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Morphological and cytological aspects of oidium formation in a basidiomycete, *Pholiota nameko*

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Pholiota nameko **produced abundant oidia on aerial hyphae from monokaryotic and dikaryotic test stocks, but oidia were rare on submerged hyphae. The oidia from the former stocks had a layer of hydrophobic protein between the cell wall and the inner cell membrane, which was absent in the oidia from the latter. The only remarkable differences in the morphological features of the oidia from monokaryotic and dikaryotic mycelia was the slightly larger size of the latter. Observation of various test stocks on slide cultures revealed that about 80% of oidia were produced from the secondary branched hypha, and about 20% from the terminal hyphal cell of the main hypha. In the former, the secondary hyphae were segmented to form several oidium cells; in the latter, a single or several oidia were formed at the terminal end of the main hypha. Most oidia from monokaryons and dikaryons had only one haploid nucleus, while the remainders were multinucleate. Among the stocks tested, most oidia had a DNA content with a haploid amount at the G1 phase of the cell cycle, but a few contained twice that amount corresponding to the G2 phase.**

Key Words——basidiomycete; mushroom; nuclear behavior; oidium formation; *Pholiota nameko*.

Asexual reproduction by means of conidia is widely distributed throughout various classes and orders of fungi (Bessey, 1964). Among conidia, the yeast-like or oidial forms generated by the rounding off and ultimate separation of vegetative cells are often called "oidia." Oidium formation is generally understood to be a process of vegetative propagation. Oidia may be disseminated by wind, insects, or other vehicles, and most of them germinate to form monokaryotic or dikaryotic mycelia in a suitable environment (Brodie, 1936). An oidium monokaryon can conjugate with another compatible monokaryon to form a dikaryotic mycelium in the natural habitats.

Oidium formation has been reported for many mushrooms, including *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray (Rao and Niederpruem, 1969), *Flammulina velutipes* (Curt.: Fr.) Singer (Brodie, 1936), *Favolus arcularius* (Fr.) Ames (Kitamoto, unpublished data), *Hypsizygus marmoreus* (Peck) Biglow (Yamanaka, 1995), and *Pholiota nameko* (T. Ito) S. Ito & Imai apud Imai (Arita, 1979). Basidiomycetous mushrooms may produce oidia or other types of asexual spores from both monokaryotic and dikaryotic mycelia, but the situation may change according to the species (Brodie, 1936). In *F. velutipes*, most oidia produced from dikaryotic mycelium have only one nucleus (Brodie, 1936; Takemaru, 1954). In the light of this, monokaryotization has primarily meaning on oidium formation. An earlier study discussed the involvement of nuclear selection in monokaryotization of dikaryotic mycelia in a bipolar basidiomycete, *P. nameko* (Masuda et al., 1995).

Research on oidia from mushrooms has mostly focused on their morphological features, but quantitative morphological and cytological studies on oidium formation are rare. This study analyzes oidium formation in *P. nameko* from morphological and cytological perspectives.

Materials and Methods

Organisms Monokaryotic stocks and their hybrid dikaryons of *P. nameko* were used. The hybrid production method has been described elsewhere (Masuda et al., 1995). Hybrid stocks were preserved on PDA slant cultures at 5°C.

Observation of oidium formation Four culture methods were used to observe oidium formation. Liquid static cultures were performed by inoculating a small piece of mycelium into a 50-ml Erlenmeyer flask containing 15 ml of PD medium (Difco). After incubation at 25°C in darkness for 2–4 wk, the cultures were agitated with a vortex mixer for about 3 min, and the fragments of mycelia were removed by filtration with a 3G2 glass filter. The oidia in suspension were washed twice with sterilized water by centrifugation for 15 min at 500 × *g*, then resuspended for morphological observation and to prepare a sample

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for electron microscopic observation. Slide culture was performed by inserting a sterilized cover glass for microscopic use at an acute angle into 15 ml of PDA medium (Nissui) in a Petri dish, 90 mm in diam. A small piece of mycelium was inoculated on the center of the medium, and incubated at 25°C in darkness for 1–2 wk to allow for hyphal growth on the glass surface. Cellophane culture was done by putting a sheet of cellophane on the surface of PDA, and incubating it to allow hyphal growth on the sheet. Agar block cultures were performed by cutting and standing a PDA block, $1 \times 1 \times 0.2$ cm, on a sterilized slide glass set in a Petri dish, inoculating, and incubating it to allow mycelial growth on the block. Oidium formation on the four culture substrates was observed at set intervals during the incubation period.

Cultivation and preparation of oidia Mycelial cultures in plastic Petri dishes were performed on 15 ml of PDA with the addition of extra agar at 0.2%. Plates were inoculated with a mycelial agar block cut from the plates for seed cultures, and incubated at 25°C in darkness for 2–4 wk. Then, 10 ml of sterilized water was injected onto the culture plate, and the colony surface was scraped with a spatula to release oidia from the mycelial mat. The oidium suspension was collected and filtered with a 3G2 glass filter to remove mycelial fragments. The resulting suspension was centrifuged at $500 \times g$ for 15 min, and the oidium sediment was resuspended in a small volume of sterilized water, then subjected to the following experimental procedures.

Measurement of oidia The size of oidia was measured with a micrometer under a microscope. The volume was calculated from the above data and compared with those obtained by the Coulter multisizer method. The oidium suspension was washed twice with a 0.08% saline solution and resuspended in the same solution to a cell concentration of 10^4–10^5 cells/ml. Cell volume was then measured with a Coulter Multisizer II (Coulter) by using a 70 μm aperture tube.

Electron microscopic observation An electron microscope was used to observe the surface structure of the oidia. They were frozen with liquid nitrogen at −196°C, freeze-etched at −100°C, and vacuum-coated with platinum and carbon. Samples were washed successively with the bleach reagent as well as 70% sulfuric acid. An electron microscope, JOEL 1200 EX, was used to observe the samples.

Giemsa staining Giemsa staining for the nuclei in oidium cells was done following the "Biotechnology laboratory manual" (Society of Fermentation and Bioengineering, 1992) with a minor modification. The oidia were fixed with cold AFA solution (50 ml of ethanol, 5 ml of glacial acetic acid, 5 ml of formaldehyde (37%) and 40 ml of distilled water) for 24 h. After washing the oidia with 1 M HCl by centrifugation, the oidium sediment was resuspended with 1 M HCl and incubated at 60°C for 10 min. The oidia in suspension were then washed successively with distilled water and 50 mM potassium phosphate buffer (pH 6.8). Staining was done for 2–24 h with a 15-fold diluted Giemsa staining solution (Merck) in the same buffer.

Determination of nuclear DNA content with propidium iodide The DNA content in the nucleus of the oidium from the test stock was determined by fluorocytometry after Takeo et al. (1993). The oidia were fixed with 70% of cold ethanol and washed with ice-cooled distilled water. They were centrifuged at $500 \times g$ for 10 min, then resuspended in water. About 1–5 ml of oidium suspension was mixed with 1 ml of a staining solution containing 5 μg propidium iodide and 0.5 mg RNase A (Sigma) dissolved in NS buffer (10.0 mM Tris, 0.25 M sucrose, 7.0 mM β-mercaptoethanol, 0.4 mM phenylmethylsufonyl fluoride, 1.0 mM EDTA, 1.0 mM $MgCl_2$, 0.1 mM $ZnCl_2$, pH 7.4). The reaction was performed for 2–4 h at 30°C, and the staining density of oidia with the fluorescent dye was measured with a fluorescent microscope (Olympus, model BHS-RFC) fitted with a fluorescent light measuring apparatus (Olympus, model OSP-1). The DNA content of the mycelia in the NX-4 strain was assigned as a reference to determine the relative values of the various samples. The RNase A in the above mixture was boiled for 10 min before use in order to inactivate the contaminating DNase.

Results

Morphology of oidia *Pholiota nameko* produced a large number of oidia from the monokaryotic and dikaryotic mycelia on PDA plates. Figure 1 shows the appearance of oidia from a monokaryotic strain, NX-6. Oidia were globular, oval or rod-shaped, but the majority was rod-shaped.

Table 1 summarizes the size and the volume of oidia from several *Pholiota* strains as measured with a micrometer and a Coulter multisizer. The average lengths of the long and short axes of oidia from monokaryons were 4.9 μm and 1.9 μm, respectively. The corresponding values for the oidia from dikaryons were 5.2 μm and 1.7 μm, respectively. The volumes of the former and the later oidia were 11.25 μm^3 and 12.21 μm^3, respectively, and these values were similar to those calculated from the length data of the oidia from both origins. The average oidium volume from monokaryotic test strains was slightly smaller than that from dikaryotic strains (cf.

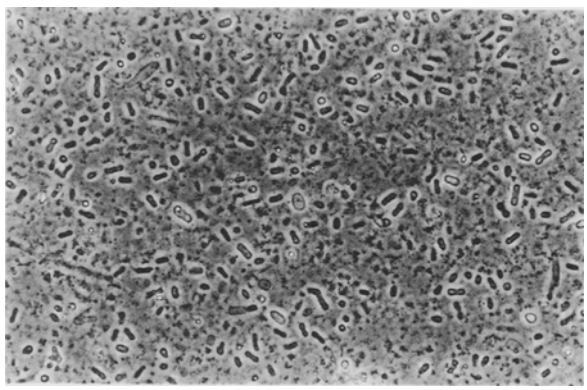


Fig. 1. Oidia from a monokaryotic strain, NX-6, in *Pholiota nemeko*.

Table 1. Average diam and volume of oidia from various monokaryotic and dikaryotic stocks in *Pholiota nameko*.

	Average diam[a] (μm)	Volume[b] (μm^3)	Pretreatment[c]
Monokaryotic stock			
NF-7	2.72±0.79	10.53	fixed
NF-7	2.90±0.70	12.76	none
NA-4	2.50±0.60	8.18	fixed
NA-4	2.77±0.57	11.12	none
NGW-20	2.82±0.86	11.73	fixed
NA-15	2.93±0.76	13.16	none
(Average)	2.77±0.71	11.25	—
Dikaryotic stock			
NA-4×NGW-19	2.75±0.66	10.88	fixed
NA-4×NGW-19	2.86±0.70	12.24	none
NGW-19×NF-7	2.80±0.73	11.48	fixed
NGW-9×NGW-19	3.01±0.80	14.27	none
(Average)	2.86±0.72	12.21	—

a) The average diam of each oidium was calculated from the data obtained with a Coulter multisizer. Each datum with the standard error was the average of ten replicates.
b) The volume of oidia was determined with a Coulter multisizer.
c) Oidia were fixed with 70% of ice-cold ethanol before determining the volume with a Coulter multisizer.

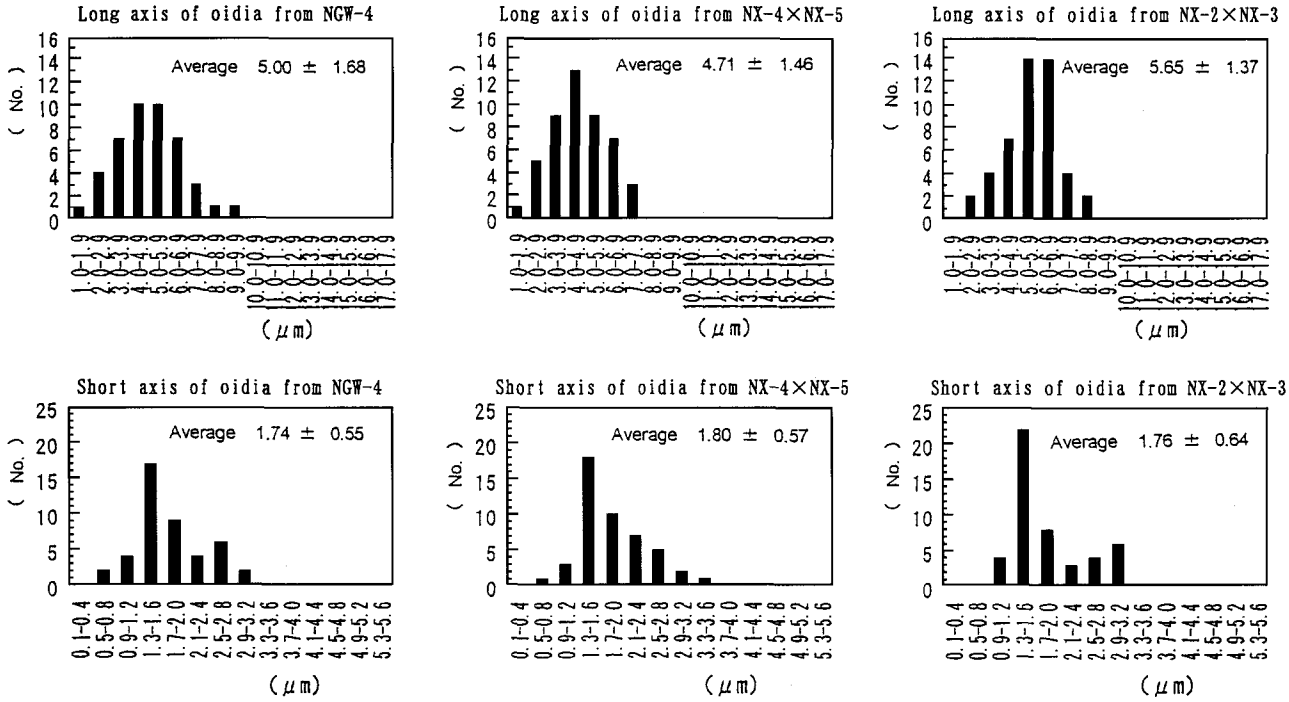
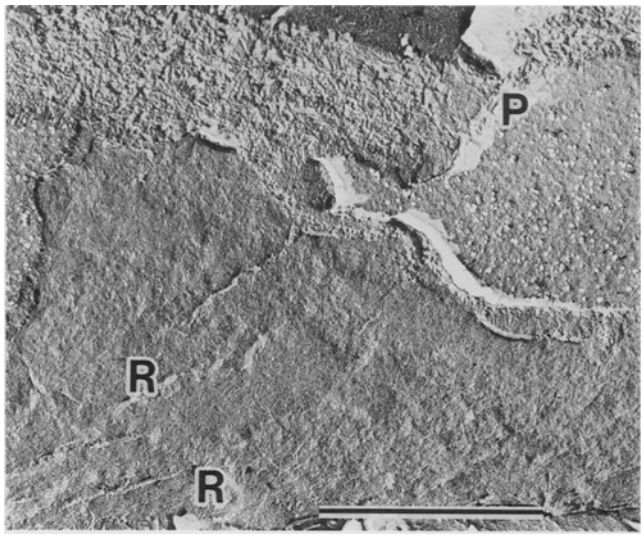


Fig. 2. Distribution of the sizes of oidia from the monokaryotic and dikaryotic strains of *Pholiota nemeko*.

NGW-4 and NX-4×NX-5, etc.).

Figure 2 shows the distributions of the oidium sizes from monokaryotic and dikaryotic mycelia. Both showed roughly normal distributions, and no significant statistical differences were found in oidium sizes between monokaryotic and dikaryotic stocks.

Oidium formation from aerial and submerged mycelia Observation of oidium formation in liquid static cultures in *P. nameko* showed that oidia were abundant on aerial hyphae, but rare on submerged hyphae. Figure 3 shows electron micrographs of oidia from submerged and aerial mycelia. The oidium cell from an aerial hypha possesses



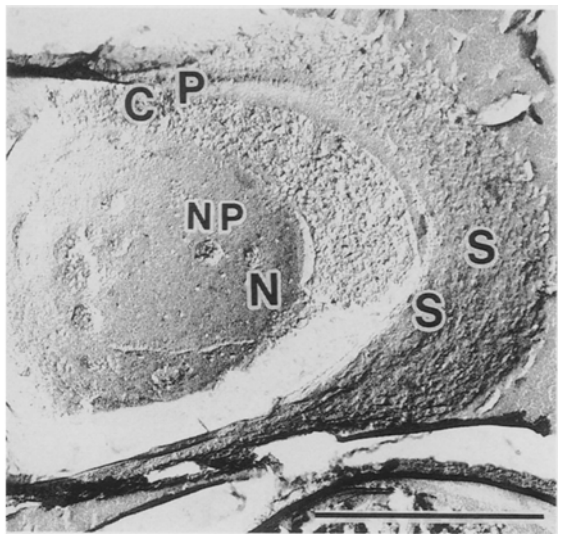


Fig. 3 (Left). Oidium originating from aerial mycelium of a monokaryotic stock, NX-6.
Note the hydrophobic rodlet layer (R). See also the P-face of the plasma membrane (P).
(Right). Freeze fractured electron micrograph of an oidium originating from submerged mycelium of a monokaryotic stock, NX-6. Note particulate wall surface (S) characteristics of submerged mycelium. See also the P-face of the plasma membrane (P), cytoplasm (C) and nucleus (N) with pores (NP). The bar indicates 1 μm.

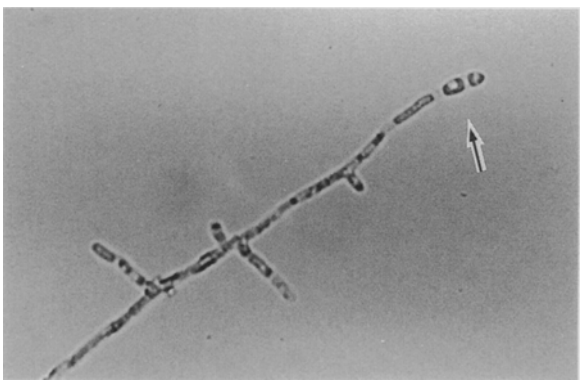


Fig. 4. Oidium formation from terminal hyphal cells of the main hypha (indicated with an arrow) in a dikaryotic stock, NGW-19 × NF-1.

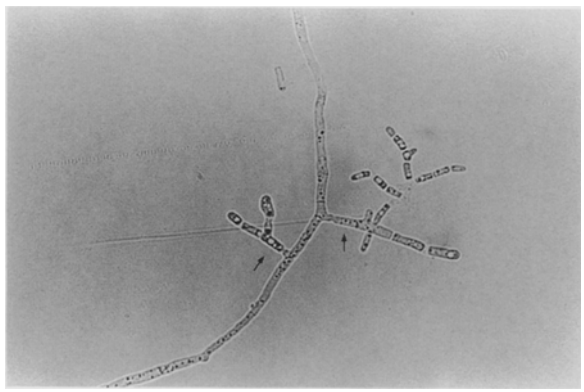


Fig. 5. Oidium formation from secondary branched hypha, which was developed vertically from the main hypha in a dikaryotic stock, NGW-19 × NF-5.

a hydrophobic rodlet layer between the cell wall and the cell membrane (Left). On the other hand, the submerged mycelium has a particulate wall surface and no protein layer. The picture also shows the P-face of the plasma membrane, cytoplasm, and nucleus with pores (Right). Therefore, it is assumed that there is some functional differentiation between the oidia of different origins.

Terminal and secondary branched hyphae as the sites of oidium formation Arita (1979) found that *P. nameko* produced two types of conidia: arthroconidia, which were formed on the lateral branches of vegetative hypha and on conidiophores that developed perpendicular to the main vegetative hypha; aleuriocondia, which were formed only from the blown-out end of the apex of conidiophores that were simple or branched with different lengths. In other words, the two types of oidiophores that are situated on the main or laterally branched hyphae had been reported. However, prior to the present study, no quantitative research on oidium formation sites and no time-chase observation of oidium formation had been done.

The slide and cellophane cultures of various test stocks showed that the oidia were produced from the terminal hyphal cells of the main hypha, and from the secondary branched hypha that developed vertically from the main hypha (Figs. 4, 5). In the former, oidium formation occurred in sequence: the first oidium developed on the terminal end of the main hypha, the hyphal cell continued to elongate, and the second oidium developed at the terminal end between the mother hypha and the first oidium. A single cell branch was most common, but in some cases two or more oval or cylindrical oidia stemmed from the same terminal hypha. This process produced a chain or a cluster of oidia. In the second pattern, the

first step in oidium formation was the branching of the secondary hypha almost perpendicularly from the main hypha, which followed by hyphal elongation. Many vacuoles were then produced in the branched hypha, and cell plasma was gradually separated and assembled in the hyphal cell. A new cell wall was then formed around each plasma pellet. Finally, the original cell wall was dissolved, and a chain of many cylindrical oidia was formed simultaneously from the secondary branched hypha.

Observations showed that about 80% of oidia were produced from the secondary branched hyphae and about 20% from the terminal hyphal cell of the main hypha (Table 2).

Number of nuclei in oidium cells Figure 6 shows the Giemsa staining of nuclei in oidium cells from *P. nameko*.

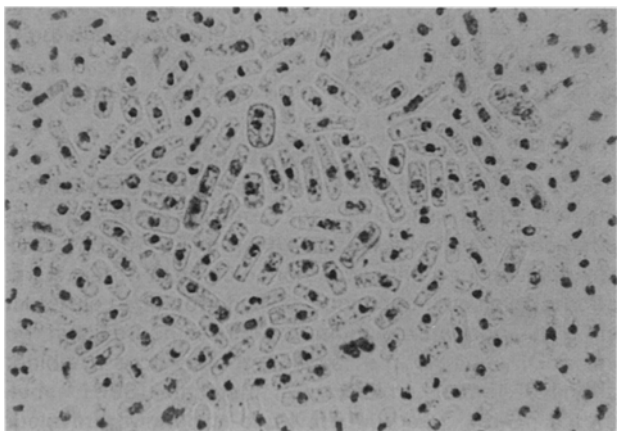


Fig. 6. Giemsa staining of the oidia from a dikaryotic stock, NGW-19×NF-1.

The numbers of nuclei in oidia from monokaryotic and dikaryotic stocks are also shown in Table 3.

About 82% of oidium cells from monokaryotic stocks had only one nucleus, while the remainder had from two to six nuclei. From dikaryotic stocks, about 69% of the oidia had one nucleus per cell, 23% had two nuclei, and the remaining 8% had three or more nuclei. These results suggest that oidium formation essentially involves the formation of multinucleate states and subsequent monokaryotization in the oidiophores. In addition, the formation of mononucleate oidia from dikaryotic hypha should involve nuclear selection before multinucleate cell formation, because most of the mononucleate oidia from the dikaryon carried one of the two nuclear types in the mother dikaryon (Cao et al., 1999)

Nuclear state of oidium cells The DNA content of oidium nuclei was measured by fluorocytometry by staining with propidium iodide and compared with the reference DNA content in a monokaryotic hyphal cell of the NX-4 mycelia. The results are shown in Table 4.

About 90% of the oidia from both monokaryotic and dikaryotic mycelia had DNA values corresponding to that of the reference, suggesting that these cells had single haploid nuclei at the G1 stage. However, the DNA content in the remaining 10% was twice in the reference value. It is, therefore, assumed that those nuclei with doubled DNA content were at the G2 stage.

Discussion

Pholiota nameko produced abundant oidia on aerial hyphae from monokaryotic and dikaryotic test stocks, but oidia were rare on the submerged hyphae. Electron microscopic observation revealed that oidia from aerial

Table 2. Percentage of oidia from branched and terminal hyphae of monokaryotic and dikaryotic mycelia of *Pholiota nameko*.[a)]

	Branch hyphae (%)	Terminal hyphae (%)	Number of test samples
Monokaryotic stock			
NGW-4	81.1	18.9	148
NX-6	82.5	17.5	166
NA-15	75.7	24.3	115
(Average)	79.8	20.2	—
Dikaryotic stock			
NGW-19×NGW-12	74.5	25.5	251
NGW-19×NF-1	83.2	16.8	113
NGW-19×NF-5	76.9	23.1	160
NX-2×NX-3	83.3	16.7	90
NX-4×NX-5	71.7	28.3	127
NF-8×NA-11	80.5	19.5	195
NF-8×NA-20	82.0	18.0	167
(Average)	78.9	21.1	—

a) The numbers of oidia from branched and terminal hyphal cells in an area of 0.01 mm^2 on the slide culture of the test stock were counted under a microscope. The number of oidia of each origin was then divided by the total number to obtain the percentage as indicated above. Each datum was an average of ten replicates.

Table 3. Number of nuclei in oidia produced from the monokaryotic and dikaryotic mycelia of *Pholiota nameko*.

	Percentage in total number of test oidium cells[a)]		
	Mononucleate	Binucleate	Polynucleate
Monokaryotic stock			
NGW-4	82.2	12.4	5.4
NX-6	82.0	14.3	3.7
NA-15	80.2	15.0	4.8
(Average)	81.5	13.9	4.7
Dikaryotic stock			
NGW-19×NGW-12	77.8	15.4	6.9
NGW-19×NF-1	74.5	17.9	7.7
NGW-19×NF-5	74.4	16.5	9.0
NX-2×NX-3	54.6	38.4	7.0
NX-4×NX-5	69.8	26.5	3.7
NF-8×NA-11	62.1	27.0	10.8
NF-8×NA-20	71.1	19.9	8.4
(Average)	69.3	23.1	7.6

a) Each datum was an average of ten replicates.

Table 4. DNA contents of mycelia and oidia produced from various stocks of *Pholiota nameko*.

	Nuclear state of hyphae	DNA contents[a)]	Number of test samples
Mycelium			
NX-4	Monokaryon	1.00 (Reference)[a)]	7
NGW-20	Monokaryon	1.04±0.03	4
Oidia at G1 stage			
HF-8	Monokaryon	1.04±0.03	173
NGW-20	Monokaryon	1.15±0.08	36
NX-4	Monokaryon	1.00±0.13	52
NX-5	Monokaryon	0.90±0.20	61
NGW-19×NF-7	Dikaryon	1.06±0.11	30
NGW-9×NGW-19	Dikaryon	0.96±0.18	46
NX-4×NX-5	Dikaryon	0.94±0.17	28
NA-15×NGW-9	Dikaryon	0.99±0.16	69
Oidia at G2 stage			
NX-4	Monokaryon	1.90±0.11	4
NX-5	Monokaryon	1.66	1
NF-8	Monokaryon	2.06	1
NA-15×NGW-9	Dikaryon	1.79±0.23	6
NGW-19×NF-7	Dikaryon	1.86	1
NGW-9×NGW-19	Dikaryon	1.61	1
NX-4×NX-5	Dikaryon	1.77	1

a) The DNA content of NX-4 monokaryotic mycelia was used as the reference. The DNA content of other samples is expressed relative to the reference.

mycelia differed surface structure from those from the submerged mycelia. A layer of hydrophobic protein was found between the cell wall and the inner cell membrane in the oidium cells from aerial mycelia, which was absent in oidia produced on submerged mycelia. Similar hydrophobic protein has been found on the wall of conidia from *Neurospora crassa* Shear & B. Dodge and *Aspergillus nidulans* (Eidam) G. Winter (Griffin, 1993). The structure and function of the hydrophobic protein layer on aerial oidia, however, is unclear.

Oidia of *P. nameko* were globular, oval, or rod-shaped, and their average size was smaller than those of *F. velutipes* (Masuda, 1996). The size of oidia might vary with the species of basidiomycetes. Oidia from monokaryotic and dikaryotic mycelia of *P. nameko* were also slightly different in size. Average volume, and the long and short axes of oidia from dikaryotic hyphae were slightly larger than those of monokaryotic hyphae. Oidia from the same stock could differ in size by a factor of about five times. This might be due to the different number of nuclei in a single oidium cell, e.g., some oidia, which seemed to be immature and not yet segmented, bore six nuclei in single oidium unit.

Oidium formation in *P. nameko* occurs in different ways at two different sites of the mycelial colony. In one case, oidia were formed at the terminal end of a main hypha, and a single or several oidia were produced one by one on the terminally positioned oidiophore cell, as described above. In the other case, they were produced at the end of a lateral hypha that arose almost perpendicularly from the main hypha. The segmentation of vacuoles as well as the separation and assembly of cell plasma was followed by the simultaneous production of several oidium units in the hypha, which functioned as oidiophore. The present study shows that about 80% of the oidia could be traced to the latter type of development and the remaining 20% to the former, in both monokaryotic and dikaryotic mycelial colonies of *P. nameko*.

In *F. velutipes*, most oidia from dikaryotic mycelia are uninucleate (Brodie, 1936; Takemaru, 1954). Arita (1968) noted that the dikaryotic mycelia of *P. nameko* produced both uninucleate and binucleate oidia with a ratio of 81:19. However, he did not observe the number of nuclei in oidium cells by staining of nuclei, but only estimated the nuclear state of oidium cells from the presence of clamp connectors on the mycelia originated from the oidia. Therefore, in his case, the "uninucleate" oidia should be homokaryotic, having one or more nuclei of the same mating type, and the "binucleate" oidia should be heterokaryotic, having two types of nuclei from the parental dikaryotic mycelia. According to the present study, about 80% and 70% of oidia from monokaryotic and dikaryotic mycelia, respectively, bore one haploid nucleus in an oidium cell, while the remainder had two or more nuclei in a cell. Some of the latter oidia germinated to form dikaryotic mycelia. Among the stocks tested, most oidia had a DNA content corresponding to the haploid amount at the G1 phase of the cell cycle, while only a few contained twice as much, corresponding to the G2 phase. In addition, more than 80% of monokaryotic oidia derived from dikaryotic stocks tested had same haploid nuclear type as one of the parental monokaryons (Cao et al., 1999). Similar results were reported for monokaryotic oidium formation from dikaryotic mycelia in *F. velutipes* (Masuda, 1996). Deviation in nuclear type has been found in monokaryotized mycelium formation from dikaryotic mycelia through nuclear selection in conjugate nuclear division of *P. nameko* (Masuda et al., 1995). Masuda et al. (1995) proposed the involvement of a cascade process in dikaryotic cell division, in which the first dividing nucleus may act as the "leading nucleus" and another nucleus as the "following nucleus." A similar type of nuclear selection might be involved in the nuclear behavior in monokaryotized oidium formation from dikaryotic mycelia of basidiomycetous mushrooms.

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Literature cited

Arita, I. 1968. Studies on the life-cycles of *Pholiota nameko* (T. Ito) S. Ito et Imai. Rept. Tottori Mycol. Inst. **6**: 49–57. (In Japanese.)

Arita, I. 1979. Cytological studies on *Pholiota nameko*. Rept. Tottori Mycol. Inst. (Japan) **17**: 1–118.

Bessey, E. A. 1964. Morphology and taxonomy of fungi. Hafner, New York.

Brodie, H. J. 1936. The occurrence and function of oidia in the Hymenomycetes. Amer. J. Bot. **23**: 309–327.

Cao, H., Yamamoto, H., Ohta, T., Takeo, K. and Kitamoto, Y. 1999. Nuclear selection in monokaryotic oidium formation from dikaryotic mycelia in a basidiomycete, *Pholiota nameko*. Mycoscience **40**: 199–203.

Griffin, D. H. 1993. Fungal physiology. John Wiley and Sons, New York.

Masuda, P. 1996. An empirical rule for genetic expression of some useful traits in edible mushrooms and the existence of dominant and recessive nuclei in heterokaryotic cells. PhD thesis, Tottori University, Tottori.

Masuda, P., Yamanaka, K., Sato, Y. and Kitamoto, Y. 1995. Nuclear selection in monokaryotization of dikaryotic mycelia of *Pholiota nameko* as described by leading and following nuclei. Mycoscience **36**: 413–420.

Rao, P. S. and Niederpruem, D. J. 1969. Carbohydrate metabolism during morphogenesis of *Coprinus lagopus* (sensu Buller). J. Bacteriol. **100**: 1222–1228.

Society of Fermentation and Bioengineering (ed.). 1992. Experiments in microbiology, pp. 37–96. Baifukan, Tokyo. (In Japanese.)

Takemaru, T. 1954. Genetics of *Collybia velutipes*. II. Dedikaryotization and its genetic implication. Jpn. J. Genet. **29**: 1–7.

Takeo, K., Tanaka, K., Taguchi, H. and Nishimura, K. 1993. Analysis of ploidy and sexual characteristics of natural isolates of *Cryptococcus neoformans*. Can. J. Microbiol. **39**: 958–963.

Yamanaka, K. 1995. Bunashimeji (*Hypsizygus marmoreus*). In: Year of mushrooms '96, (ed. by Ohashi, H.), pp. 54–58. Noson-Bunkasha, Tokyo. (In Japanese.)