

## The number of nuclei in basidiospores of 63 species of ectomycorrhizal Homobasidiomycetes

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**Abstract:** The production of even a limited number of heterokaryotic spores would be advantageous for establishing new individuals after long distance dispersal. While *Suillus* and *Laccaria* species are known to produce binucleate, heterokaryotic spores, this condition is poorly studied for most ectomycorrhizal fungi. To begin addressing this matter the number of nuclei in basidiospores was recorded from 142 sporocarps in 63 species and 20 genera of ectomycorrhizal (EM) fungi. The mean proportion of binucleate basidiospores produced by sporocarps within a species ranged from 0.00 to 1.00, with most genera within a family showing similar patterns. Basidiospores from fungi in *Amanita*, Cortinariaceae and *Laccaria* were primarily binucleate but were likely still homokaryotic. Basidiospores from fungi in Boletaceae, *Cantharellus*, Rhizopogonaceae, Russulaceae, Thelephorales and *Tricholoma* were primarily uninucleate, but binucleate basidiospores were observed in many genera and in high levels in *Boletus*. Further research is needed to relate basidiospore nuclear number to reproductive potential in ectomycorrhizal species.

**Key words:** DAPI, primary succession, spore dispersal

### INTRODUCTION

I investigated the number of nuclei contained in basidiospores of ectomycorrhizal fungi in the Homobasidiomycetes. It is assumed that a dikaryotic thallus typically forms from the fusion of hyphae from two haploid basidiospores of opposite mating type (Gardes et al 1990, Kropp and Fortin 1988). Although data are lacking for most EM species many Basidiomycetes produce binucleate basidiospores (Buller 1924, Duncan 1970, Kühner 1977, Mueller and Ammirati 1993). To date the only EM genera with species that are known to produce dikaryotic spores are *Suillus*, *Laccaria* and *Hydnagium*, the latter genus being a hypogeous relative of *Laccaria* (Bonello et al 1998,

Jacobson and Miller 1994, Mueller et al 1993, Treu and Miller 1993). Dikaryotic spores produced through secondary homothallism may increase the success of establishing new individuals dispersing to uncolonized areas, a strategy with clear parallels in plants that produce viable seed through self-fertilization. Although a self-fertilizing strategy negatively affects a population because of inbreeding, it lets individuals establish new populations after long distance dispersal (Jain 1976). Here I report the nuclear condition of mature basidiospores from more than 60 species of EM fungi and review the literature to assess the potential for these species to produce self-fertile basidiospores. These results are discussed with respect to EM ecology during primary succession.

### MATERIALS AND METHODS

The fungi were collected as part of a project investigating ectomycorrhizal ecology in a primary successional system on the coast of Oregon (Ashkannejhad and Horton 2006). In this system isolated shore pine (*Pinus contorta* Dougl. ex Loud var. *contorta*) seedlings are establishing at least 100 m from existing ectomycorrhizal (EM) fungal networks associated with surrounding forests. Under such conditions establishing plants are inoculated by propagules (spores, sclerotia or hyphal fragments) dispersed by wind and animals (Allen 1987, Allen et al 1984).

*Site description.*—The study area is located along the central coast of Oregon at 43°5'N latitude and 124°1'W longitude. The area is part of the Oregon Dunes National Recreation Area in the Suislaw National Forest. Most of the collections were made along the edge of the forests where *Pinus contorta* dominates, but other ectomycorrhizal hosts are commonly observed including *Pseutosuga menziesii* Mirb. Franco, *Picea sitchensis* Bong. Carr., *Tsuga heterophylla* Rag. Sarg., *Salix hookeriana* Barr., *Arctostaphylos uva-ursi* (L.) Spreng. and *A. columbiana* Piper. Further details of the study area are published elsewhere (Ashkannejhad and Horton 2006).

*Sporocarp collections.*—Sporocarps were collected in fall 1999 and 2000. Care was taken to avoid sampling sporocarps from the same genet by collecting specimens from different plots (separated by hundreds of meters) for the first four sporocarps of a species. Species were identified with available keys in the library of J.M. Trappe at Oregon State University and through the help of experts in the different groups of fungi (see acknowledgments).

Basidiospores were deposited on microscope slides by placing the slides beneath the hymenium surface within 8 h

TABLE I. Percentage of binucleate spores by species

Species	Family or order	n	Mean	SE	Minimum	Maximum
<i>Amanita muscaria</i>	Amanitaceae	2	98	1	97	98
<i>Boletus edulis</i>	Boletaceae	5	73	15	24	100
<i>Boletus subtomentosus</i>	Boletaceae	4	8	4	1	16
<i>Boletus zelleri</i>	Boletaceae	1	0		0	0
<i>Leccinum manzanitae</i>	Boletaceae	2	2	2	0	3
<i>Cantharellus formosus</i> var. <i>roseocanus</i>	Cantharellaceae	3	0	0	0	0
<i>Cortinarius aurantiobasis</i>	Cortinariaceae	2	99	1	98	100
<i>Cortinarius croceus</i>	Cortinariaceae	2	100	0	100	100
<i>Cortinarius orange</i>	Cortinariaceae	2	99	1	98	100
<i>Cortinarius semisanguineus</i>	Cortinariaceae	2	100	1	99	100
<i>Cortinarius seriocybe</i> gp.	Cortinariaceae	1	96		96	96
<i>Cortinarius seriocybe</i> gp. violet	Cortinariaceae	1	100		100	100
<i>Cortinarius telemonia</i> gp.	Cortinariaceae	2	99	1	98	100
<i>Cortinarius traganus</i>	Cortinariaceae	1	99		99	99
<i>Cortinarius vanduzerensis</i>	Cortinariaceae	2	100	0	100	100
<i>Hebeloma</i> sp. 1	Cortinariaceae	1	95		95	95
<i>Hebeloma</i> sp. 2	Cortinariaceae	1	98		98	98
<i>Inocybe lacera</i>	Cortinariaceae	2	96	2	94	97
<i>Inocybe</i> sp.	Cortinariaceae	1	100		100	100
<i>Inocybe sambucina</i>	Cortinariaceae	3	96	2	93	98
<i>Chroogomphus rutilus</i>	Rhizopogonaceae	3	2	1	1	4
<i>Chroogomphus</i> sp.	Rhizopogonaceae	1	1		1	1
<i>Chroogomphus vinicolor</i>	Rhizopogonaceae	1	0		0	0
<i>Rhizopogon fuscorubens</i>	Rhizopogonaceae	3	0	0	0	0
<i>Rhizopogon occidentalis</i>	Rhizopogonaceae	3	0	0	0	0
<i>Rhizopogon subcaerulescens</i>	Rhizopogonaceae	4	0	0	0	0
<i>Rhizopogon evadens</i>	Rhizopogonaceae	6	0	0	0	1
<i>Suillus brevipes</i>	Rhizopogonaceae	4	1	1	0	2
<i>Suillus lakei</i>	Rhizopogonaceae	2	6	5	1	10
<i>Suillus tomentosus</i>	Rhizopogonaceae	11	2	1	0	5
<i>Suillus umbonatus</i>	Rhizopogonaceae	4	1	0	1	2
<i>Lactarius deliciosus</i> -like	Russulaceae	2	3	3	0	6
<i>Lactarius rufus</i>	Russulaceae	1	0		0	0
<i>Russula cascadiensis</i>	Russulaceae	4	0	0	0	1
<i>Russula consobrina</i> cf.	Russulaceae	1	0		0	0
<i>Russula fragilis</i>	Russulaceae	1	0		0	0
<i>Russula pectinoides</i>	Russulaceae	1	3		3	3
<i>Russula</i> sp.	Russulaceae	4	1	1	0	3
<i>Russula</i> sp. brown violet	Russulaceae	1	0		0	0
<i>Russula</i> sp. green brown	Russulaceae	1	0		0	0
<i>Russula</i> sp. orange	Russulaceae	1	2		2	2
<i>Bankera fuligineo-alba</i>	Thelephorales	1	2		2	2
<i>Boletopsis subsquamosus</i>	Thelephorales	1	0		0	0
<i>Hydnellum conrescens</i>	Thelephorales	1	0		0	0
<i>Hydnellum scrobiculatum</i>	Thelephorales	1	0		0	0
<i>Phellodon niger</i>	Thelephorales	1	0		0	0
<i>Sarcodon fuscoindicum</i>	Thelephorales	1	3		3	3
<i>Sarcodon imbricatum</i>	Thelephorales	2	0	0	0	0
<i>Sarcodon</i> sp.	Thelephorales	2	3	1	2	3
<i>Thelephora americana</i>	Thelephorales	2	4	3	1	7
<i>Laccaria bicolor</i>	Tricholomataceae	3	99	1	97	100
<i>Laccaria laccata</i>	Tricholomataceae	4	96	1	93	97
<i>Laccaria</i> sp.	Tricholomataceae	1	93		93	93
<i>Tricholoma flavovirens</i>	Tricholomataceae	4	2	1	0	4
<i>Tricholoma imbricatum</i>	Tricholomataceae	4	0	0	0	0
<i>Tricholoma luteomaculosum</i>	Tricholomataceae	1	1		1	1

TABLE I. Continued

Species	Family or order	n	Mean	SE	Minimum	Maximum
<i>Tricholoma magnivelare</i>	Tricholomataceae	4	0	0	0	1
<i>Tricholoma mutabile</i>	Tricholomataceae	5	1	0	0	1
<i>Tricholoma</i> sp.	Tricholomataceae	1	0		0	0
<i>Tricholoma sejunctum</i>	Tricholomataceae	1	1		1	1
<i>Tricholoma ustale</i>	Tricholomataceae	2	0	0	0	0
<i>Tricholoma vaccinum</i>	Tricholomataceae	1	0		0	0

of collection. Sporocarps and slides were placed in a paper or wax paper bag overnight. The next morning the sporocarps were removed, and a second microscope slide was placed over the slide with the spore print, taped down and labeled. Basidiospores of hypogeous fungi (*Rhizopogon* spp.) were collected by placing a thin section of the mature gleba between two microscope slides. Spores were stored at room temperature for up to 4 mo before the nuclei were observed. Most sporocarps and spore prints were preserved and vouchered except for degraded specimens. The vouchers are maintained at SUNY-ESF.

*Spore preparation and nuclear visualization.*—DAPI staining procedure followed (Coleman et al 1981) with McIlvaine's dye solvent at pH 4.4 as summarized here. Basidiospores were fixed by scraping them off the microscope slide into a 1.5 mL Eppendorf tube with 70% ethanol, vortexed briefly, spun briefly in a microcentrifuge and left 1 h at room temperature. The samples then were spun in a microcentrifuge at 13 000 g for 1 min, and the ethanol was poured off. Material from the spore pellet was placed on a fresh microscope slide and left to dry under a fume hood (ca. 5 min). One drop of DAPI stain (0.5 µg/mL) was applied to the spore mass and a cover slip was placed over the material. Cover slips were sealed with clear nail polish. Spores were viewed after 1–2 h with a Leica DMRB fluorescence microscope, under 400× magnification. The number of nuclei in 100–200 basidiospores from each sporocarp was recorded, with sporocarps serving as replicate samples.

#### RESULTS

For most species either uni- or binucleate basidiospores predominate (TABLE I). An exception to this rule is seen in *Boletus edulis*, in which variable but consistently moderate to high levels of binucleate basidiospores were observed from individual sporocarps. Note that binucleate basidiospores were observed for many species in which uninucleate basidiospores predominated. Note also that in Tricholomataceae, sporocarps from some species yielded 0 binucleate basidiospores ("Minimum") while others yielded only binucleate basidiospores ("Maximum"), as indicated by the relatively high standard error for this group.

Sporocarps in these genera tended to produce

uninucleate basidiospores: *Bankera*, *Boletopsis*, *Boletus*, *Cantharellus*, *Chroogomphus*, *Hydnellum*, *Lactarius*, *Leccinum*, *Rhizopogon*, *Russula*, *Phellodon*, *Sarcodon*, *Suillus*, *Thelephora* and *Tricholoma*. In contrast sporocarps in these genera tended to produce binucleate basidiospores: *Amanita*, *Cortinarius*, *Hebeloma*, *Inocybe* and *Laccaria*.

These generic patterns influence patterns at higher taxonomic levels. Sporocarps in Rhizopogonaceae, Russulaceae, Thelephorales and Tricholomataceae (except *Laccaria*) produce mostly uninucleate basidiospores and fungi in Cortinariaceae produce mostly binucleate basidiospores. The mixed condition observed in Boletaceae is strongly influenced by *Boletus edulis*, but *B. subtomentosus* also produced a moderate amount of binucleate basidiospores. In the case of Tricholomataceae the two spore types separate into *Laccaria* and *Tricholoma*.

#### DISCUSSION

These results are in agreement with cytological data (Duncan and Galbraith 1972, Hibbett and Thorn 2001, Kühner 1977, Mueller et al 1993). Post meiotic mitosis in most of the uninucleate nontheleporoid genera in this list is thought to occur in the spore, with one daughter nucleus migrating back into the basidium (Mueller and Ammirati 1993). Hibbett and Thorn (2001) suggest that theleporoid genera such as *Boletopsis*, *Hydnellum* and *Sarcodon* probably follow this pattern as well but do not rule out that postmeiotic mitosis may occur in the sterigmata with one daughter nucleus migrating into the basidium. Regardless of the mechanisms involved uninucleate, homokaryotic basidiospores cannot establish a dikaryon upon germination without fusing with hyphae containing compatible nuclei.

Binucleate spores in *Amanita*, *Cortinarius* and *Hebeloma* are formed when postmeiotic mitosis follows migration of a single nucleus into a spore without back migration of the daughter nuclei (Mueller and Ammirati 1993). It is reasonable to assume that *Inocybe*, another member of Cortinariaceae in this study, follows the same pattern. While fungi in Cortinariaceae and *Amanita* produce bi-

nucleate basidiospores, the basidiospores are homokaryotic and functionally equivalent to uninucleate homokaryotic basidiospores. These multiple nuclei might provide an increased metabolic capability upon germination.

Binucleate spores in some *Laccaria* species had been thought to form when postmeiotic mitosis occurs in the basidium followed by migration of multiple nuclei into the spores (Shih et al 1986, Tommerup et al 1991). However Mueller et al (1993) and Mueller and Ammirati (1993) report that postmeiotic mitosis actually occurred in the basidiospores in four-spored species of *Laccaria* and further that earlier reports documenting mitosis occurring in basidia of four-spored species might have missed the fact that such basidia collapsed before mature basidiospores formed. The basidiospores observed here were mature because they were collected from spore prints, but the location of mitosis was not identified. However all of these species had four-spored basidia, suggesting that their binucleate spores were likely the result of postmeiotic mitosis after the migration of a single nucleus into the spores and were therefore homokaryotic.

I observed many of the species producing mostly uninucleate spores also produced a few binucleate spores. Variation in the number of nuclei packaged in spores within a species has been reported in a variety of dark-spored nonectomycorrhizal taxa (Buller 1924, Kühner 1977). I found that *Boletus* showed a high level of variation in the proportion of binucleate basidiospores produced by single sporocarps (24–100%). Thirteen species of *Boletus* other than those in this study were reported to produce only uninucleate basidiospores (Duncan 1970; Duncan and Galbraith 1972; Yoon and McLaughlin 1986, 1987). Kerrigan and Ross (1987) reported that cold temperatures induce variation in the number of spores borne on basidia, which led the authors to conclude that basidiospore number is not fixed in *Agaricus*. The number of basidiospores born on basidia may not be fixed for other genera as well, and this could lead to variation in the number of nuclei observed here in *Boletus* and the other species.

The mating types of the nuclei in the occasional binucleate spore produced by uninucleate species remains unclear. Spores of most EM fungi unfortunately cannot be induced to germinate and are otherwise difficult to maintain in culture allowing mating studies. Thus many of the advances in our understanding of spore biology in pathogenic and saprotrophic fungi (Raper 1966) have not been made for most EM fungi. However work has been successful for *Suillus*, *Laccaria* and *Hydnagium*. It appears that the small percentage of binucleate basidiospores

produced by species of *Suillus* are indeed heterokaryotic (Bonello et al 1998, Jacobson and Miller 1994, Treu and Miller 1993). In the case of *Laccaria* and its hypogeous relative *Hydnagium*, clamped hyphae in single spore isolates from the two-spored species are an indication of the production of heterokaryotic spores (Tommerup et al 1991). The other uninucleate species in the present study remain intriguing because even a small number of self-fertile spores could be advantageous for colonization after long distance dispersal, analogous to the reproductive assurance hypothesis proposed by Jain (Jain 1976). When applied to plants this hypothesis suggests that a single seed can establish a new individual after long distance dispersal, and through self-fertilizing, a new viable population. The same would apply to Basidiomycetes but with the additional mechanism that a single dikaryotic spore would support the establishment of the new individual. Research should be conducted incorporating cultural (when possible), cytological and genetic approaches to directly assess the mating types found in these infrequent binucleate spores. Further, attention should be directed to species such as *Thelephora*, and *Tomentella* that are commonly observed in disturbed habitats, nurseries and plantations. It cannot be ruled out that these fungi may be successful in these settings because of unique aspects of their basidiospore biology.

*Basidiospore dispersal ecology.*—This study was part of an investigation into mechanisms contributing to successful establishment of EM fungi in pioneering pines, and the broader scope of the project can be used to inform our understanding of the importance of spore nuclear status of fungi establishing after long distance dispersal (>100 m from fruit bodies). Although I found a surprising number of species producing binucleate basidiospores in the area, only *Suillus* and *Rhizopogon* dominated on the primary successional pines (Ashkannejhad and Horton 2006). *Suillus* and *Rhizopogon* may produce some heterokaryotic spores (see *R. evadens*, TABLE I). However functioning basidiospores of both *Suillus* and *Rhizopogon* are dispersed by the millions by deer, as demonstrated with fecal pellets for EM inoculum in seedling bioassays (Ashkannejhad and Horton 2006). Mammals are known to eat ectomycorrhizal fungi, and their fecal pellets can contain large quantities of basidiospores (Claridge et al 1992, Colgan and Claridge 2002, Fogel and Trappe 1978, Johnson 1996, Kotter and Farentinos 1984, Launchbaugh and Urness 1992, Maser et al 1978, Maser and Maser 1988). In addition to eating single sporocarps, mammals

likely sample multiple sporocarps in an area, increasing the probability of outcrossing for preferred species. Species whose basidiospores are dispersed in fecal pellets apparently are successful in this habitat regardless of the nuclear condition of their basidiospores. Whether the nuclear status affects the establishment of species not dispersed by mammals remains to be determined.

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## LITERATURE CITED

- Allen MF. 1987. Re-establishment of mycorrhizas on Mount St. Helens: migration vectors. *Trans Brit Mycol Soc* 88:413–417.
- , MacMahon JA, Anderson DC. 1984. Reestablishment of Endogonaceae on Mount St Helens: survival of residuals. *Mycologia* 76:1931–1038.
- Ashkannejhad S, Horton TR. 2006. Ectomycorrhizal ecology under primary succession on the coastal sand dunes: interactions involving *Pinus contorta*, suilloid fungi and deer. *New Phytologist* 169:345–354.
- Bonello P, Bruns TD, Gardes M. 1998. Genetic structure of a natural population of the ectomycorrhizal fungus *Suillus pungens*. *New Phytologist* 138:533–542.
- Buller AHR. 1924. *Researches on fungi*. Vol. III: the production and liberation of spores in Hymenomycetes and Uredineae. London: Longman, Greene & Co.
- Claridge AW, Tanton MT, Seebeck JH, Cork SJ, Cunningham RB. 1992. Establishment of ectomycorrhizae on the roots of two species of *Eucalyptus* from fungal spores contained in the faeces of the long-nosed potoroo (*Potorous tridactylus*). *Austral J Ecol* 17: 207–217.
- Coleman AW, Maguire MJ, Coleman JR. 1981. Mithramycin and 4'-6-Diamidino-2-phenylindole (DAPI)-DNA staining for fluorescence microspectrophotometric measurement of DNA in nuclei, plastids and virus particles. *J Histochem Cytochem* 29:959–968.
- Cogan WI, Claridge AW. 2002. Mycorrhizal effectiveness of *Rhizopogon* spores recovered from faecal pellets of small forest-dwelling mammals. *Mycol Res* 106(3): 314–320.
- Duncan EG. 1970. Post-meiotic events in boleti. *Trans Brit Mycol Soc* 54:367–370.
- , Galbraith MH. 1972. Post-meiotic events in the homobasidiomycetidae. *Trans Brit Mycol Soc* 58(3): 387–392.
- Fogel R, Trappe JM. 1978. Fungus consumption (mycophagy) by small animals. *Northw Sci* 52:1–31.
- Gardes M, Wong KKY, Fortin A. 1990. Interaction between monokaryotic and dikaryotic isolates of *Laccaria bicolor* on roots of *Pinus banksiana*. *Symbiosis* 8:233–250.
- Hibbett DS, Thorn RG. 2001. Homobasidiomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA, eds. *The Mycota VII: systematics and evolution part B*. New York: Springer-Verlag.
- Jacobson KM, Miller OK Jr. 1994. Postmeiotic mitosis in the basidia of *Suillus granulatus*: Implications for population structure and dispersal biology. *Mycologia* 86(4):511–516.
- Jain SK. 1976. The evolution of inbreeding in plants. *An Rev Ecol Syst* 7:469–495.
- Johnson CN. 1996. Interactions between mammals and ectomycorrhizal fungi. *Tree* 11(12):503–507.
- Kerrigan RW, Ross IK. 1987. Dynamic aspects of basidiospore number in *Agaricus*. *Mycologia* 79:204–215.
- Kotter MM, Farentinos RC. 1984. Formation of ponderosa pine ectomycorrhizae after inoculation with feces of tassel-eared squirrels. *Mycologia* 76:758–760.
- Kropp BR, Fortin JA. 1988. The incompatibility system and relative ectomycorrhizal performance of monokaryons and reconstituted dikaryons of *Laccaria bicolor*. *Can J Bot* 66:289–294.
- Kühner R. 1977. Variation of nuclear behaviour in the Homobasidiomycetes. *Trans Brit Mycol Soc* 68(1): 1–16.
- Launchbaugh KL, Urness PJ. 1992. Mushroom consumption (mycophagy) by North American cervids. *Gr Basin Natural* 54(4):321–327.
- Maser C, JM Trappe, RA Nussbaum. 1978. Fungal-small mammal interrelationships with emphasis on Oregon coniferous forests. *Ecology* 59:799–809.
- , Maser Z. 1988. Interactions among squirrels, mycorrhizal fungi and coniferous forests in Oregon. *Gr Basin Natural* 48:358–369.
- Mueller GJ, Mueller GM, Shih L, Ammirati JF. 1993. Cytological studies in *Laccaria* (Agaricales) I. Meiosis and post-meiotic mitosis. *Am J Bot* 80:316–321.
- Mueller GM, Ammirati JF. 1993. Cytological studies in *Laccaria* (Agaricales). II. Assessing phylogenetic relationships among *Laccaria*, *Hydangium*, and other Agaricales. *Can J Bot* 80(3):322–329.
- Raper JR. 1966. *Genetics of sexuality in higher fungi*. New York: The Ronald Press Co. 283 p.
- Shih L, Mueller GM, Ammirati JF. 1986. Basidial cytology of *Laccaria* species (Agaricales). *Mycol Soc Am Newsl* 37:42.
- Tommerup IC, Bougher NL, Malajczuk N. 1991. *Laccaria fraterna*, a common ectomycorrhizal fungus with monosporic and bi-sporic basidia and multinucleate spores—

- comparison with the quadristerigmate, binucleate *L. laccata* and the hypogeous relative *Hydnagium carneum*. Mycol Res 95:689–698.
- Treu R, Miller OK Jr. 1993. Nuclear status of two *Suillus* species. Mycologia 85(1):46–50.
- Yoon KS, McLaughlin DJ. 1986. Basidiosporogenesis in *Boletus rubinellus* II. Late spore development. Mycologia 78(2):185–197.
- . 1987. Meiosis and postmeiotic mitosis in *Boletus rubinellus*. Kor J Bot 30:225–247.