fig. 32) which effectively disrupts any symplyastic continuum.

A post-meiotic mitosis occurs during basidiosporogenesis to yield two nuclei, one of which remains in the basidiospore while the other descends into the sterigma (Fig. 9). An adventitious septum is laid down separating the spiculum from the vacuolated metabasidial compartment (Fig. 8). Discharged basidiospores germinate either by the production of secondary ballistospores (Figs. 25, 26) or germ tubes (Figs. 27, 28). Axenic cul-

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Figs. 12, 13. TEM of germinating probasidia (PB) and developing metabasidium (MB) in E. austroamericana. 12. Probasidium containing fusion nucleus in pachytenie of meiotic prophase I. Note synaptonemal complexes (arrows) and metabasidium emergence (arrowheads), ×5200. 13. Fusion nucleus (N) in diploplane of meiotic prophase I migrating out of the probasidium and into the metabasidium. Note absence of synaptonemal complexes, ×4500. Fig. 14. TEM of probasidial fusion nucleus in pachytenie containing synapsed homologues showing recombination nodule (open arrow), indicative of chiasmata formation, and a nuclear envelope attachment site (dark arrow), ×13,500. Fig. 15. TEM of synaptonemal complex illustrating the two lateral elements (arrowheads), each supporting two sister chromatids (C), and separated by a central ladder-like element (white arrow), ×39,500. Fig. 16. TEM of developing metabasidium containing migrating late diploplane nucleus (N) showing distal nucleolar region harboring the nucleolus (Nu) and condensed chromatin (arrows). Note the absence of synaptonemal complexes, ×9300. Fig. 17. TEM of prophase I spindle pole body (SPB) during the zygotene-pachytene transition, prior to exiting of the fusion nucleus from the probasidium. The SPB is situated on the nuclear envelope (NE) and consists of a pair of layered disks separated by a middle piece (MP). Heterochromatin (C) is seen to associate with the SPB. A ribosome-free zone (arrowheads) surrounds the cytoplasmic face of the SPB. Note laterally associated cytoplasmic microtubules (arrow), ×80,000.
ture of mono- and polysporous isolates of *E. muscicola* proved unsuccessful, yielding only simple, unbranched germ tubes (Fig. 27) which degenerated after 6 days.

The development of somatic dikaryotic branches in *E. muscicola* involves rupture of the outer wall layers (Fig. 29). This was studied primarily in the dikaryotic vegetative branch which subtends each apical probasidium (Figs. 10, 11, 29). This branch does not itself become another probasidium and may represent a hyphidium.

Septa in *E. muscicola* (Figs. 30–32) are simple with a single pore surrounded by an abruptly flattened narrow rim. Characteristic osmiophilic pulley-wheel pore occlusions develop soon after septal maturation and are present in vegetative (Fig. 30) and probasidial (Fig. 31) septal pores. An organelle-free zone, delimited by microbodies, surrounds each side of the septal pore in vegetative and probasidial septa (Figs. 29–31). A wall develops over occluded pores, evidenced by serial sections, in mature metabasidial septa (Fig. 32), further insuring the individuality of each metabasidial compartment.

**DISCUSSION**

Studies on ultrastructural features of nuclear division and the associated SPB (Bourett and McLaughlin, 1986; Heath, 1978, 1986; Heath and Heath, 1976; McLaughlin, 1987; O'Donnell and McLaughlin, 1981a–c, 1984a, b), as well as septal morphology (Khan and Kimbrough, 1982), are becoming increasingly important as indicators of fungal phylogeny. These apparently conserved cellular events, such as meiosis and mitosis, imply a degree of evolutionary stability useful for judging natural relationships (Heath, 1978, 1980b, 1986; Kubai, 1978). Heath (1980a) has established 18 characters to compare nuclear division and SPB behavior amongst the lower eukaryotes. However, due to their general uniformity within a division, they offer little assistance for understanding the basidiomycetes. Certain ultrastructural features (Bourett and McLaughlin, 1986) and possibly patterns of basidial development (McLaughlin, 1982; Oberwinkler, 1982; O'Donnell and McLaughlin, 1984b), however, may provide the necessary variation to permit delimiting of some orders within the basidiomycetes.

**SPB substructure and behavior.**—Details of nuclear division and SPB ultrastructure have served to separate the ascomycetes from the basidiomycetes (Heath, 1978, 1986; Wells, 1977). The Uredinales, however, share many features with the ascomycetes, such as the intranuclear spindle composed of a central bundle of non-kinetochore microtubules and the discoidal SPBs, which become inserted in a close-fitting pore in the nuclear envelope during division (Ashton and Moens, 1979; Heath, 1978; Heath and Heath, 1976; O'Donnell and McLaughlin, 1981c).

Several SPB types have been reported for the basidiomycetes (Heath, 1980a). The discoid SPBs found in the rusts and simple-septate auricularioid basidiomycetes differ significantly from the nonstratified subspherical or ellipsoidal SPB in *Auricularia fuscosuccinea* (Mont.) Farl. (McLaughlin, 1981) and the Homobasidiomycetes (Heath, 1978; McLaughlin, 1971; Thielke, 1982b). This reinforces the view, based on septal ultrastructure, that the Auriculariales *sensu lato* are heterogeneous, composed of at least two very disparate groups (Bandoni, 1984; Khan and Kimbrough, 1982; McLaughlin, 1980, 1981).

The prophase SPB in *E. muscicola* closely resembles that found in *Helicobasidium mompa* Tanaka (Bourett and McLaughlin, 1986) and *Helicogloea* sp. (McLaughlin, 1987). The prophase SPB in all three simple-septate auricularioid fungi so far analyzed share certain features with the Uredinales and ascomycetes, namely, a...
SPB consisting of two stratified disks separated by an osmiophilic middle piece. As the two disks separate in late prophase (prometaphase) to initiate the spindle, the middle piece is rapidly lost in *E. muscicola*, while in *Puccinia malvacearum* Bert. ex Mont. (O'Donnell and McLaughlin, 1981a) it is retained until mid-metaphase. Late in division the SPB disks achieve their largest size and are composed of four layers in *P. malvacearum* (O'Donnell and McLaughlin, 1981c) and five in *H. mompa* (Bourett and McLaughlin, 1986). Due to the lack of a precisely medial section through the telophase SPB in *E. muscicola* and insufficient numbers of telophase nuclei analyzed, an unequivocal count could not be made.

During interphase and prophase a ribosome-free zone surrounds the cytoplasmic side of each SPB disk in *E. muscicola*, a general feature reported for many ascomycetes and basidiomycetes (Heath, 1978). As in mitotic interphase-prophase in *H. mompa* (Bourett and McLaughlin, 1986) and *Helicogloea* sp. (McLaughlin, 1987), the interphase-prophase I SPB in *E. muscicola* was not associated with multivesicular bodies, unlike the SPB in *P. malvacearum* (O'Donnell and McLaughlin, 1981c). However, during later stages of nuclear division multivesicular bodies are seen to surround the spindle poles in all three simple-septate auricularioid taxa as in the rusts and other basidiomycetes (Heath, 1978; O'Donnell and McLaughlin, 1981b).

**Nuclear envelope and ER cap.** —The nuclear envelope in the simple-septate ballistosporic auricularioid fungi, rusts and ascomycetes remains intact throughout division, with the exception of polar fenestrae (Bourett and McLaughlin, 1986; Heath, 1978; McLaughlin, 1987; O'Donnell and McLaughlin, 1981c), whereas the nuclear envelope in the Homobasidiomycetes is quite labile, displaying discontinuities during division (Wells, 1977; Heath, 1978, 1986; Thielke, 1982b). The degree of association between the interphase-prophase SPB and the nuclear envelope seems variable in the basidiomycetes. In the Uredinales and the simple-septate auricularioid fungi the disks are intimately associated with the nuclear envelope (Bourett and McLaughlin, 1986; O'Donnell and McLaughlin, 1981a), while a looser association exists with the globoid SPB in more advanced Heterobasidiomycetes (McLaughlin, 1981; Taylor, 1985) and the Homo-basidiomycetes (Girbardt, 1971; McLaughlin, 1971).

Kubai (1978) has presented a plausible argument for the evolution of the fungal spindle. In the rusts and ascomycetes, SPB disk separation and spindle initiation are nuclear envelope-associated phenomena, whereas in the more advanced heterobasidiomycetous and homobasidiomycetous groups SPB separation and spindle initiation appear to be independent of the envelope, occurring in the cytoplasm. The simple-septate ballistosporic auricularioid taxa (E. muscicola, H. mompa and *Helicogloea* sp.) seem somewhat intermediate in that disk separation and spindle initiation appear to be associated with the envelope, as in the ascomycetes and rusts, but to a lesser degree (Bourett and McLaughlin, 1986; McLaughlin, 1987).

During mid-metaphase a characteristic ER cap develops over each spindle pole, enclosing the SPBs, in *E. muscicola*, *H. mompa* (Bourett and McLaughlin, 1986) and *Helicogloea* sp. (McLaughlin, 1987). This structure appears to be unique to these simple-septate ballistosporic auricularioid basidiomycetes. The ER cap is not
continuous with the nuclear envelope, merely overlapping it at each pole. As division proceeds the ER cap becomes increasingly perforate, allowing astral microtubules to pass through until its dissolution in late telophase.

ER caps have been reported in the Homobasidiomycetes (Girbardt, 1968; Settliff et al., 1974; Thielke, 1982a), but these are continuous with the nuclear envelope (Bourett and McLaughlin, 1986). The metaphase SPB in the Homobasidiomycetes and in some advanced Heterobasidiomycetes (Heath, 1978; Taylor, 1985; Thielke, 1982a) sits beyond the plane of the nuclear envelope in a pocketed region delimited by the ER cap. This is unlike the rusts and simple-septate aauricularioid fungi in which the SPB resides in a tight or loose association, respectively, within the fenestrated nuclear envelope (Bourett and McLaughlin, 1986; McLaughlin, 1987; O'Donnell and McLaughlin, 1981c).

**Nucleolus.** — It is not clear whether the nucleolus in *E. muscicola* is pocketed throughout the later stages of division, as in *H. mompa* (Bourett and McLaughlin, 1986), or whether it is dispersed as in the Uredinales (O'Donnell and McLaughlin, 1981a). However, during prophase, concomitant with nuclear migration into the metabasidium, the fusion nucleus becomes polarized into nucleolar and karyokinetic regions, as in *H. mompa* (Bourett and McLaughlin, 1986), a feature less pronounced in the rusts and absent in the ascomycetes (Bourett and McLaughlin, 1986; Heath, 1978; Taylor and Wells, 1979; Wells, 1977).

**Wall and septa.** — A notable feature which further serves to separate the simple-septate auricularioid taxa from the rusts involves wall-breakage phenomena. As in *H. mompa* (Bourett and McLaughlin, 1986), *Helicogloeas* sp. (McLaughlin, unpubl.) and *E. muscicola* initiate vegetative branches by breakage of the outer hyphal wall. Similar wall breakage events during branch initiation have been observed in some members of the Atractiellales (McLaughlin, 1987). This has not been recorded in the Uredinales except in urediosporogenesis (Harder, 1984; Littlefield and Heath, 1979).

Sterigmal initiation in *E. muscicola* involves rupture of the outer metabasidial wall and has been documented for *A. fuscouscinea* (McLaughlin, 1980) and *Ustilago maydis* (DC.) Corda (O'Donnell and McLaughlin, 1984b). This feature may represent a convergent pattern of development. However, unlike *A. fuscouscinea* (McLaughlin, 1981), metabasidial maturation in *E. muscicola* and the rusts (McLaughlin and O'Donnell, 1981) displays no sequential pattern of basidial vacuolation. Each metabasidial compartment behaves independently, presumably due to the walling off of the septal pores between compartments. Such asynchrony in basidial development has been interpreted as a 'primitive feature (McLaughlin et al., 1985). The maturation of the metabasidium in *E. muscicola* appears to be independent of the subtending hyphae, being separated by a vacuolated probasidium. This is reminiscent of metabasidial maturation in the advanced rusts, where the teliospore functions in dissemination (Savile, 1979).

In *A. fuscouscinea* (McLaughlin, 1980) metabasidial maturation is dependent on the subtending hyphae and a symplastic continuum exists via non-occluded metabasidial septa.

Sterigmal septa in *E. muscicola* resemble those reported for rusts (see O'Donnell and McLaughlin, 1984b). They also occur in some simple-septate auricularioid basidiomycetes (Talbot, 1968). Secondary septa occur in metabasidia of *E. muscicola* and have been reported in other orders (O'Donnell and McLaughlin, 1948b).

**Septal ultrastructure.** — Septal ultrastructure in *E. muscicola* differs from earlier reports (Khan and

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**Fig. 29.** Wall breakage (arrowheads) associated with vegetative branch (VG) initiation in *Eoeronotum muscicola.* An apical probasidium (PB) is associated with a subtending dikaryotic vegetative branch. Note organelle-free zones delimited by microbodies (arrows), ×22,000. Figs. 30–32. TEM of the septal pore apparatus in *E. muscicola.* 30. Near medial section through a mature vegetative septum illustrating the organelle-free zone (Z), delimited by microbodies (Mb), surrounding each side of the pore (P), ×38,000. 31. Medial section through a mature septum subtending the probasidium (PB). An omniloph-style pulley-wheel pore occlusion (P) is present as are organelle-free zones (Z) delimited by microbodies (Mb). Note the abruptly flattened rim which surrounds the pore, ×40,000. 32. Medial section through a mature metabasidial septum. The upper compartment is vacuolated while the lower compartment shows a wall deposition over the pore plug (P), ×44,000.
Kimbrorough, 1980, 1982) in the presence of an organelle-free zone surrounding the vegetative and probasidial septal pores. The single-pored septum in *E. muscicola* is abruptly flattened to a narrow rim surrounding the pore and possesses an osmiophilic pulley-wheel pore occlusion soon after maturity. Roughly similar septa characterize the simple-septate basidiomycetous orders, *i.e.*, Septobasidiales (Dykstra, 1974), Uredinales (Harder, 1984; Littlefield and Bracker, 1971), Atractiellales (Oberwinkler and Bandoni, 1982a, b) and the simple-septate ballistosporic auricularioid basidiomycetes (Bandoni, 1984; Bourett and McLaughlin, 1986; Khan and Kimbrough, 1980; Oberwinkler and Bandoni, 1982a). Caution should be exercised, however, in interpreting septal data and especially in advancing phylogenetic deductions based solely on the septal pore apparatus. For instance, according to Khan and Kimbrough (1982) the septa in *E. muscicola* are intermediate between the Septobasidiales, which apparently lack microbodies, and rust septa, which possess organelle-free zones. Several septal types have been analyzed in *E. muscicola*—vegetative, probasidial and metabasidial—and, depending upon their stage of maturation, microbodies, zones of exclusion and pulley-wheel pore occlusions may or may not be present. The septa found in *H. mompa* (Bourett and McLaughlin, 1986) and *Helicogloea* sp. (McLaughlin, 1987) are similar to *E. muscicola* and all three share features with the rusts.

**Phylogenetic considerations.—** Persoon (1801), Karsten (1868) and Fries (1821, 1874) were the first to make reference to a simple clavarioid hymenomycete parasitic upon moss gametophytes. However, it was Atkinson (1902) who first described the transversely septate metabasidium and established *Eocronaria* atk. to stress the superficial resemblance to the telial horn found in the uredinalean genus *Cronaria*. An extensive study of the life history and cytology led Fitzpatrick (1918a, b) to establish *E. muscicola* (Fr.) Fitz. and to reemphasize the pivotal taxonomic position this fungus occupies between the Uredinales and the phytoparasitic Auriculariales on archaic host groups. Numerous authors have since adhered to the view according to which *E. muscicola* is believed to be an extant representative of the ancestral complex postulated to have given rise to the Uredinales (Dietel, 1928; Gaumann, 1964; Hennen and Burtica, 1980; Hiratsuka and Sato, 1980; Khan and Kimbrough, 1980, 1982; Leppik, 1955; Oberwinkler, 1982).

However, it has remained problematic to postulate the progenitors of the extant primitive fern-rusts in the Pucciniales (Uredinopsis, *Milesina, Hylaloporsa*) due to their complex heteroecious and macrocyclic life cycles (Hiratsuka and Sato, 1982). Autoecism and microcyclic reductions are considered derived from the primitive multisporic life cycle (Jackson, 1931). Although the puccinialstraceous rusts on archaic hosts display the most complex life cycle known, it is obvious that rust evolution must, at some time, have progressed from the simple to the complex (Arthur, 1929; Jackson, 1931). To resolve this apparent contradiction, Hennen and Burtica (1980) have suggested that simple septate "autoecious auricularioid rusts" (*Eocronarium, Jola, Herpobasidium, Platycarpa*), parasitic on mosses and ferns, represent archaic rust lineages with originally unexpanded life cycles, having never acquired the auxiliary anamorphs and hosts characteristic of the puccinialstraceous rusts. No examples of uredinioid, aeciod or pycniod spore states or alternate hosts have been reported for these simple-septate auricularioid fungi, although anamorphs are known (Kendrick and Watling, 1979). Jülich (1982) has established the *Eocronariaceae*, in the *Uredinales*, to accommodate *E. muscicola*; however, this proposal is based on insufficient evidence and has been severely criticized (Bandoni, 1984; Boehm and McLaughlin, 1988). Savile (1955, 1976) has presented a plausible argument for the derivation of the basidiomycetes from the ascomycetes via a *Taphrina*-like precursor. The Uredinales seem to be intermediate between the two divisions, possessing such ascomycete features as the simple septum (Khan and Kimbrough, 1982), similar ultrastructural aspects of nuclear division and SPB (Heath, 1978, 1986) and conidiogeny (Hughes, 1970; Savile, 1979), while clearly basidiomycetous in the metabasidium and ballistosporic discharge mechanism (Mc Laughlin et al., 1985).

Interestingly, details of nuclear division in the rusts are very homogeneous, from *Uredinopsis* and *Coleosporium* (McLaughlin, unpub.) to *Puccinia* (O'Donnell and McLaughlin, 1981c) and *Uromyces* (Heath and Heath, 1976). This may reflect the stable environment afforded by the host (Heath, 1986; Savile, 1976). The looser nu-
clear envelope-SPB association reported for the simple-septate auricularioid fungi (Bourett and McLaughlin, 1986) suggests either that they are more recent than the rusts or that nuclear division is continuing to evolve in an archaic group. It is not yet clear whether a hiatus exists between nuclear division patterns in the rusts and the simple-septate auricularioid fungi or whether a continuum will be found. Too few taxa have been studied. However, the three genera analyzed to date, Helicobasidium (Bourett and McLaughlin, 1986), Helicogloea (McLaughlin, 1987) and Eocronartium, on dicotyledons, wood and mosses, respectively, show wide substrate preferences.

The simple-septate auricularioid fungi encompass many nutritional modes from saprotrophy to biotrophy. Eocronartium muscicola, unlike the rusts, does not produce haustoria but, rather, makes use of gametophytic archegonial nutritional sinks via the exploitation of the host transfer cell apparatus (Boehm and McLaughlin, 1988). This mode of biotrophy is unlike Herpobasidium and Platycarpa, auricularioid fern parasites, which produce elongate coiled hyphae (Oberwinkler and Bandoni, 1984) resembling those found in the Septobasidiaceae (Couch, 1938) and the pucciniastreaceous rusts (Moss, 1926).

Much emphasis has been placed on the relative age of the host as reflective of the age of the parasite and vice versa (Ando, 1984; Anikster and Wahl, 1979; Bennell and Henderson, 1985; Leppik, 1955, 1967; Savile, 1968, 1971a, b, 1975), presumably because initial ingress and establishment of the parasite occurs early in the evolution of the host at a time of great genetic plasticity (M. C. Heath, 1986). The simple-septate auricularioid moss parasites (Eocronartium and Jola) have continually occupied a basal position in the phylogenies of the Heterobasidiomycetes due, primarily, to the presumed phylogenetic antiquity of the host group (Dietel, 1928; Hennen and Buritsica, 1980; Fitzpatrick, 1918a; Leppik, 1955; Oberwinkler, 1982; Savile, 1955; Stanley, 1940).

All of the moss hosts reported for E. muscicola (Boehm and McLaughlin, 1988) are in the Bryales, subclass Bryidae, with no representatives in the more primitive Polystichales and Tetraphidales. The first undisputed fossils attributable to the Bryidae occur in the Permian, but these form genera cannot be assigned to any extant groups (Vitt, 1984). Fossils resembling extant genera and species, however, appear in the Tertiary (Miller, 1980), suggesting a Cretaceous radiation for the modern Bryidae, concomitant with the angiosperm expansion (J. A. P. Janssens, pers. comm.; Vitt, 1984).

Of the 21 reported moss hosts for E. muscicola, 14 are in the Hypninae, 4 in the Leucodontinae, 2 in the Fissidentinae and 1 in the Pottiinae (Boehm and McLaughlin, 1988). The Hypninae and Leucodontinae are believed to have evolved with the break-up of Laurasia and Gondwanaland, respectively, during the Jurassic-Cretaceous transition, while the Fissidentinae and, to a lesser extent, the Pottiinae represent somewhat older lineages (Vitt, 1984). If Savile (1955) and Leppik (1973) are correct in assuming the precursors of the pucciniastreaceous fern-fir rusts to have evolved in the lower Carboniferous, on extinct Marattiales, then E. muscicola may be quite recent in comparison. The Osmundaceae, supporting extant species of Uredinopsis, are believed to have arisen from the Marattiales during the Permian with modern representatives becoming established by the late Mesozoic–early Cenozoic (R. M. Lloyd, pers. comm.). It is therefore erroneous to consider E. muscicola archaic simply because it parasitizes mosses.

What phylogenetic deductions can be drawn from the ultrastructural data on nuclear division and basidial development in E. muscicola? Features of the septal pore apparatus apparently point to a close relationship with the Uredinales, especially since zones of exclusion, which previously separated uredinalean septa from E. muscicola (Khan and Kimbrough, 1982) are reported here. However, despite septal similarities, which are shared by other heterobasidiomycetous groups, the details of nuclear division (including the SPB, and its relation to the nuclear envelope during division), ER cap association, nucleolus behavior and wall breakage phenomena distinguish E. muscicola from the rusts. These same characteristics have been used to distinguish H. mompa and Helicogloea sp. from the rusts (Bourett and McLaughlin, 1986; McLaughlin, 1987).

The Auriculariales Schroeter emend. Bandoni (Bandoni, 1984) presently exclude the simple-septate auricularioid taxa (Eocronartium, Helicobasidium, Helicogloea, Herpobasidium, Insolebiasidium, Jola, Mycogloea, Neotyphula, Paraphelaria, Platycarpa, Platygloea, Pitechetelium, Xenogloea). Simple septa have been recorded for all taxa except Cystobasidium, Platycarpa and Pitechetelium (Bourett and McLaughlin, 1986;
Khan and Kimbrough, 1982; McLaughlin, unpubl.; Oberwinkler and Bandoni, 1982a); however, it is not clear whether they represent a homogeneous group (Bandoni, 1984). The Cystobasidiaceae was established by Gaumann (1926) to accommodate those auricularioid taxa possessing a thin- to thick-walled probasidium; however, Helicobasidium was retained in the Auriculariaceae because, like Auricularia, it did not possess a definite probasidium (Gaumann, 1964). The Cystobasidiaceae Gaumann has been proposed as a temporary repository for the simple-septate auricularioid fungi until sufficient evidence has accumulated to unite or segregate these organisms into a separate order or orders distinct from the Auriculariales Schroeter emend. Bandoni (Boehm and McLaughlin, 1988; Oberwinkler and Bandoni, 1982a).

In summary, E. muscicola cannot be accommodated in the Uredinales, as proposed by Jülich (1982) and Hennem and Burratica (1980), based on intrinsic differences in the mode of biotrophy (Boehm and McLaughlin, 1988) and ultrastructural aspects of nuclear division and branching. Eocronartium muscicola shares enough similarities with H. mompa and Helicogloeoida sp. to suggest that a distinct group of simple-septate ballistosporic auricularioid Heterobasidiomycetes is beginning to emerge, distinct from the Uredinales and the Auriculariales sensu stricto. Currently, there is no existing order which can satisfactorily accommodate these organisms (Bandoni, 1984); however, it is premature to establish a new heterobasidiomycetous order until more taxa have been analyzed.

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