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# THE SECOTIOID FORM OF *LENTINUS TIGRINUS*: GENETICS AND DEVELOPMENT OF A FUNGAL MORPHOLOGICAL INNOVATION[1]

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Secotioid fungi resemble gasteromycetes, but are presumably closely related to agaricoid fungi. *Lentinus tigrinus* is a wood-decaying mushroom that has both a secotioid and an agaricoid form. We examined ontogeny and heritability of the secotioid phenotype in *L. tigrinus* with a combination of formal genetic crosses, scanning electron microscopy, and macroscopic observation of cultured sporocarps. For F1 analysis, we crossed single-spore isolates (SSIs) representing four mating types derived from a secotioid dikaryon and four mating types from an agaricoid dikaryon. All F1 sporocarps had typical agaricoid morphology. For F2 analysis, 200 SSIs from one F1 sporocarp and 100 SSIs from another F1 sporocarp were backcrossed to tester SSIs from sporocarps produced by the parental secotioid dikaryon. Ratios of secotioid to agaricoid dikaryons thus produced were 47:49 and 84:109, which confirms previous reports that the secotioid phenotype is conferred by a recessive allele at a single locus ($\chi^2 = 0.0417$, $P > 0.05$, $\chi^2 = 3.2383$, $P > 0.05$, respectively). Early ontogeny of the secotioid form is indistinguishable from that of the agaricoid form. Later, the hymenophore is obscured by a weft of hyphae that proliferates from the margins of the developing lamellae. Longevity of the sporocarps and rate and duration of sporocarp growth are approximately equal in the secotioid and agaricoid forms. Developmental evolution of the secotioid form is interpreted as an example of von Baerian differentiation, rather than paedomorphosis, which has been implicated in evolution of other secotioid taxa.

Mushrooms are produced in diverse groups of basidiomycetes and appear to be elegantly adapted to their sole function of aerial spore dispersal. In some families of predominantly mushroom-forming basidiomycetes there are also gasteromycete-like secotioid species that do not form exposed gills or tubes, but rather produce their spores internally on contorted lamellar plates or in locules. Despite their macromorphological differences, close relationships between certain secotioid and agaricoid (mushroomlike) fungi have been proposed on the basis of anatomical features (e.g., Thiers, 1984; Singer, 1986). Polyphyletic derivation of secotioid fungi from diverse hymenomycete ancestors has been argued on the basis of morphological characters and parsimony (Thiers, 1984). Recently, molecular phylogenetic studies have supported the view that certain secotioid taxa have been derived from within the Boletales (Bruns et al., 1989; Baura, Szaro, and Bruns, 1992), Tricholomataceae (Pine and Mueller, 1993), and Coprinaceae (Hopple, 1990). Nevertheless, some authors have maintained that secotioid fungi are a plesiomorphic, paraphyletic group from which various lineages of hymenomycetes have been independently derived (e.g., Singer, 1951, 1986). Regardless of their polarity, secotioid-agaricoid transformations certainly represent major innovations in fungal morphology, with potentially profound influences on spore dispersal, breeding systems, and ecology. In this paper, we examine the developmental and genetic bases of the secotioid phenotype in the mushroom, *Lentinus tigrinus* (Bull.:Fr.) Fr.

*Lentinus tigrinus* is a common wood-decaying basidiomycete that typically has toothed, decurrent lamellae, and a white spore print. *Lentinus tigrinus* has a broad, generally Laurasian distribution, but also extends to the tropics in both the New and Old World (Corner, 1981; Pegler, 1983; Redhead, 1988). In addition to the typical agaricoid form, there is also a secotioid form that occurs throughout central and eastern North America (Fig. 1). The secotioid form has a membranous weft of hyphae, previously termed a veil (Lyman, 1907), that encloses the hymenophore, which may become contorted to varying degrees. The secotioid and agaricoid forms are otherwise anatomically very similar. Indeed, some mycologists may object to calling the atypical, veiled form of *L. tigrinus* secotioid on the grounds that it has curved sterigmata and forcible spore discharge, whereas secotioid fungi in the strict sense lack forcible spore discharge. Nevertheless, for lack of a better term we will continue to call this fungus secotioid.

The secotioid form was first noted by Lea (1849) who identified the unusual collections as *L. tigrinus,* despite their enclosed, contorted lamellae. Berkeley (1845, p. 302) examined Lea's American collections and agreed that they represented "a very curious, but monstrous state of our European species." Morgan (1895, p. 37) considered the secotioid form to be a "normal production," rather than a monster, and therefore erected a new genus for the secotioid form which he named *Lentodium squamulosum* Morgan. Lyman (1907) and Snell (1923) upheld Morgan's concept of *L. squamulosum* on the grounds that the secotioid form could be regularly produced from spores in

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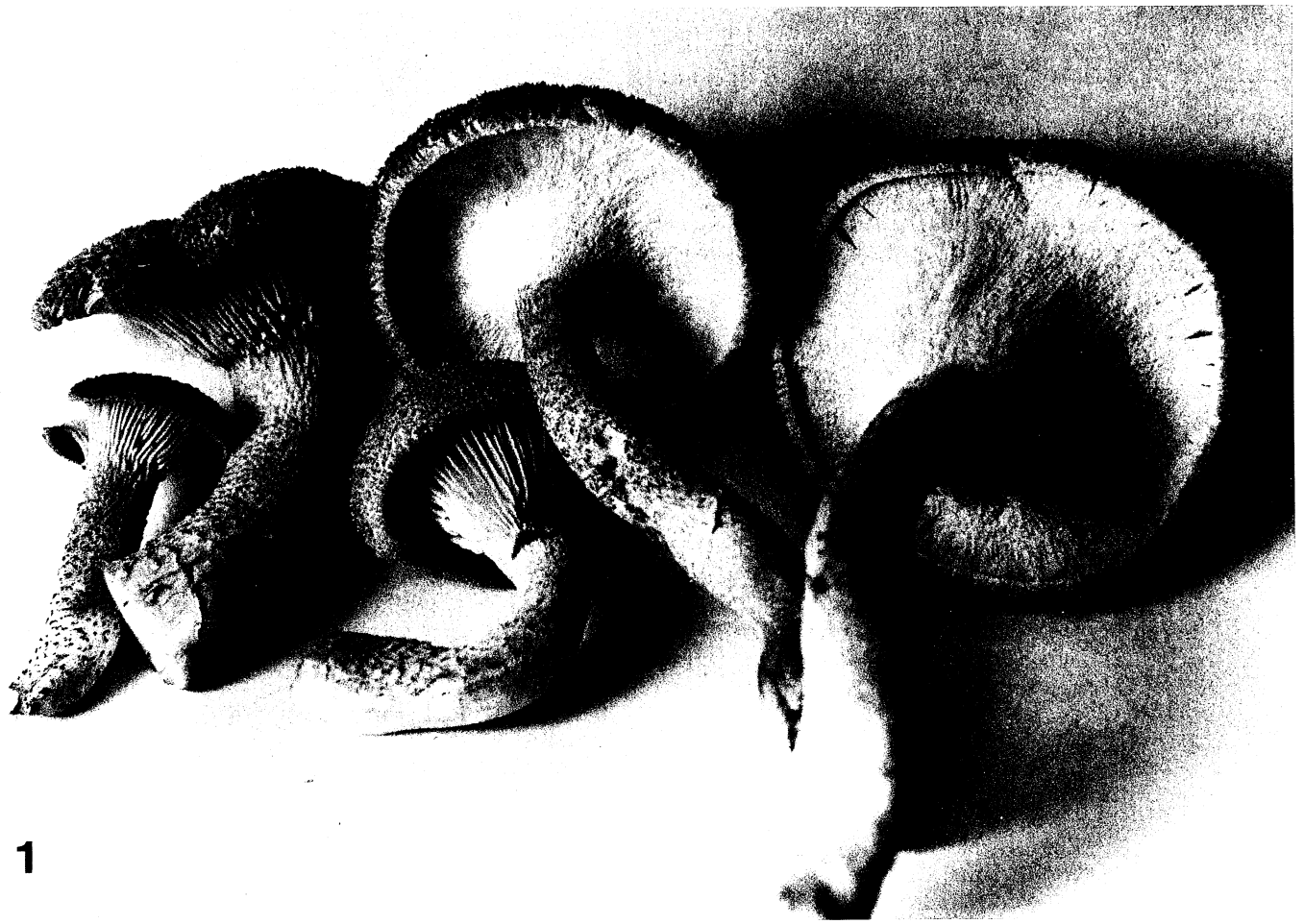


Fig. 1. Cultured agaricoid (left) and secotioid (right) sporocarps of *L. tigrinus* isolates D.744 and D.787, respectively. Note the radial slits in the veil of the larger secotioid sporocarp. Approximately twice natural size.

culture. This view was challenged by Harper (1921) who suggested that the weft of hyphae that distinguished *Lentodium* was the result of attack by a parasite such as *Hypomyces*.

The dispute over *Lentodium* was largely settled by Rosinski and Robinson (1968) who demonstrated that single-spore isolates (SSIs) of *L. squamulosum* and *L. tigrinus* were mating-compatible and that the F1 sporocarps thus produced had typical lamellate hymenophores. On the basis of these results, Rosinski and Robinson proposed that the secotioid form be recognized as *Panus tigrinus* Fr. var. *squamulosus* (Morgan) Rosinski and Robinson. (Placement of *L. tigrinus* in *Panus* Fr. follows Singer [1986, and earlier editions].) Later, Rosinski and Faro (abstract, 1968) reported F2 backcrosses that suggested that the secotioid morphology in *L. tigrinus* was controlled by a recessive allele at a single locus. Citing these observations, Pegler, in his monograph of *Lentinus* (1983), placed *P. tigrinus* var. *squamulosus* under synonymy with *L. tigrinus*.

Because it fruits easily in culture, *L. tigrinus* has been popular not only for genetic studies, but also for studies on sporocarp physiology and development (e.g., Faro, 1972; Bobbitt and Crang, 1974, 1975; Hibbett, Murakami, and Tsuneda, 1993a). In the present study, our primary goal was to confirm and expand upon the results of Rosinski and Robinson (1968, 1969) and Rosinski and Faro (1968) regarding the genetic basis of the secotioid phenotype. We also were interested in reconsidering ontogeny of the secotioid form in terms of evolutionary modifications to the developmental program of the typical agaricoid form of *L. tigrinus*. Our previous observations of sporocarp development in *L. tigrinus* and other *Lentinus* species (Hibbett, Murakami, and Tsuneda, 1993a) provide a comparative basis for interpreting derivation of the secotioid phenotype. The typical form of *L. tigrinus* is gymnocarpic (Bobbitt and Crang, 1975; Hibbett, Murakami, and Tsuneda, 1993a; contrary to Kühner, 1925, and Pegler, 1983). In other words, the lamellae form on the surface of the sporocarp and are never enclosed by veils or other protective structures (Watling, 1985). Implicit in the present study is the assumption that the secotioid form is derived from the gymnocarpic, agaricoid form in *Lentinus*. This premise is supported by previous molecular phylogenetic studies in which the gymnocarpic polypore, *Polyporus arcularius* Batsch:Fr., has been shown to be a close outgroup candidate to *Lentinus* (Hibbett and Vilgalys, 1991, 1993).

TABLE 1. Wild *L. tigrinus* dikaryons used in this study.

| Isolate number[a] | Hymenophore type | Geographic origin |
|---|---|---|
| D.787 | Secotioid | North Carolina |
| D.744 | Agaricoid | North Carolina |
| DAOM 54158 | Secotioid | Michigan |

[a] D-isolates were obtained from Dr. Rytas Vilgalys, Department of Botany, Duke University, Durham, North Carolina. DAOM isolate was obtained from Dr. Scott Redhead, Biosystematics Research Centre, Ottawa, Ontario. All isolates have been deposited in the culture collection of the Tottori Mycological Institute.

## MATERIALS AND METHODS

***Culturing and developmental morphology***—The three dikaryotic isolates of *L. tigrinus* used in this study (Table 1) and voucher sporocarps produced in culture are deposited at the Tottori Mycological Institute (TMI). Isolates DAOM-54158 and D.787 were derived from secotioid sporocarps, and isolate D.744 was derived from an agaricoid sporocarp. Culturing and fruiting procedures were essentially the same as those that we previously reported for *Lentinus* (Hibbett, Murakami, and Tsuneda, 1993a). Sporocarps were produced either in a moist chamber in the laboratory at ambient light and temperature conditions (approximately 25–30 C), or in a mushroom fruiting room with a 12-hour fluorescent light cycle. Occasionally, a second flush was produced by scraping the spawn blocks clean after the first fruiting and replacing them in the fruiting areas.

Scanning electron microscopy (SEM) was used to observe developmental morphology of sporocarps from the three original dikaryotic isolates, following the same protocols for sample preparation, observation, and photography that we used previously for *Lentinus* and *Panus* Fr. (Hibbett, Murakami, and Tsuneda, 1993a, b). Sporocarps produced in the crossing studies were examined macroscopically. We also examined a total of 71 herbarium collections of the secotioid form of *L. tigrinus* from the holdings at MICH, NY, FLAS, WTU, SFSU, and FH (collection data are available on request).

***Genetic crosses***—*Lentinus tigrinus* has previously been reported to have a tetrapolar mating system (mating ability is determined by two unlinked loci; Nobles, 1965; Rosinski and Robinson, 1968). To obtain monokaryons of all four mating types, up to 25 SSIs from cultured sporocarps of D.787 and D.744 were crossed in all possible intrastrain combinations on MEA (1.25% malt-extract, 2.0% agar) plates and scored for clamp connections under light microscopy.

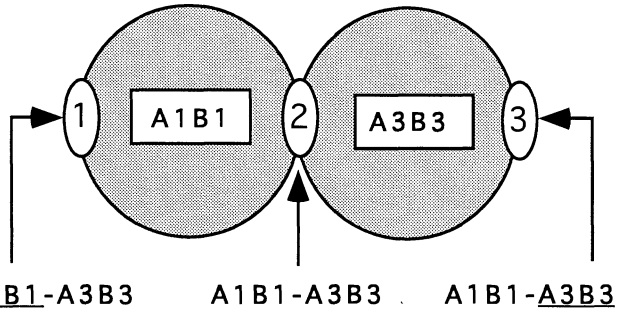


Fig. 2. Diagram of mating between monokaryons from D.787 and D.744 (shaded). After monokaryons had grown together (plasmogamy), dikaryons were sampled from points 1, 2, and 3.

For F1 analysis, monokaryons, representing the four mating types of D.787 and D.744, were crossed in all possible interstrain combinations on MEA plates. To assess the role of the mitochondrial genome in determining sporocarp morphology, dikaryons were sampled from the central contact zone, halfway between the two inoculation points, as well as from points distal to the contact zone, behind the inoculation points (Fig. 2). In other words, for a cross between A1B1 and A3B3 monokaryons, three dikaryons would be sampled for fruiting: A1B1–A3B3 from the central contact zone, and *A1B1*–A3B3 (putatively possessing the A1B1 mitochondrial genome) and A1B1–*A3B3* (with the A3B3 mitochondrial genome) from the distal zones. Thus, 48 dikaryons were obtained, each of which was transferred to sawdust media, fruited, and observed macroscopically for typical or secotioid morphology.

For F2 analysis, SSIs from F1 sporocarps were backcrossed to a D.787 tester monokaryon and fruited in duplicate (Table 2). Backcross 1 used 110 SSIs from a single F1 sporocarp and backcross 2 used 210 SSIs from another F1 sporocarp. To determine mating types, 200 of the SSIs used in backcross 2 were crossed to two monokaryotic mating type testers and examined microscopically for clamp connections (Table 3).

Four SSIs were obtained from a DAOM-54158 sporocarp and crossed to two tester monokaryons from both D.744 and D.787. Crosses were examined microscopically for clamp connections and hyphal morphology, and dikaryons were transferred to sawdust media for fruiting (Table 4).

TABLE 2. Results of F2 backcrosses between isolates D.787 (secotioid) and D.744 (agaricoid).

| | Backcross 1 | Backcross 2 |
|---|---|---|
| F1 parent dikaryon mating type | A2B1–A4B4 | A2B1–A3B3 |
| Tester monokaryon mating type | A3B3 | A4B4 |
| Total F2 progeny | 96 | 193 |
| F2 agaricoid progeny[a] | 49/48 | 109/96.5 |
| F2 secotioid progeny[a] | 47/48 | 84/96.5 |
| $\chi^2$ | 0.0417[b] | 3.2383[b] |

[a] Observed/expected values for 1:1 segregation.
[b] $P < 0.05$.

## RESULTS

***Developmental morphology***—*D.744*—Development of the agaricoid isolate D.744 was described previously by Hibbett, Murakami, and Tsuneda (1993a).

*D.787*—Vegetative growth and initiation of fruiting in the secotioid isolate D.787 were similar to that of the agaricoid isolate D.744 (Hibbett, Murakami, and Tsuneda, 1993a). Solitary to clumped primordia began to appear approximately 12–16 days after inoculation of sawdust media. Initially, primordia were short, conical,

TABLE 3. Analysis of linkage between F2 sporocarp morphology and mating types of SSIs used in backcross 2.

| Mating type[a] | F2 morphology | | Mating compatibility[b] | |
|---|---|---|---|---|
| | Secotioid | Agaricoid | A2B2 | A1B1 |
| A2B1 | 21 | 36 | − | − |
| A3B3 | 18 | 25 | + | + |
| A2B3 | 22 | 20 | − | + |
| A3B1 | 18 | 27 | + | − |
| | Observed/Expected[c] | | | |
| All A2 | 43/49.5 | 56/49.5 | | |
| All A3 | 36/44 | 52/44 | | |
| | $\chi^2 = 7.6644$[d] | | | |
| All B1 | 39/51 | 63/51 | | |
| All B3 | 40/42.5 | 45/42.5 | | |
| | $\chi^2 = 2.4117$[d] | | | |

[a] Inferred mating genotypes of monokaryotic progeny from F1 sporocarp derived from A2B1–A3B3 hybrid. Recombinant mating alleles excluded.

[b] Determined by presence (+) or absence (−) of clamp connections in crosses on MEA.

[c] Under null hypothesis that mating type loci and secotioid locus are unlinked, with independent assortment.

[d] $P > 0.05$.

TABLE 4. Sporocarp morphologies of F1 hybrids between secotioid isolates DAOM-54158 and D.787 and agaricoid isolate D.744.

| | DAOM monokaryons and sporocarp morphologies[a] | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| D.787 monokaryons | | | | |
| A3B3 | sec | sec | sec | sec |
| A4B4 | sec | sec | sec | sec |
| D.744 monokaryons | | | | |
| A1B1 | aga | inc | inc | inc |
| A2B2 | aga | aga | aga | inc |

[a] sec = secotioid sporocarp; aga = agaricoid sporocarp; inc = incompatible cross, no sporocarps produced.

and pale grey. As the primordia elongated, they became darkly pigmented at the apex. Pileus initiation generally began on the second or third day after primordium initiation. The hymenophore was first visible on the stipe apex about 1 day after pileus initiation (Fig. 3). Initially, the hymenophore was composed of irregular, crested ridges of hyphae approximately 15–30 μm wide oriented more or less parallel to the stipe axis, with occasional anastomoses and dichotomies (Figs. 3–5). This is identical to the young hymenophore of typical *L. tigrinus* that we observed previously and is also very similar to the early hymenophore of *L. crinitus* (which is also agaricoid; Hibbett, Murakami, and Tsuneda, 1993a).

Differentiation of veil tissue began approximately 3 days after primordium initiation, when individual lamellae had just become macroscopically visible. The veil developed as hyphae at the margins of the young lamellae began to proliferate and overarch the spaces between the lamellae (Figs. 6–10). Soon the veil hyphae formed a continuous covering over the hymenophore (Figs. 6, 7, 11, 12). The growth of the veil began near the margin of the pileus and advanced toward the hymenophore at the stipe apex, which is the first-formed portion of the hymenophore (Figs. 6, 7). Often the veil did not completely obscure the hymenophore in a narrow zone at the stipe apex, which thus remained exposed throughout the life of the sporocarp.

The lamellae continued to develop in a nearly normal fashion. At maturity, almost all of the hymenophore was enclosed, but individual lamellae were separate and distinct structures (Fig. 12). However, the lamellae were somewhat misshapen with frequent anastomoses or bifurcations (Fig. 12). Unlike the smooth lamellae of the typical form (Hibbett, Murakami, and Tsuneda, 1993a, figs. 21, 25), the radial surfaces of the lamellae of the secotioid form had numerous venose or platelike outgrowths (Figs. 13, 14). The hymenium itself appeared normal and covered the entire surface of the hymenophore. At the base of the hymenophore there were tangential cross-bridges between the lamellae that delimited more or less rectangular spaces, as in typical *L. tigrinus* (Figs. 15, 16; Hibbett, Murakami, and Tsuneda, 1993a, figs. 24, 25). Finally, there were numerous hyphal pegs produced on the lamellae (Figs. 12–14) that are indistinguishable from those produced by the typical form.

The veil was approximately 15–20 μm thick in most areas (Fig. 12). The outer surface of the veil was composed of a loose, irregular weft of smooth generative hyphae with slight apical swellings (Figs. 17, 18). Macroscopically, faint longitudinal striations corresponding to the arrangement of lamellae under the veil could usually be observed (Fig. 1). Longitudinal slits sometimes formed in the veil tissue along the striations near the margin of the pileus

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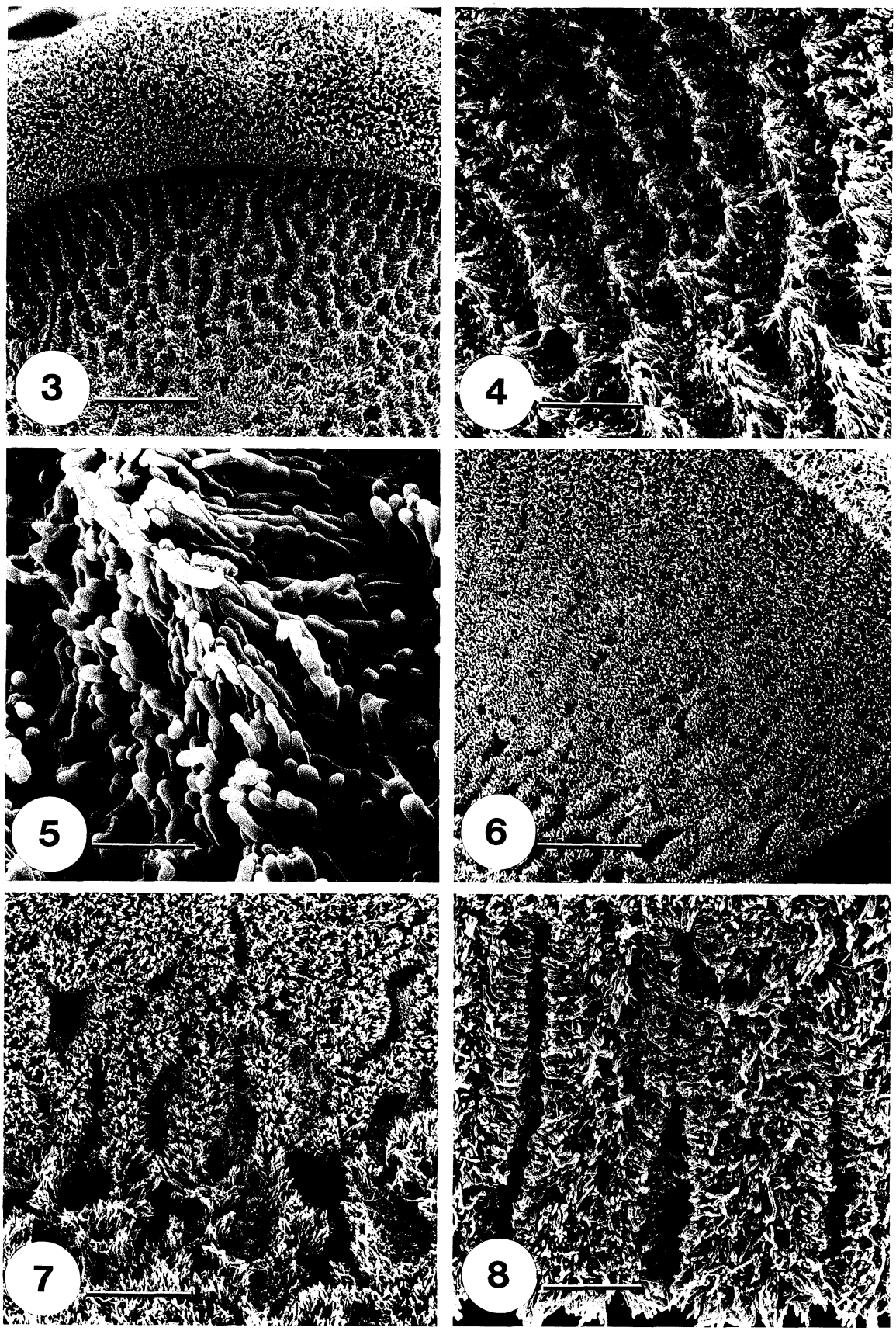
Figs. 3–8. Early sporocarp ontogeny in secotioid isolate D.787. **3.** Early hymenophore is composed of irregular, crested ridges of hyphae over the stipe apex. At this stage, the hymenophore is identical to that of typical *L. tigrinus*. There are no veils or other protective tissues. Bar = 400 μm. **4.** Detail of young hymenophore. Bar = 100 μm. **5.** A single young lamella showing apical growth of surface hyphae to form hymenophore. Bar = 20 μm. **6.** Later developmental stage. The veil tissues have formed over the distal portions of the hymenophore. At the stipe apex parts of the hymenophore remain exposed. Note the faint radial striations in the veil that correspond to spaces between the underlying lamellae. Bar = 50 μm. **7.** Detail from same sporocarp as in Fig. 6 showing veil advancing over hymenophore toward stipe apex. Bar = 200 μm. **8.** Tangential vertical section through young lamellae near zone of veil development. The lamellae are distinct and separate even to their bases. Bar = 75 μm.

Figs. 9–14. Later hymenophore and veil development in D.787. **9.** Tangential section through hymenophore near zone of veil development. Note that hyphae at the margins of the lamellae are proliferating outward. Bar = 20 μm. **10.** Tangential section through hymenophore in zone of veil development. The hyphae at the margins of the lamellae have overarched the intralamellar space and have begun to interweave. Bar = 20 μm. **11.** Tangential section through mature hymenophore and veil. The veil has completely enclosed the hymenophore. Bar = 150 μm. **12.** Tangential section through mature hymenophore and veil. There are some anastomoses and other irregularities in the lamellae, but they are clearly recognizable as lamellae (compare to Figs. 27, 28). Hyphal pegs protrude from the lamellae. Bar = 400 μm. **13.** Radial section through hymenophore showing lamellar face with venose outgrowths. Bar = 150 μm. **14.** Detail from Fig. 13 showing hyphal pegs and hymenium covering venose outgrowths from lamella. Bar = 75 μm.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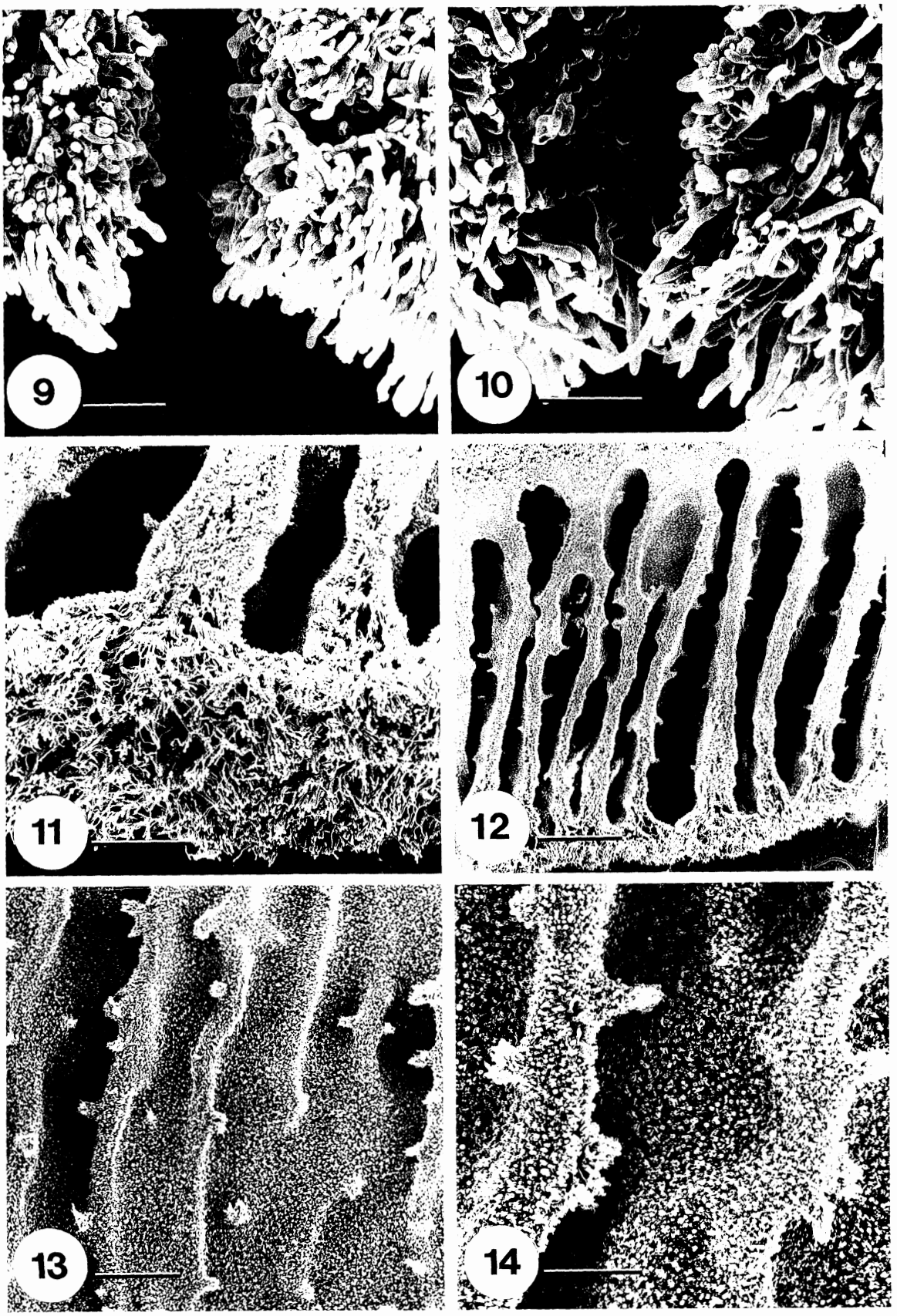
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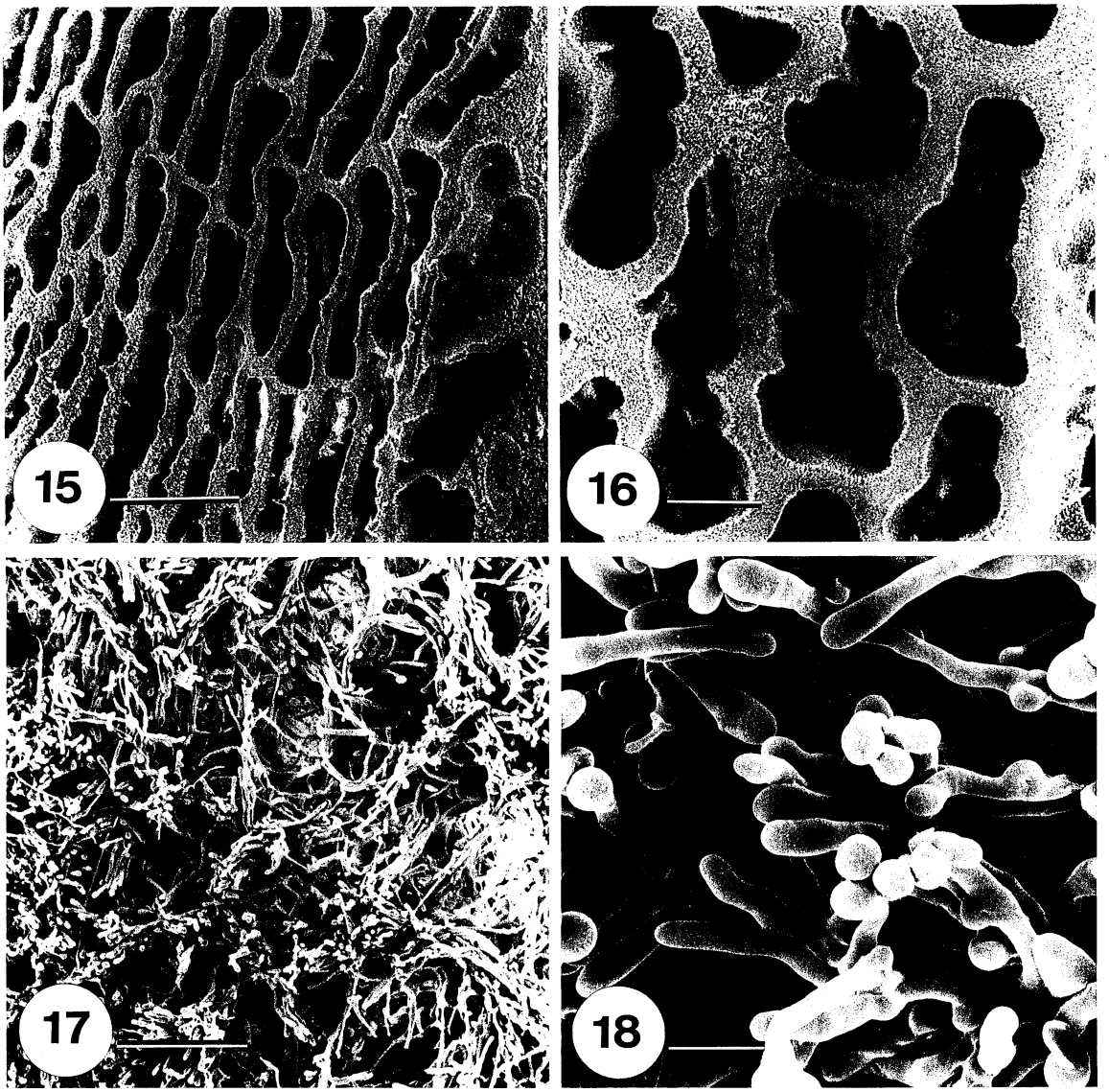


Figs. 15–18. Mature hymenophore and veil morphology in D.787. **15.** Scalp section through hymenophore showing subporoid arrangement of hymenophore at the base (compare to Fig. 28). Bar = 50 μm. **16.** Closer view of regular, rectangular chambers at base of hymenophore. The subporoid structure is also observed in typical *L. tigrinus* and has been hypothesized to be a vestige of polyporoid ancestry (Pegler, 1983; Hibbett, Murakami, and Tsuneda, 1993a). Bar = 250 μm. **17.** Surface view of veil. Bar = 100 μm. **18.** Detail from Fig. 17 showing tips of generative hyphae that form veil. Bar = 10 μm.

in older sporocarps (Fig. 1). In a few extreme cases, the slits extended through the pileus, which then took on a lobate aspect. The inner surface of the veil was lined by a hymenium that was continuous with and indistinguishable from the hymenium of the lamellae, except that no hyphal pegs were present (Figs. 11, 12). Lyman (1907) described and illustrated conidia that were formed on sporocarps of the secotioid form of *L. tigrinus,* but we never observed them.

In terms of overall shape and stature, duration of growth,

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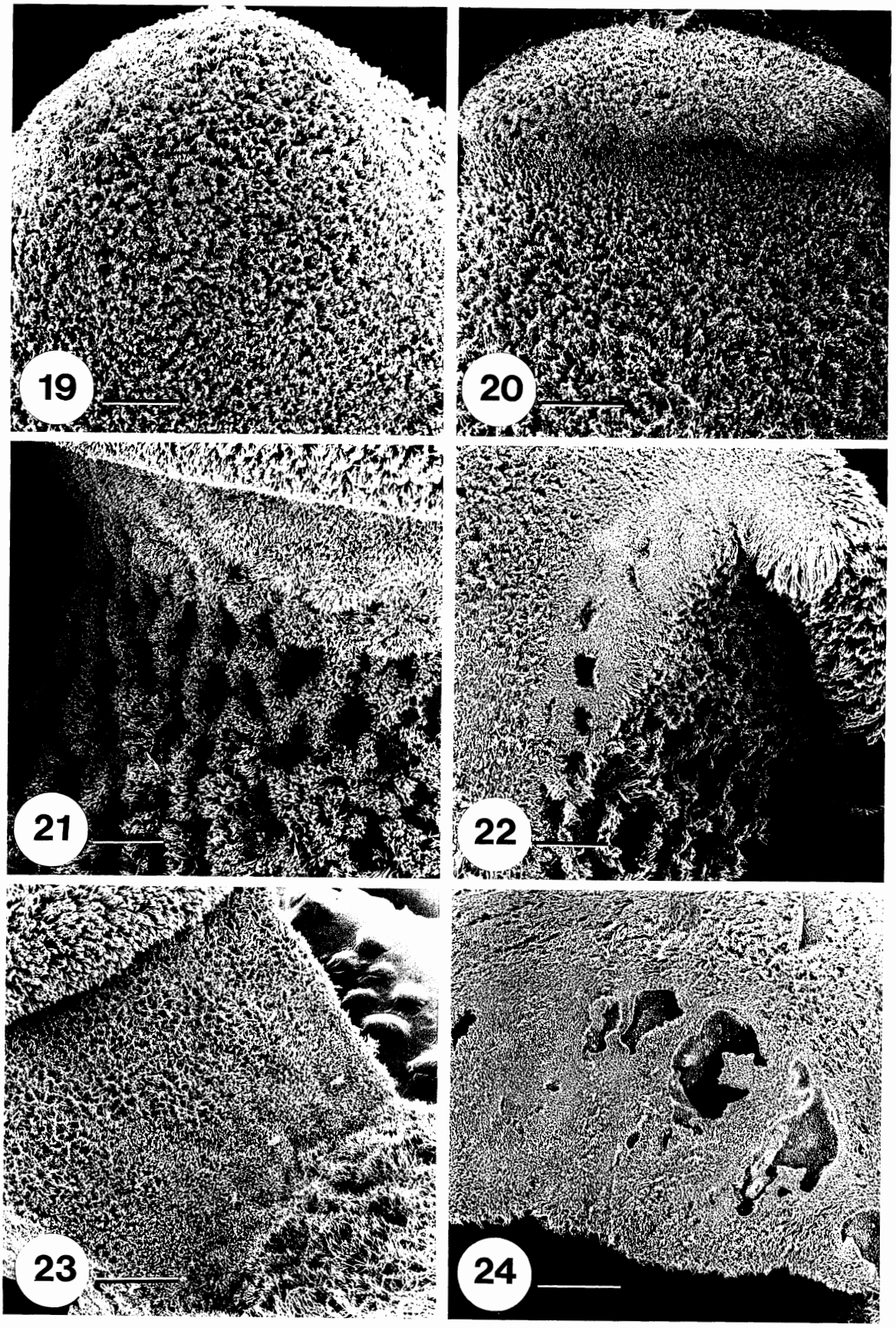
Figs. 19–24. Early ontogeny of secotioid isolate DAOM-54158. **19.** Primordium. Bar = 125 μm. **20.** Early stage of pileus differentiation. There are no hymenophore elements visible. Bar = 200 μm. **21.** Later developmental stage. The veil has started to form over the distal portions of the young hymenophore. Bar = 200 μm. **22.** Radial section through hymenophore and veil at approximately same stage as in Fig. 21. The hymenophore is exposed at the stipe apex only. At this stage, the development of the veil is already more luxuriant than in D.787 sporocarps. Bar = 200 μm. **23.** Later developmental stage. The veil has completely covered the hymenophore. Bar = 400 μm. **24.** Radial section through veil and hymenophore at approximately same stage as Fig. 23. The hymenophore is loculate with thick veil tissue and no discernible lamellae (but see Fig. 25). Bar = 400 μm.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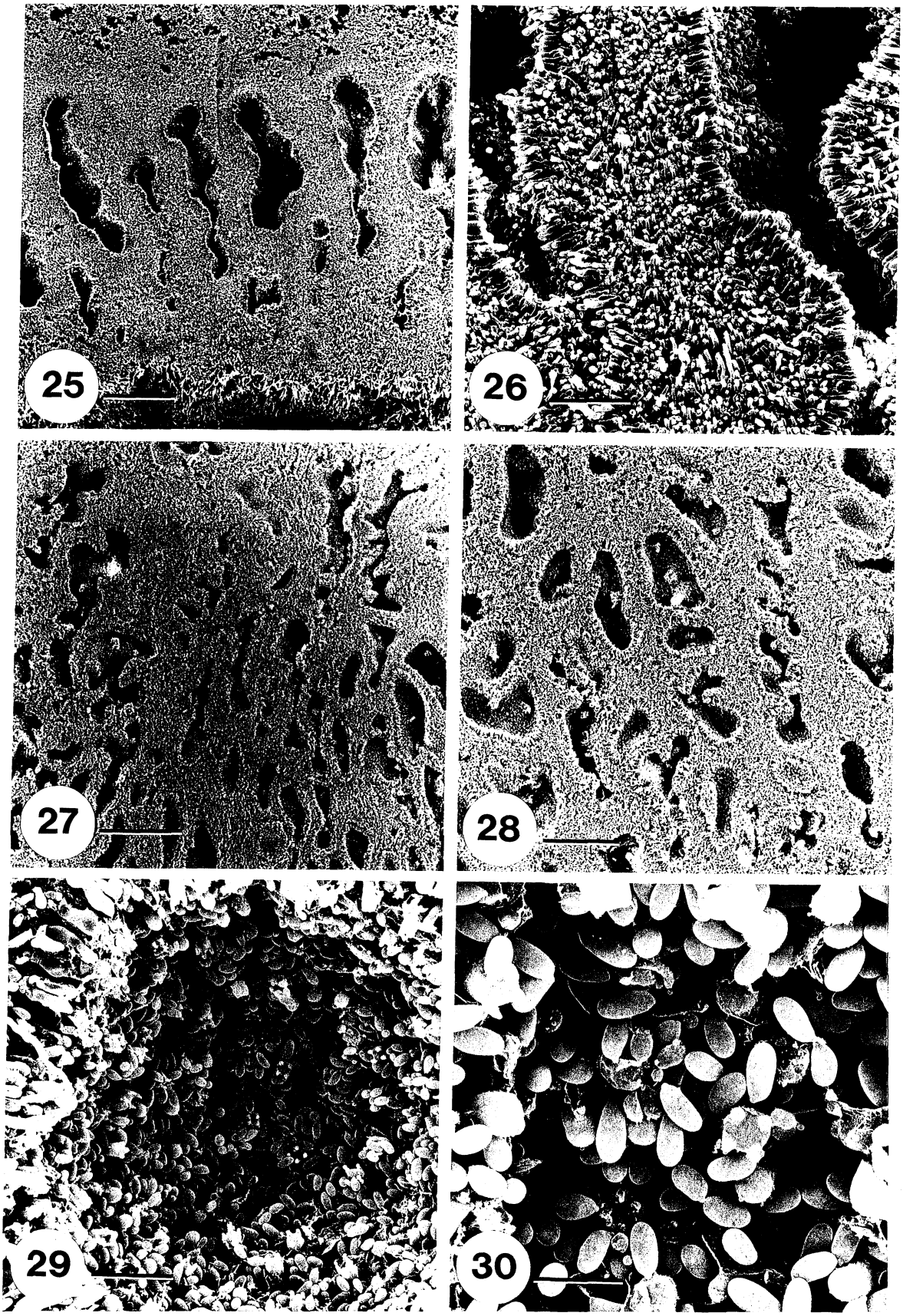
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and longevity, the D.787 sporocarps were similar to those of the agaricoid form (Fig. 1). Sporocarps usually matured in about 5 days and persisted for up to 10 days.

*DAOM-54158*—Vegetative growth, primordium initiation, and early differentiation of the stipe, pileus, and hymenophore were essentially the same as in D.787 and D.744 (Figs. 19, 20). Again, veil tissue began to develop from the distal portions of the hymenophore and grew toward the stipe apex (Figs. 21, 22). However, from the outset the growth of the veil was more luxuriant in DAOM-54158 than in D.787 (Figs. 22–26). Individual lamellae could just barely be differentiated early in the ontogeny of DAOM-54158 (Figs. 25, 26). Later, the hymenophore became so convoluted that the lamellae could not be resolved. Instead, the hymenophore became loculate, with irregular hymenium-lined pockets scattered through the confluent mass of veil and tramal tissue (Figs. 27–30). At maturity, these pockets became filled with apparently normal, discharged ballistospores (Figs. 29, 30). Hyphal pegs that had emerged from the hymenium could still be observed (Figs. 27, 28).

The overall rate and duration of growth and shape and proportions of the sporocarps in DAOM-54158 were similar to those of D.787. However, the veil and hymenophore tissue frequently became so thick that the pilei lost their normal form and took on a lumpy, irregular shape with an uplifted, undulate margin. Longitudinal slits and striations like those observed in D.787 were rarely observed in DAOM-54158 sporocarps.

***Herbarium materials***—The collections of the secotioid form of *L. tigrinus* that we examined are distributed from Michigan and southern Ontario south to Mississippi and Texas, and from the Atlantic coast west to Arizona (Fig. 31). The Arizona collection (Sycamore Canyon, Santa Cruz Co., Aug. 7, 1980, Bigelow 18180 [NY]) represents a significant westward range extension from that reported for the species by Redhead (1988). Otherwise, the range of the secotioid form in North America overlaps with that of the agaricoid form (Redhead, 1988). Herbarium label data and published (although sometimes anecdotal) accounts indicate that the secotioid form of *L. tigrinus* occurs in pure populations (Morgan, 1895; Lyman, 1907) or in mixtures with the typical form (Peck, 1909; Kauffman, 1918; Harper, 1921). Seven of the herbarium collections that we examined included mixtures of secotioid and agaricoid sporocarps. We made four collections of the secotioid form and three collections of the agaricoid form on a single day along a 1-mile stretch of the Ipswich River in Massachusetts.

*Lentinus tigrinus* is noted for its variability in sporocarp size and proportions (Harper, 1921; Pegler, 1983). We observed such variation among collections of the secotioid form, as well as variation in the apparent severity of the secotioid phenotype. There was variation in the development of striations and slits in the veil, anastomoses and contortions of the lamellae, deviation of the pileus from the typical shape, etc. However, there was no apparent correlation between geographic distribution and variation in expression of the secotioid phenotype.

Fig. 31. Distribution of collections of the secotioid form of *L. tigrinus* examined in this study.

***Genetic crosses***—As anticipated, monokaryons from both D.744 and D.787 fell into four mating types, which is consistent with previous reports of a tetrapolar mating system in *L. tigrinus* (Nobles, 1965; Rosinski and Robinson, 1968). Mating type alleles from D.744 were designated A1, A2, B1, and B2. Those from D.787 were designated A3, A4, B3, and B4.

F1 dikaryons from interstrain crosses of D.744 and D.787 fully colonized the sawdust media and began to produce primordia within 11–16 days. All but one (*A2B2–A3B4*) of the dikaryons fruited, and all of these produced typical, agaricoid sporocarps that were indistinguishable from those produced by the D.744 dikaryon.

For backcross 1, 110 SSIs from an A2B1–A4B4 F1 sporocarp were crossed to an A3B3 monokaryon (Table 2). The F2 dikaryons thus produced were fruited and gave rise to 49 agaricoid sporocarps and 47 secotioid sporocarps (Table 2). We encountered contamination problems in the mushroom fruiting room; 42 of the F2 dikaryons only fruited in one replicate, and 14 of the F2 dikaryons failed to fruit at all. In almost all cases, the failed fruitings were producing primordia when they became contami-

←

Figs. 25–30. Immature and mature hymenophore anatomy of secotioid isolate DAOM-54158. **25.** Vertical tangential section through hymenophore of immature sporocarp at approximately same developmental stage as in Fig. 24. Lamellae were not discernible in radial section in Fig. 24, but here in tangential section there are vertically elongate locules that may be vestiges of lamellar structures. Bar = 200 μm. **26.** Detail from Fig. 25 showing hymenium-lined locules of hymenophore and irregular trama. Bar = 40 μm. **27.** Radial section through loculate mature hymenophore. Note hyphal pegs emergent from hymenium (and in Fig. 28). Bar = 400 μm. **28.** Scalp section through mature hymenophore (compare to Fig. 15). Bar = 250 μm. **29.** Detail from Fig. 28 showing locule filled with discharged spores. Bar = 20 μm. **30.** Detail from Fig. 29 showing discharged ellipsoid, asymmetric basidiospores. Bar = 7 μm.

nated. Fifty-four dikaryons fruited in both replicates, and in all cases there was agreement between replicates.

Backcross 2 used 210 SSIs from an A2B1–A3B3 sporocarp that were crossed to an A4B4 monokaryon (Table 2). The F2 dikaryons produced 109 agaricoid sporocarps and 84 secotioid sporocarps (Table 2). Contamination was again a problem; 65 F2 dikaryons fruited in only one replicate, and 17 F2 dikaryons failed to fruit in either replicate.

Mating types of 200 of the SSIs from the A2B1–A3B3 F1 sporocarp that were used in backcross 2 were determined by crossing them with A2B2 and A1B1 mating type tester monokaryons (Table 3). Frequencies of mating types were: A2B1, 30%; A2B3, 22%; A3B1, 24%; and A3B3, 24% (these estimates exclude the possibility of recombination at mating type loci). Out of the 200 SSIs that were scored for mating type, 187 were successfully dikaryotized and fruited in backcross 2.

We observed variation in the development of the secotioid phenotype among the F2 progeny. Some F2 secotioid sporocarps had entirely enclosed hymenophores whereas others had pronounced longitudinal slits in the veil. Nevertheless, all mature sporocarps produced in the F1 and F2 crosses could be unambiguously scored as either secotioid or agaricoid. Similar results were reported by Rosinski and Robinson (1968). Replicates of F2 dikaryons fruited at different times, and locations often displayed differences in the extent of pileus expansion, stipe elongation, and, in the case of secotioid sporocarps, veil development.

Four SSIs from DAOM-54158 were mated with A3B3 and A4B4 SSIs from D.787 and A1B1 and A2B2 SSIs from D.744 (Table 4). All eight dikaryons produced by crosses between DAOM-54158 and D.787 produced secotioid sporocarps, the lamellae of which were distinct and separable under the dissecting microscope, and thus resembled sporocarps produced by the D.787 parental dikaryon. Four of the dikaryons produced in crosses between DAOM-54158 and D.744 SSIs fruited, and each produced typical agaricoid sporocarps that were indistinguishable from those produced by the parental D.744 dikaryon. However, the other four dikaryons did not fruit. In the nonfruiting crosses, the colony macromorphology on MEA plates showed a thin, appressed mycelium in the zone of contact between the two monokaryons. Microscopically, copious coralloid branchings could be discerned in the zone of contact in the nonfruiting crosses. We did not observe clamp connections in the contact zones of the nonfruiting crosses. Away from the contact zone, the colony morphology was typical for *L. tigrinus,* with fluffy, white aerial mycelium, and numerous chlamydospores (Lyman, 1907; Martin, 1956; Nobles, 1965). Three of the incompatible crosses occurred with an A1B1 SSI from D.744, and one occurred with an A2B2 SSI (Table 4).

## DISCUSSION

Our results support the conclusion by Rosinski and Faro (1968) that the secotioid phenotype in *L. tigrinus* is controlled by a recessive allele at a single locus (Table 2). Because F1 progeny of D.787 (North Carolina) and DAOM-54158 (Michigan) had the secotioid phenotype, we suggest that the same locus is responsible for the secotioid phenotype in both isolates. We can now add that the mitochondrial genome appears not to affect expression of this putatively nuclear gene, and that it is apparently not linked to mating type loci (Table 3).

Rosinski and Robinson (1968) suggested that the secotioid form of *L. tigrinus* arose as a result of a single mutation. Our observations are consistent with this hypothesis. It is also possible that there have been two or more independent mutations at the same locus that have resulted in loss of gene function, and that have thus created multiple nonhomologous, recessive alleles. However, the absence of the secotioid form of *L. tigrinus* in Europe and Asia suggests that the locus that governs the secotioid phenotype is not subject to frequent mutation. We therefore think that the simplest explanation for the observed geographic distribution of the secotioid form of *L. tigrinus* and the morphology of F1 progeny of D.787 and DAOM-54158 is that the mutation that confers the secotioid phenotype is uniquely derived and that there has been little or no gene flow between American populations that carry the secotioid gene and populations of *L. tigrinus* on other continents.

Although the potential to develop the secotioid morphology is probably inherited at a single locus, there appear to be environmental and genetic factors that can affect the degree of expression of the secotioid phenotype. We observed the effects of environmental factors by fruiting certain dikaryons in both the mushroom fruiting room and the laboratory. Some secotioid dikaryons that had conspicuous radial slits in the veil when fruited in the mushroom fruiting room had completely closed veils when fruited in the laboratory. Other aspects of sporocarp morphology, such as stipe length, also varied among fruitings of individual dikaryons (cf. Bougher, Tommerup, and Malajczuk, 1993, for another example of developmental plasticity in secotioid fungi). We observed the effects of genetic factors by comparing sporocarps from D.787 and DAOM-54158 dikaryons, and their F1 hybrids. Expression of the secotioid phenotype was consistently milder in D.787 than in DAOM-54158; in D.787, individual lamellae could always be distinguished whereas in DAOM-54158 there were no recognizable lamellae in the mature hymenophore. Hymenophore and veil morphology in sporocarps derived from F1 crosses between D.787 and DAOM-54158 resembled those in D.787 sporocarps. Lyman (1907, figs. 131, 132) presented photomicrographs of sporocarps of the secotioid form of *L. tigrinus* whose hymenophores are intermediate between the extremes of D.787 and DAOM-54158. Further formal genetic studies, using an expanded sample of isolates, might reveal additional loci involved in production and regulation of aspects of the secotioid phenotype in *L. tigrinus.*

Thiers (1984) and Bruns et al. (1989) have suggested that evolution of some secotioid fungi has proceeded by initial macromutations with strong effects on developmental morphology in hymenomycete ancestors, followed by more gradual evolution, via natural selection, for gasteromycete-like features, such as a loculate hymenophore, loss of forcible spore discharge, etc. Following these models, it is tempting to speculate that certain secotioid populations of *L. tigrinus* have undergone genetic divergence—by either natural selection or random processes—

toward a gasteroid morphology, while other populations have retained a more plesiomorphic, agaricoid morphology. However, to evaluate this hypothesis it will be necessary to achieve a greater understanding of developmental genetics, population and breeding biology, and intraspecific genealogy in *L. tigrinus*. We mention this here only to encourage future research, which we feel might provide insights into processes of morphological diversification in fungi.

The switch from the agaricoid to the secotioid phenotype could have profound influences on ecology and evolution of *L. tigrinus* populations. Intuitively, it seems likely that the secotioid phenotype would lower the fitness of individuals of *L. tigrinus* by reducing the capacity for aerial spore dispersal and perhaps by reducing outcrossing. However, the effect of the secotioid phenotype on fitness is unknown; it could actually be neutral or positive. As in many basidiomycetes, the relative importance of basidiospores and asexual propagules for establishing and maintaining *L. tigrinus* populations is unknown. Recent studies on giant *Armillaria* clones (Smith, Bruhn, and Anderson, 1992) have demonstrated clearly that some basidiomycetes can spread extensively by purely vegetative growth. *Lentinus tigrinus* does not form rhizomorphs like those of *Armillaria,* but its dikaryotic and monokaryotic mycelia produce chlamydospores (Lyman, 1907; Tsuneda, Thorn, and Hibbett, 1993) whose role in dispersal and colonization remains to be determined.

Previous authors (e.g., Thiers, 1984) have suggested that secotioid fungi are well adapted to arid environments, in which they are often found. In *L. tigrinus,* the presence of the veil reduces the ratio of surface area to volume and might therefore increase desiccation tolerance. However, *L. tigrinus* is typically found not in dry habitats, but on logs adjacent to or emergent from water (Redhead, 1988, and others). Noting this, Rosinski and Robinson (1968) suggested that the secotioid sporocarps might be well adapted to dispersal of spores by water. In other secotioid fungi, mycophagy by rodents has been suggested as a mechanism for spore dispersal (Thiers, 1984; Bruns et al., 1989), but this has not been observed in *L. tigrinus*. Snell (1923) reported that spores from below the veil of a 5-year-old herbarium collection germinated at high frequency and suggested that the protection provided by the veil increases spore longevity (there are no comparable data on longevity of spores from agaricoid sporocarps). Whatever its functional attributes, the secotioid form of *L. tigrinus* has been frequently collected since the late nineteenth century. If the allele that confers the secotioid form did arise by a single mutational event, then it has been able not only to persist, but to spread across much of North America (Fig. 31). Collectively, this evidence suggests that the secotioid phenotype in *L. tigrinus* is an evolutionarily successful innovation (cf. Martin, 1956). This stands in contrast to the idea that secotioid fungi are necessarily poorly adapted aberrations that must evolve into more refined gasteromycete-like forms or else be doomed to extinction (e.g., Baura et al., 1992). Although such may often be the case, the occurrence of stipitate, epigeous, secotioid taxa in diverse families of basidiomycetes (e.g., *Podaxis* Desv. and *Montagnea* Fr. in Coprinaceae, *Thaxterogaster* Singer in Cortinariaceae, *Longula* Zeller in Agaricaceae, etc.) suggests that evolution of secotioid forms has been a common and successful mode of sporocarp morphological evolution (cf. Thiers, 1984; Miller and Miller, 1988).

Evolution of certain secotioid fungi has previously been interpreted as involving paedomorphosis, resulting from an arrest of an ancestral ontogeny (Gould, 1977; Thiers, 1984; Bruns et al., 1989; Baura, Szaro, and Bruns, 1992). Indeed, the commonly applied definition of secotioid fungi as resembling unopened agarics (e.g., Miller and Miller, 1988) has an inherent implication of paedomorphosis. We do not contest that paedomorphosis has been important in evolution of some secotioid fungi, but it has not been involved in evolution of the secotioid form of *L. tigrinus*. At no point in the ontogeny of agaricoid *L. tigrinus* is there a structure like the veil of the secotioid form (Bobbitt and Crang, 1975; Hibbett, Murakami, and Tsuneda, 1993a). Typical *L. tigrinus* is gymnocarpic (Bobbitt and Crang, 1975; Hibbett, Murakami, and Tsuneda, 1993a), and therefore a hymenophore that is enclosed at maturity could not be derived by paedomorphosis. Only hemiangiocarpy, in which an initially enclosed hymenophore becomes exposed at maturity (Watling, 1985), can lead from an exposed to an enclosed mature hymenophore strictly by paedomorphosis. Finally, we note that the secotioid form of *L. tigrinus* grows to the same size and proportions as the typical form, in about the same amount of time. For these reasons, we feel that the developmental modifications that are involved in derivation of the secotioid form from the typical agaricoid form are best interpreted as a realization of von Baer's law of differentiation (Gould, 1977) which predicts that derived ontogenies parallel ancestral ontogenies in early stages, and then deviate to produce novel features in later stages. Secotioid fungi are doubtless a highly polyphyletic group. Other secotioid taxa have probably been derived by different classes of developmental changes, possibly including paedomorphosis.

We do not know the reason for the incompatibility reactions between certain SSIs of DAOM-54158 and D.744 (Table 4). For the incompatibility to be due to shared mating type alleles, it would be necessary for the DAOM-54158 and D.744 dikaryons to have at least two mating type alleles in common (incompatibility of certain DAOM-54158 SSIs with either A1B1 or A2B2 mating type SSIs requires presence of one A1 or B1 allele *and* one A2 or B2 allele in the DAOM-54158 dikaryon). Given the geographic separation of the isolates (Michigan and North Carolina), this seems unlikely. An alternate explanation is that there is partial vegetative incompatibility between certain North American populations of *L. tigrinus,* but more extensive crossing studies will be needed to resolve this issue.

Agaricoid-secotioid transformations provide fascinating demonstrations of morphological and ecological evolution in fungi. Among secotioid fungi, the secotioid form of *L. tigrinus* is unique because it is still mating compatible with the typical agaricoid form, and because a specific locus has been identified that confers the secotioid phenotype. Developmental mutants have already provided insights into mechanisms of morphogenesis in culture studies of *Agaricus bisporus, Coprinus cinereus, Schizophyllum commune,* and other basidiomycetes (see papers in Moore et al., 1985; Reijnders, 1991, and references

therein). *Lentinus tigrinus* also fruits well in culture and could therefore be an excellent system for understanding the molecular-developmental basis for evolution of secotioid fungi.

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