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Postmeiotic nuclear behavior in *Lentinus*, *Panus*, and *Neolentinus*

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Abstract: We investigated nuclear behavior during basidiosporogenesis in *Lentinus*, *Panus*, and *Neolentinus* in an attempt to discover characters for taxonomic segregation of lentinoid-pleurotoid fungi. In *Lentinus tigrinus* and *Panus lecomtei* somatic hyphae are binucleate. Karyogamy and meiosis occur in the basidia, meiotic products migrate into spores and postmeiotic mitosis occurs; this is followed by back-migration of one nucleus from each spore into the basidium. Mature spores are consequently uninucleate and discharged basidia are quadrinucleate. In *Neolentinus lepideus* many somatic cells are multinucleate. Karyogamy, meiosis, and postmeiotic mitosis occur as in *L. tigrinus* and *P. lecomtei*, but there is no back-migration, mature spores are binucleate, and discharged basidia are anucleate. The patterns of nuclear behavior in *L. tigrinus* and *P. lecomtei* correspond to type C nuclear behavior as defined by Duncan and Galbraith, whereas that of *Neolentinus* corresponds to type D nuclear behavior. *Lentinus* and *Panus* cannot be distinguished on the basis of postmeiotic nuclear behavior, but both can be distinguished from *Pleurotus* which has been reported to have type A nuclear behavior *sensu* Duncan and Galbraith.

Key Words: cytology, *Lentinus*, *Neolentinus*, nuclear behavior, *Panus*

INTRODUCTION

The number of nuclei per basidiospore (usually one or two) has been widely applied as a taxonomic character in the Agaricales (e.g., Kühner, 1980). Superficially, this would appear to be an ideal character for phylogenetic analyses; it can be observed in virtually all basidiomycetes, and it is easily coded into discrete

states. However, the apparent simplicity of this character conceals a diversity of ontogenetic patterns in basidiosporogenesis that result in uninucleate or binucleate spores. Duncan and Galbraith (1972) observed three patterns that can produce uninucleate spores, which they termed the A, B, and C types of postmeiotic nuclear behavior, and one pattern that produces binucleate spores, which they termed the D type. In recent years, two additional patterns have been reported, which Mueller and Ammirati (1993) have designated the E and F types of nuclear behavior. The various patterns are differentiated by the following criteria: 1) presence or absence of postmeiotic mitotic divisions; 2) location of the postmeiotic mitosis; 3) presence or absence of back-migration of one daughter nucleus derived from the postmeiotic mitosis into the basidium (not applicable in patterns where postmeiotic mitosis occurs within the basidium); and, 4) presence or absence of migration of all postmeiotic mitotic nuclei into spores (applicable only in patterns where postmeiotic mitosis occurs in basidia) (TABLE I). Patterns A, B, C, and E typically result in uninucleate basidiospores, whereas patterns D and F typically result in binucleate basidiospores (the number of nuclei per spore also depends in part on the number of spores produced per basidium). Distribution of the various nuclear behavior types in the Agaricales was reviewed by Mueller and Ammirati (1993).

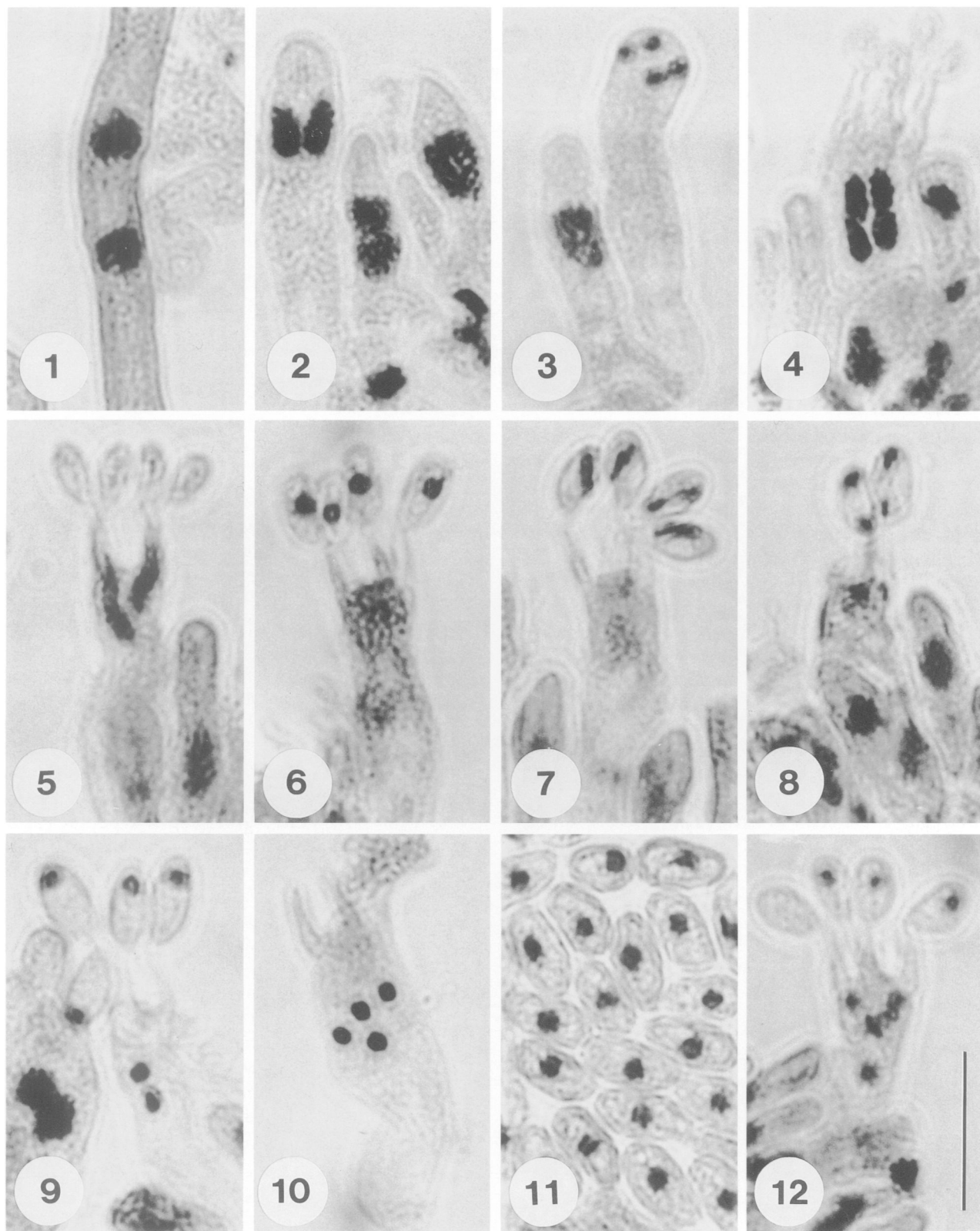
The diversity of nuclear behavior types suggests that it is incorrect to score all uninucleate spores or all binucleate spores as homologous. We think that it is preferable to recognize nuclear behavior during basidiosporogenesis as a single character with six character states that correspond to the A, B, C, D, E, and F types of postmeiotic nuclear behavior. Thus, this would be an explicitly developmental character in which the ontogenetic transformations themselves define the character states, even though the mature morphologies may be identical.

An alternative to coding nuclear behavior as a single character would be to recognize each of the variable aspects that define the different nuclear behavior types as an independent character. This would reduce a single, complex character with six states to four simple characters, three with two states, and one with three states (TABLE I). The apparent independence of the variable aspects of nuclear behavior (TABLE I) sup-

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FIGS. 1–12. *Lentinus tigrinus* somatic nuclei and basidiosporogenesis. 1. Dikaryotic hypha in hymenophoral trama. 2. Prefusion dikaryotic basidium (center), diploid basidium immediately after karyogamy (right), and anaphase I basidium (left). 3. Anaphase II basidium. The nuclei have migrated to the apex of the basidium and are more condensed than those in FIG. 2. 4. Meiotic tetrad. The nuclei have descended to the central part of the basidium and have relaxed somewhat compared to those in FIG. 3. Sterigmata and spores have begun to form. 5. Meiotic products migrating into sterigmata. 6. Uninucleate

TABLE I. Definition of nuclear behavior types and distribution among lentinoid-pleurotoid fungi

Nuclear behavior type ^a	Postmeiotic mitosis (PMM)	Location of PMM ^b	Back migration of PMM products ^b	All nuclei from PMM enter spore ^b	Lentinoid-pleurotoid examples
A	+	basidia	NA	—	<i>Pleurotus eryngii</i> ^c <i>P. ferulae</i> Lanzi ^c <i>P. nebrodensis</i> Inzenga ^c
B	+	sterigmata	+	NA	None
C	+	spores	+	NA	<i>Lentinus tigrinus</i> <i>Panus lecomtei</i>
D	+	spores	—	NA	<i>Neolentinus adhaerens</i> ^d <i>N. lepideus</i>
E	—	NA	NA	NA	None
F	+	basidia	NA	+	None

^a *Sensu* Duncan and Galbraith (1972) and Mueller and Ammirati (1993).

^b NA = not applicable.

^c Slézac (1984).

^d Kühner et al. (1962).

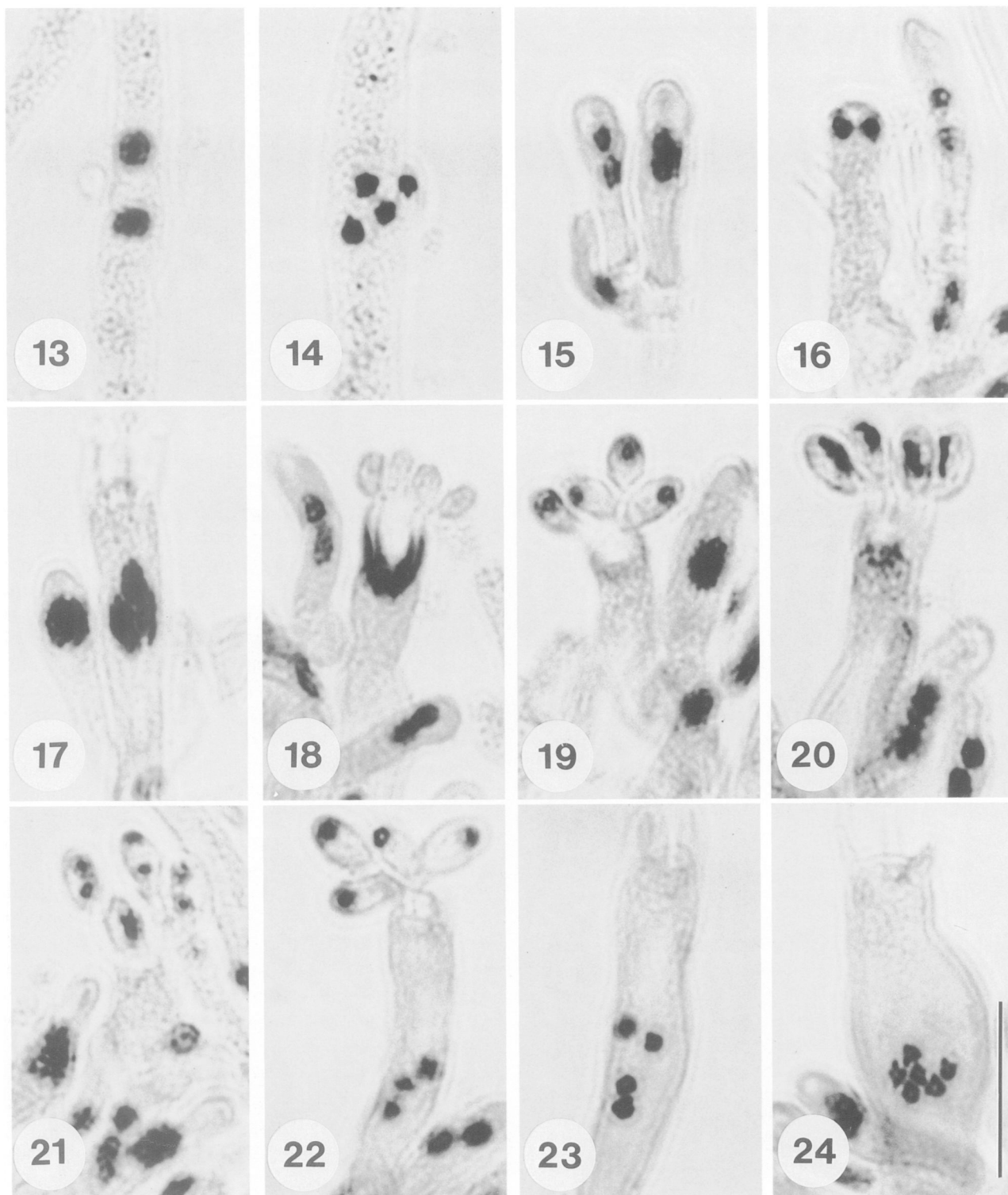
ports the four-character coding. To simplify the present discussion, we prefer to treat the entire ontogeny as a single character. However, the four-character coding might yield better phylogenetic resolution than the single-character coding, and it should be considered if this character is used in cladistic analyses.

We were interested in using patterns of postmeiotic nuclear behavior to help resolve taxonomic distinction of the lentinoid-pleurotoid fungi, *Lentinus* Fr., *Panus* Fr., *Neolentinus* Redhead & Ginns, and *Pleurotus* (Fr.) Kummer. Considerable controversy surrounds the taxonomy of these genera [for brief, recent reviews, see Redhead and Ginns (1985), Hibbett (1993), Hibbett and Vilgalys (1993), and Rune (1994)]. Recent studies aimed at clarifying relationships of the lentinoid-pleurotoid fungi have employed cultural characters, mating systems, anatomy (Redhead and Ginns, 1985; Johnson and Methven, 1994; Hibbett and Thorn, 1994), phylogenetic analyses of molecular and morphological characters (Hibbett and Vilgalys, 1993; Molina et al., 1992), and comparative developmental morphology (Hibbett et al., 1993a, b). The general picture emerging from these studies supports the monophyly of *Lentinus s.str.*, typified by *L. tigrinus* (Bull. : Fr.) Fr., *Panus s.str.*, typified by *P. conchatus* (Bull. : Fr.) Fr., *Neolentinus*, typified by *N. lepideus* (Fr.) Redhead & Ginns, and *Pleu-*

rotus, typified by *P. ostreatus* (Jacq. : Fr.) Kummer, but suggests that these lineages are not closely related to one another. This stands in contrast to previous classifications in which the lentinoid-pleurotoid fungi have been placed into a small number of putatively closely related genera (e.g., Pegler, 1975, 1983; Kühner, 1980; Corner, 1981; Singer, 1986).

Neolentinus has binucleate spores and can therefore be segregated from *Lentinus*, *Panus*, and *Pleurotus*, all of which have uninucleate spores (Kühner, 1980; Redhead and Ginns, 1985). D type nuclear behavior has been reported for *Neolentinus adhaerens* (A.&S. : Fr.) Redhead & Ginns (Kühner et al., 1962). *Neolentinus lepideus* has been cited as having D type nuclear behavior (Kühner, 1980, *vide* Mueller and Ammirati, 1993), but this was due to an oversight; there have been no published descriptions of nuclear behavior in *N. lepideus* (G. M. Mueller, pers. comm.). A type nuclear behavior has been reported for three putative species of the *Pleurotus eryngii* (DC. : Fr.) Quél. complex (Slézac, 1984), but there have been no reports for *Lentinus* or *Panus*. The primary objective of this study was, therefore, to determine the type of postmeiotic nuclear behavior that occurs in *Lentinus* and *Panus*. If we could find any differences between *Lentinus*, *Panus*, and *Pleurotus*, then it could help to further delimit these

spores on anucleate basidium. 7. Postmeiotic mitosis occurring in spores. 8. Two binucleate spores on a nucleate basidium. The other two spores have presumably been dislodged. Note that nuclei are at opposite ends of spore. 9. Uninucleate spores on quadrinucleate basidium following back-migration of proximal nuclei derived from postmeiotic mitosis. 10. Discharged quadrinucleate basidium. 11. Mature uninucleate spores. 12. Anomalous basidium with three uninucleate spores, one anucleate spore, and a pentanucleate basidium. One meiotic product may have failed to enter the spore and gone through postmeiotic mitosis in the basidium. Bar = 10 μ m. All figures are to same scale.



FIGS. 13–24. *Panus lecomtei* somatic nuclei and basidiosporogenesis. 13. Dikaryotic hypha in hymenophoral trama. 14. Mitotic division in clamp connection in trama. 15. Prefusion diploid basidium (left), and diploid basidium after karyogamy (right). 16. Anaphase I basidium (left) and prefusion basidium (right). 17. Basidium with meiotic tetrad and developing sterigmata and spores (right). 18. Meiotic products entering spores. 19. Uninucleate spores on anucleate basidium. 20. Postmeiotic mitosis occurring in spores. 21. Binucleate spores on anucleate basidium. 22. Uninucleate spores on quadrinucleate basidium following back-migration of proximal nuclei derived from postmeiotic mitosis. The nuclei remaining in the spores are still in the distal part of the spore. 23. Discharged quadrinucleate basidium. 24. Anomalous basidium with six nuclei and three sterigmata. Bar = 10 μ m. All figures are to same scale.

controversial genera. To expand on the previous report for *N. adhaerens* (Kühner et al., 1962), we also examined *N. lepideus*.

MATERIALS AND METHODS

Material for staining was obtained from fresh, cultured sporocarps of *L. tigrinus* (isolate D.744) and *Panus lecomtei* (Fr.) Corner (isolate D.616, as *Panus rudis* Fr.), and a field-collected sporocarp of *N. lepideus* (DSH 92-006). Protocols for in vitro sporocarp production in *L. tigrinus* and *P. lecomtei* were described previously (Hibbett et al., 1993a, b). Voucher cultures are deposited at TMI and DUKE. Voucher sporocarps of *L. tigrinus* and *P. lecomtei* are deposited at TMI; the voucher for *N. lepideus* is in the personal collection of DSH.

Nuclei were visualized by acid hydrolysis-Giemsa staining (after Aist, 1969). Fresh, hand-sectioned pieces of lamellae were fixed in 99.5% ethanol-glacial acetic acid (3:1 by volume) for 2 or more h, washed for 10 min in 35% ethanol, washed for 10 min in cool tap water, hydrolyzed for 6–8 min (bracketed in 1 min increments) in 1 N HCl at 60 C, washed for 10 min in cool tap water, and transferred to approximately 1 ml 0.067 M PO_4 buffer (Wako, Osaka, Japan) for approximately 10 min. Each vial received 9–11 drops of Giemsa stain (Merck, Darmstadt) and was stained overnight. Samples were stored in Giemsa- PO_4 buffer for up to 6 months.

Fragments of stained lamellae were squashed beneath a coverslip, which was ringed with nail polish to prevent drying, and observed under oil immersion. Observations were recorded with Fuji Neopan film.

RESULTS AND DISCUSSION

LENTINUS TIGRINUS.—Tramal hyphae and prefusion basidia were binucleate (FIGS. 1, 2). Prefusion basidia were cylindric to tapering, with nuclei oriented one directly above the other in the center of the basidium, parallel to the longitudinal axis of the basidium (FIG. 2). Following karyogamy, diploid nuclei initially occupied the same location as the prefusion nuclei and later migrated towards the distal portion of the basidium (FIGS. 2, 3). The spindle in anaphase I was oriented perpendicular to the long axis of the basidium (FIG. 2). Telophase I nuclei were generally slightly more condensed than prefusion nuclei. As meiosis progressed, the basidia became more clavate and exserted from the hymenium (FIG. 3). Anaphase II occurred at the extreme distal portion of the basidium, with the spindles oriented at an oblique angle to the longitudinal axis of the basidium (FIG. 3). Telophase II nuclei were initially highly condensed, but relaxed

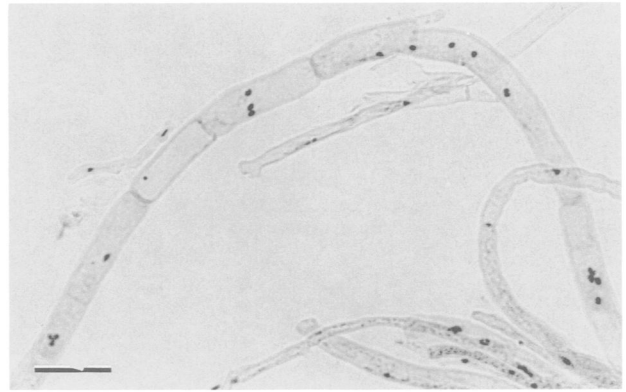
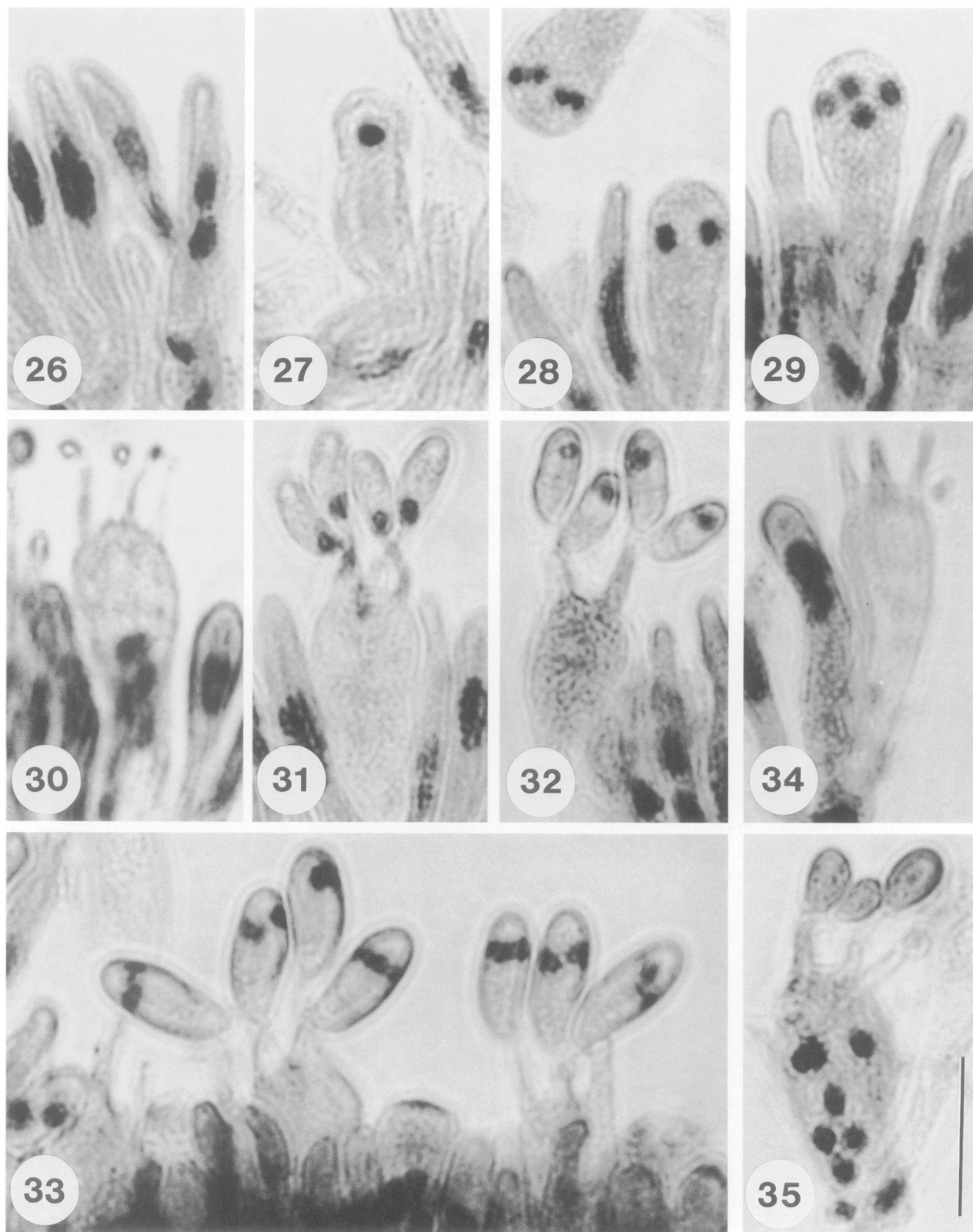


FIG. 25. *Neolentinus lepideus* multinucleate, clampless somatic hyphae in hymenophoral trama. Bar = 25 μm .

somewhat and descended to the center of the basidium (FIG. 4). While the meiotic tetrad was in the center of the basidium, the sterigmata and spores began to form (FIG. 4). The four meiotic products migrated towards the apex of the basidium, elongated, and migrated into the basidiospores (FIG. 5), which thus became uninucleate (FIG. 6). Postmeiotic mitosis occurred in the basidiospores (FIG. 7), which became transiently binucleate (FIG. 8). The mitotic spindle was oriented parallel to the long axis of the spore and the binucleate spores had one nucleus in the distal part of the spore and the other near the base (FIG. 8). The proximal nucleus in each basidiospore migrated back into the basidium and eventually came to lie in the middle of the basidium where they remained highly condensed (FIGS. 9, 10). The remaining nucleus in each spore moved to the center of the spore and appeared to relax slightly (FIG. 11). Discharged basidia were quadrinucleate (FIG. 10). Occasional anomalous basidia were observed. These generally seemed to result from failure of meiotic products to enter spores (FIG. 12), production of too few or too many spores, or failure of products of postmeiotic mitosis to return to the basidium.

PANUS LECOMTEI.—Results were essentially identical to those of *L. tigrinus* (FIGS. 13–24). Postmeiotic mitosis occurred in the basidiospores, with the mitotic spindle parallel to the longitudinal axis of the spore (FIGS. 20, 21). This was followed by back-migration of one nucleus from each spore (FIG. 22), resulting in uninucleate mature spores and quadrinucleate discharged basidia (FIG. 23). Anomalous basidia were also observed (FIG. 24).

NEOLENTINUS LEPEDEUS.—Hyphae in the trama of the hymenophore contained irregular numbers of nuclei. The variation was greatest in certain inflated tramal hyphae, which had from one to seven highly con-



FIGS. 26–35 *Neolentinus lepideus* basidiosporogenesis. 26. Dikaryotic prefusion basidia (right) and diploid basidia that have undergone karyogamy (left). 27. Diploid basidium with condensed nucleus. 28. Anaphase I basidium (lower right) and anaphase II basidium (upper left). 29. Telophase II basidium. 30. Tetrad of relaxed meiotic products that has descended to lower part of basidium. Sterigmata and spores have begun to form. 31. Meiotic products migrating into spores. 32. Uninucleate spores on anucleate basidium. 33. Two basidia with spores in various stages of postmeiotic mitosis. One spore has been dislodged

densed nuclei (FIG. 25). Simple and clamped septa were observed. The presence of only a single nucleus in some cells suggests that some of the simple septa may be adventitious. Karyogamy and meiosis proceeded very much like those in *L. tigrinus* and *P. lecomtei*, except that prophase I and anaphase I nuclei were more condensed than in *L. tigrinus* and *P. lecomtei* (FIGS. 26–34). Postmeiotic mitosis occurred in the spores (FIG. 33), but there was no back-migration. Unlike *L. tigrinus* and *P. lecomtei*, the mitotic spindle was transversely oriented relative to the longitudinal axis of the spore (FIG. 33). Mature spores were binucleate (FIG. 33) and the discharged basidia were anucleate (FIG. 34). Anomalous basidia were observed (FIG. 35).

The patterns of nuclear behavior in *L. tigrinus* and *P. lecomtei* represent type C nuclear behavior, as defined by Duncan and Galbraith (1972), whereas that of *N. lepideus* is type D nuclear behavior (TABLE I). Our observations of *N. lepideus* agree with the previous report from *N. adhaerens* (Kühner et al., 1962).

Postmeiotic nuclear behavior may be useful for resolving limits of lentinoid-pleurotoid genera. *Lentinus* and *Panus* have C type nuclear behavior, and thus can be distinguished from *Pleurotus* which has A type nuclear behavior, even though all three genera have uninucleate spores. *Neolentinus* can be distinguished from the other genera because it alone has binucleate spores and D type nuclear behavior. In addition, the multinucleate condition of somatic hyphae that we observed in *N. lepideus* was unique among the species investigated. Kühner et al. (1962) also observed multinucleate cells in *N. adhaerens*, but only in monokaryotic hyphae. Nobles (1948) observed that the tips of growing hyphae in the “advancing zone” in cultures of *N. lepideus* (as *L. lepideus* Fr.) and *N. kauffmanii* (Smith) Redhead & Ginns (as *L. kauffmanii* Smith) are simple-septate, but that side branches further back from the tips are clamped, as are hyphae in all parts of cultures of *L. tigrinus*. These observations suggest that cytology of mycelia and fruiting bodies may provide additional characters for segregation of *Neolentinus* from other lentinoid-pleurotoid fungi.

Unfortunately, nuclear behavior cannot address the boundaries between *Lentinus* and *Panus*. However, nuclear behavior may be informative about relationships of *Lentinus* and *Panus* at a more inclusive level. In a cladistic context, all putative synapomorphies constitute evidence for the existence of particular mono-

phyletic groups. The presence of C type nuclear behavior in *Lentinus* and *Panus* may therefore suggest that they are part of a larger clade that does not include *Neolentinus* or *Pleurotus*. However, if C type nuclear behavior is a symplesiomorphy, then it does not constitute evidence that the group containing *Lentinus* and *Panus* is monophyletic. We do not yet have a hypothesis of polarity for this character and it is, therefore, not possible to determine which of the states is plesiomorphic and which are derived.

We do not mean to imply that a phylogenetic classification for lentinoid-pleurotoid fungi can be based solely on nuclear behavior, or any other single character. Indeed, Mueller and Ammirati (1993) noted that the distribution of certain nuclear behavior types across morphologically divergent families, and the diversity of nuclear behavior types within certain families suggest that this character has considerable homoplasy. In addition, nuclear behavior has been sampled in only a few exemplar species of lentinoid-pleurotoid genera (TABLE I). It may be premature to generalize the few results obtained so far to entire genera. Nevertheless, we feel that nuclear behavior shows promise as a phylogenetic character. We therefore recommend that nuclear behavior be examined in more species of lentinoid-pleurotoid fungi, and that its phylogenetic significance be evaluated in terms of congruence with other independent characters.

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LITERATURE CITED

- Aist, J. R. 1969. The mitotic apparatus in fungi, *Ceratocystis fagacearum* and *Fusarium oxysporum*. *J. Cell Biol.* 40: 120–135.
- Corner, E. J. H. 1981. The agaric genera *Lentinus*, *Panus*, and *Pleurotus*. *Beih. Nova Hedwigia* 69: 1–189.
- Duncan, E. G., and M. H. Galbraith. 1972. Post-meiotic events in the Homobasidiomycetidae. *Trans. Brit. Mycol. Soc.* 58: 387–392.
- Hibbett, D. S. 1993. Towards a phylogenetic classification

from the basidium on the right. Note that the mitotic spindles are transversely oriented relative to the longitudinal axis of the spores (compare to FIGS. 7 and 20). A mature binucleate spore is at the lower left. 34. Anucleate discharged basidium. 35. Anomalous basidium with partially formed spores and eight nuclei. Bar = 10 μ m. All figures are to same scale.

- for shiitake: taxonomic history and molecular perspectives. *Rept. Tottori Mycol. Inst.* 30: 30–42.
- , S. Murakami, and A. Tsuneda. 1993a. Hymenophore development and evolution in *Lentinus*. *Mycologia* 85: 428–443.
- , ———, and ———. 1993b. Sporocarp ontogeny in *Panus* (Basidiomycotina): evolution and classification. *Amer. J. Bot.* 80: 1336–1348.
- , and R. G. Thorn. 1994. Nematode-trapping in *Pleurotus tuberregium*. *Mycologia* 86. (In press)
- , and R. Vilgalys. 1993. Phylogenetic relationships of *Lentinus* (Basidiomycotina) inferred from molecular and morphological characters. *Syst. Bot.* 18: 409–433.
- Johnson, J. E., and A. S. Methven. 1994. *Panus conchatus*: cultural characters and mating data. *Mycologia* 86: 146–150.
- Kühner, R. 1980. Les hyménomycètes agaricoïdes, étude générale et classifications. *Bull. Soc. Linn., Lyon*, Numéro spécial. 1027 pp.
- , D. LAMOURE, AND M.-L. FICHET. 1962. *Lentinus adhaerens* A. & S. ex Fr., morphologie, caryologie, sexualité. *Bull. Trimestriel Soc. Mycol. France* 78: 254–277.
- Molina, F. I., P. Shen, and S. C. Jong. 1992. Molecular evidence supports the separation of *Lentinula edodes* from *Lentinus* and related genera. *Canad. J. Bot.* 70: 2446–2452.
- Mueller, G. J., G. M. Mueller, L.-H. Shih, and J. F. Ammirati. 1993. Cytological studies in *Laccaria* (Agaricales). I. Meiosis and postmeiotic mitosis. *Amer. J. Bot.* 80: 316–321.
- Mueller, G. M., and J. F. Ammirati. 1993. Cytological studies in *Laccaria* (Agaricales). II. Assessing phylogenetic relationships among *Laccaria*, *Hydnangium*, and other Agaricales. *Amer. J. Bot.* 80: 322–329.
- Nobles, M. K. 1948. Studies in forest pathology. IV. Identification of cultures of wood-rotting fungi. *Canad. J. Res., Sect. C.* 26: 281–431.
- Pegler, D. N. 1975. The classification of the genus *Lentinus* Fr. (Basidiomycota). *Kavaka* 3: 11–20.
- . 1983. The genus *Lentinus*. *Kew Bull., Addit Ser.* 10: 1–281.
- Redhead, S. A., and J. H. Ginns. 1985. A reappraisal of agaric genera associated with brown rots of wood. *Trans. Mycol. Soc. Japan.* 26: 349–381.
- Rune, F. 1994. *Neolentinus*—a well-founded genus in Pleurotaceae that includes *Heliocybe*. *Mycol. Res.* 98: 542–544.
- Singer, R. 1986. *The Agaricales in modern taxonomy*. 4th ed. Koeltz Scientific Books, Koenigstein, Germany. 981 pp.
- Slézec, A.-M. 1984. Variabilité du nombre chromosomique chez les pleurotes des ombellifères. *Canad. J. Bot.* 62: 2610–2617.