Mycoscience 40: 199–203, 1999

Short Communication

Nuclear selection in monokaryotic oidium formation from dikaryotic mycelia in a basidiomycete, *Pholiota nameko*

Cao Hui[1], Hideki Yamamoto[1], Tomoko Ohta[1], Kanji Takeo[2] and Yutaka Kitamoto[1],*

[1] Department of Bioscience and Biotechnology, Faculty of Agriculture, Tottori University, 4–101, Minami, Koyama-cho, Tottori 680–0945, Japan

[2] Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, 1–8–1, Inohana, Chuo-ku, Chiba 260–8673, Japan

Accepted for publication 15 October 1998

The effect of nuclear dominance in monokaryotic oidium formation from dikaryotic mycelia in *Pholiota nameko* was examined. Over 90% of oidium isolates from dikaryotic mycelia were monokaryotic. Although only one parental nuclear type was recovered from an average of about 80% in these isolates, the nuclear selection process in oidium formation seems essentially to produce split nuclear type composition in oidium products. The hierarchy of relative dominance among the nuclear types of the parental dikaryons in monokaryotic oidium formation was determined. The two hierarchies in nuclear selection between monokaryotic oidium formation and monokaryotic mycelium formation coincided at a level of at least 75%.

Key Words——basidiomycete; monokaryotization; nuclear selection; oidium formation; *Pholiota nameko*.

Many basidiomycetous mushrooms produce oidia or other asexual spores from both monokaryotic and dikaryotic mycelia. Oidium formation occurs in such mushrooms as *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray (Rao and Niederpruem, 1969), *Flammulina velutipes* (Curt.: Fr.) Singer (Brodie, 1936; Takemaru, 1954), *Favolus arcularius* (Fr.) Ames (Kitamoto, unpublished data), *Hypsizygus marmoreus* (Peck) Bigelow (Yamanaka, 1995), and *Pholiota nameko* (T. Ito) S. Ito & Imai in Imai (Arita, 1979). Most oidia from dikaryotic mycelium of *F. velutipes* (Brodie, 1936; Takemaru, 1954; Masuda, 1996) and *P. nameko* (Cao et al., 1999) are in a monokaryotic state. Although both parental nuclear types of monokaryotic oidia were isolated from the majority of hybrid dikaryons, one type was recovered in a much higher rate (85%) in the oidia from most hybrids (Masuda, 1996). Therefore, the process of spontaneous monokaryotization and the occurrence of dominant nucleus selection might be involved in oidium formation from dikaryotic mycelia in various mushrooms. A similar monokaryotization process in monokaryotic mycelia from dikaryotic mycelia in *P. nameko* has been reported (Masuda et al., 1995).

We have previously demonstrated that *P. nameko* produced abundant oidia on aerial hyphae from monokaryotic and dikaryotic test stocks, but rarely did so on submerged hyphae (Cao et al., 1999). Observation of various test stocks on slide cultures revealed that about 80% of oidia were produced from a secondary branched hypha and about 20% from the terminal hyphal cell of the main hypha. About 82% and 70% of the oidia from monokaryons and dikaryons, respectively, had only one haploid nucleus, while the remainders were multinucleate. Among the stocks tested, most oidia had a DNA content corresponding to a haploid at the G1 phase of the cell cycle, while a few contained twice as much, corresponding to the G2 phase.

In the present study, we have sought an empirical rule for dominant nucleus selection in monokaryotic oidium formation from dikaryotic mycelia in *P. nameko*. We have also compared the hierarchies of relative dominance in the selection of one of the two nuclei of dikaryotic cells in monokaryotization between mycelium monokaryotization and oidium monokaryotization in this mushroom.

Monokaryotic line stocks of *P. nameko* were obtained by single spore isolation from the fruit-bodies of various wild strains, as detailed elsewhere (Masuda et al., 1995). The incompatibility factors of the oidium monokaryotic stocks were determined by crossing them against tester stocks.

Mycelial cultures were conducted in plastic Petri dishes (Iwaki, 90×15 mm) containing 12 ml of PDA medium (Nissui). Plates were inoculated with a mycelial agar block (3×3×3 mm) excised from the stock cultures and incubated at 20–22°C in the dark. The dikaryotic stocks used for oidium production were prepared by reciprocal crossing of two compatible monokaryotic

* Corresponding author.

stocks. The inoculation for crossing was done by planting two different monokaryotic stocks 4 mm apart in the center of a PDA plate. After incubation for 10–14 d at 20°C, the colony on the PDA plate was inspected under a microscope for the formation of clamp connections as evidence for dikaryotization. Dikaryotic mycelium thus confirmed was transplanted onto a PDA slant for the preparation of hybrid stocks.

Stocks of oidium isolates were prepared as follows. Small pieces of mycelia were excised from a dikaryotic stock slant and inoculated onto 15 ml of PDA medium in a 50-ml Erlenmeyer flask. The cultures were incubated at 25°C for 2 wk in the dark to allow for oidium formation on aerial hyphae. Then 4 ml of sterilized water was poured into the flask, and the culture was scraped with a sterile spatula to release oidia. The resulting suspension of mycelial fragments and oidium cells was transferred into a Corning centrifuge tube and agitated vigorously for 1 min with a vortex mixer. The suspension was passed through a 3G2 glass filter (Iwaki Glass) to remove mycelial fragments, and the filtrate containing oidia was centrifuged at 2,500 rpm for 10 min. The oidium sediment was suspended in a small volume of distilled water, then diluted with water to 10^2-10^3 cells/ml. The oidium concentration was determined by counting the number of oidia with a hemocytometer (Nippon Rinsho Kikaikogyo). A 0.1-ml portion of oidium suspension was added to 2 ml of molten soft PDA agar at 45°C, mixed thoroughly, and poured onto a plate containing 12 ml of solidified PDA, which was then incubated at 25°C. The oidia usually germinated after 3 d and formed mycelial colonies. When the colonies exceeded 2 mm in diam, they were excised from the agar plate and planted on PDA slants to prepare oidium isolate stocks. The presence or absence of clamp connections on hyphae was confirmed under a microscope, and stocks were classified accordingly as monokaryotic or dikaryotic.

Nuclear type of monokaryotic oidium isolates was determined by using incompatibility factors as nuclear markers. The monokaryotic stocks from oidia were crossed with tester strains with the same incompatibility factor composition as each of the two parental monokaryotic stocks. Incompatibility with a tester parental monokaryon was taken to indicate that the test stock had the same nuclear type as the tester.

Cao et al. (1999) demonstrated that the oidia from dikaryotic mycelia of *P. nameko* contained various numbers of nuclei per unit cell, and that about 69% of oidia had a single nucleus per cell, 23% had two nuclei, and the remaining 8% had three or more nuclei. Most oidia had the DNA content of a haploid nucleus, but a few had twice that amount, corresponding to a diploid nucleus. In addition, most oidium cells were produced by the repeated partitioning of multinucleate branched hyphae (oidiophores) of dikaryotic mycelia. These results suggested that most oidia were monokaryotic, while the remainders were homokaryotic with two or more of the same nucleus, or heterokaryotic with two compatible nuclei in one cell unit. In this study, the oidium isolates from several hybrid stocks were cultured on PDA medium, and the formation of clamps was examined to determine the proportion of homokaryotic oidia produced by dikaryotic mycelia. It was found that over 90% of the germinating oidia from various dikaryotic stocks did not have clamps (data not shown), and that two-thirds of the binucleate or polynucleate oidia were estimated to be in a homokaryotic state.

Mycelium monokaryotization from dikaryotic mycelia has been reported in such mushrooms as *Typhula trifolii* Rostrup (Nobles, 1937), *Polyporus pseudoboletus* Speg. (Furtado, 1966), *P. nameko* (Arita 1964; Masuda et al., 1995), *Coprinus disseminatus* (Pers.: Fr.) S. F. Gray (Butler, 1972), and *Armillariella mellea* (Vahl: Fr.) Kummer (Korhonen and Hintikka, 1974). Masuda et al. (1995) found that most of the monokaryotized mycelium isolates of *P. nameko* had nuclear types similar to only one of the parental monokaryotic stocks, while the replicates isolated from a few dikaryotic hybrids showed split nuclear type compositions. They estimated the hierarchy of relative dominance among nuclei of 18 parental monokaryotic stocks in the monokaryotization of their crossing products, and summarized their findings in a figure (Masuda et al., 1995). Accordingly, we examined whether relative dominance in nuclear selection could be observed in monokaryotic oidium formation from the dikaryotic mycelia of this mushroom by using incompatibility factors as nuclear markers.

The monokaryotic oidium isolates from each of the dikaryotic hybrids produced by crossing among the eighteen monokaryotic stocks were back-crossed to parental monokaryotic stocks to determine their nuclear type. As shown in Table 1, one parental nuclear type was recovered in a frequency of 50–90% in the isolates from 84 % of the hybrids, and 16% of the hybrids produced monokaryotic oidia similar to only one of the parental stocks. Monokaryotization in oidium formation from dikaryotic mycelia, therefore, essentially involves the process of nuclear selection, and the nuclear selection process seems to produce essentially a split nuclear-type composition in oidium products.

The table also indicates that relative dominance is active in the selection of one of the two nuclei of the dikaryotic cells in monokaryotic oidium formation. For the NA-11 × NF-1 hybrid, 90% of the monokaryotic oidium progenies had an NA-11 nucleus, but the same nucleus was not selected as the dominant one in the oidia from the NA-20 × NA-11 hybrid. Thus, the NA-11 nucleus could be either dominant or recessive in monokaryotic oidium formation from two hybrid dikaryons. Figure 1 shows the hierarchy of the nuclei of 18 parental monokaryotic stocks in the mycelium monokaryotization of their crossing products, together with their relative dominance in the monokaryotic oidium formation from the corresponding hybrids (cf. Masuda et al., 1995). If a half of 12 test hybrids in Table 1 showing 50% recoveries in nuclear selection could be roughly divided into the stocks that were followed the hierarchy rule, the two hierarchies of nuclear selection in mycelium and oidium monokaryotizations coincided at a level of at least 75%. These results suggest that the same rule in

Table 1. Analysis of nuclear types of the monokaryotized oidium isolates from various dikaryotic mycelium in *Pholiota nameko*.

Mated stock		Predominant nuclear type of oidium isolates[a]		Mated stock		Predominant nuclear type of oidium isolates[a]	
NX-3 ×	NA-4	NA-4	(7/10)	NGW-19 ×	NA-11	NA-11	(10/10)
	NGW-19	NGW-19	(9/10)		NX-2	NX-2	(7/10)
	NX-5	NX-5	(9/10)		NGW-9	NGW-19	(10/10)
	NF-5	NF-5	(7/10)		NA-15	NGW-19	(8/10)
	NF-8	NX-3	(6/10)				
	NX-6	NX-3	(10/10)	NX-5 ×	NF-5	NX-5	(9/10)
	NA-20	NX-3	(7/10)		NX-6	NX-5	(8/9)
	NX-4	NX-3	(8/10)		NA-11	NX-5	(10/10)
	NF-1	NX-3	(8/10)		NX-4	NX-5	(8/9)
	NX-2	NX-3	(9/10)		NF-1	NX-5	(8/10)
	NA-11	NX-3	(10/10)		NX-2	NX-5	(9/10)
	NGW-20	NX-3	(10/10)		NA-15	NX-5	(5/10)
	NA-15	NX-3	(5/10)				
				NF-5 ×	NF-8	NF-5	(7/10)
NA-4 ×	NGW-12	NA-4	(7/10)		NA-11	NF-5	(5/10)
	NGW-19	NA-4	(7/10)		NX-4	NF-5	(8/10)
	NF-5	NA-4	(8/10)		NGW-9	NF-5	(7/10)
	NF-7	NA-4	(9/10)		NA-15	NF-5	(5/10)
	NF-8	NA-4	(6/10)				
	NA-20	NA-4	(7/10)	NF-7 ×	NX-4	NX-4	(10/10)
	NX-4	NA-4	(10/10)		NX-2	NF-7	(6/10)
	NF-1	NA-4	(8/10)		NGW-9	NGW-9	(10/10)
	NGW-20	NA-4	(8/10)		NA-15	NA-15	(6/10)
	NGW-9	NA-4	(5/10)				
	NA-15	NA-4	(8/10)	NF-8 ×	NA-20	NF-8	(8/10)
					NA-11	NF-8	(10/10)
NX-1 ×	NGW-19	NX-1	(7/10)		NX-4	NF-8	(9/10)
	NX-5	NX-1	(9/10)		NX-2	NF-8	(5/10)
	NF-7	NX-1	(9/10)		NGW-9	NGW-9	(6/10)
	NF-8	NX-1	(9/10)		NA-15	NA-15	(5/10)
	NX-6	NX-1	(8/10)				
	NA-20	NX-1	(9/10)	NX-6 ×	NX-4	NX-4	(9/10)
	NX-2	NX-1	(7/10)		NGW-9	NGW-9	(10/10)
	NGW-9	NGW-9	(6/10)				
	NA-15	NA-15	(6/10)	NA-20 ×	NA-11	NA-20	(10/10)
NGW-12 ×	NGW-19	NGW-12	(5/9)	NA-11 ×	NF-1	NA-11	(9/10)
	NX-5	NX-5	(10/10)		NX-2	NX-2	(9/10)
	NF-5	NF-5	(7/10)		NGW-9	NA-11	(6/10)
	NF-7	NGW-12	(8/10)		NA-15	NA-15	(7/10)
	NF-8	NF-8	(8/10)				
	NX-6	NGW-12	(5/10)	NX-4 ×	NF-1	NF-1	(9/10)
	NA-20	NGW-12	(9/10)		NX-2	NX-4	(9/10)
	NA-11	NGW-12	(6/10)				
	NX-4	NGW-12	(10/10)	NF-1 ×	NGW-9	NF-1	(8/10)
	NF-1	NGW-12	(7/10)		NA-15	NA-15	(5/10)
	NX-2	NGW-12	(7/10)				
	NGW-20	NGW-12	(10/10)	NX-2 ×	NGW-9	NX-2	(8/10)
	NA-15	NA-15	(6/10)				
				NGW-20 ×	NGW-9	NGW-20	(7/10)
NGW-19 ×	NX-5	NGW-19	(5/10)		NA-15	NA-15	(5/10)
	NF-5	NGW-19	(5/10)				
	NF-7	NGW-19	(8/10)	NGW-9 ×	NA-15	NGW-9	(9/10)
	NF-8	NGW-19	(8/10)				

a) Ten oidium isolates from each hybrid dikaryon were examined for their nuclear type.

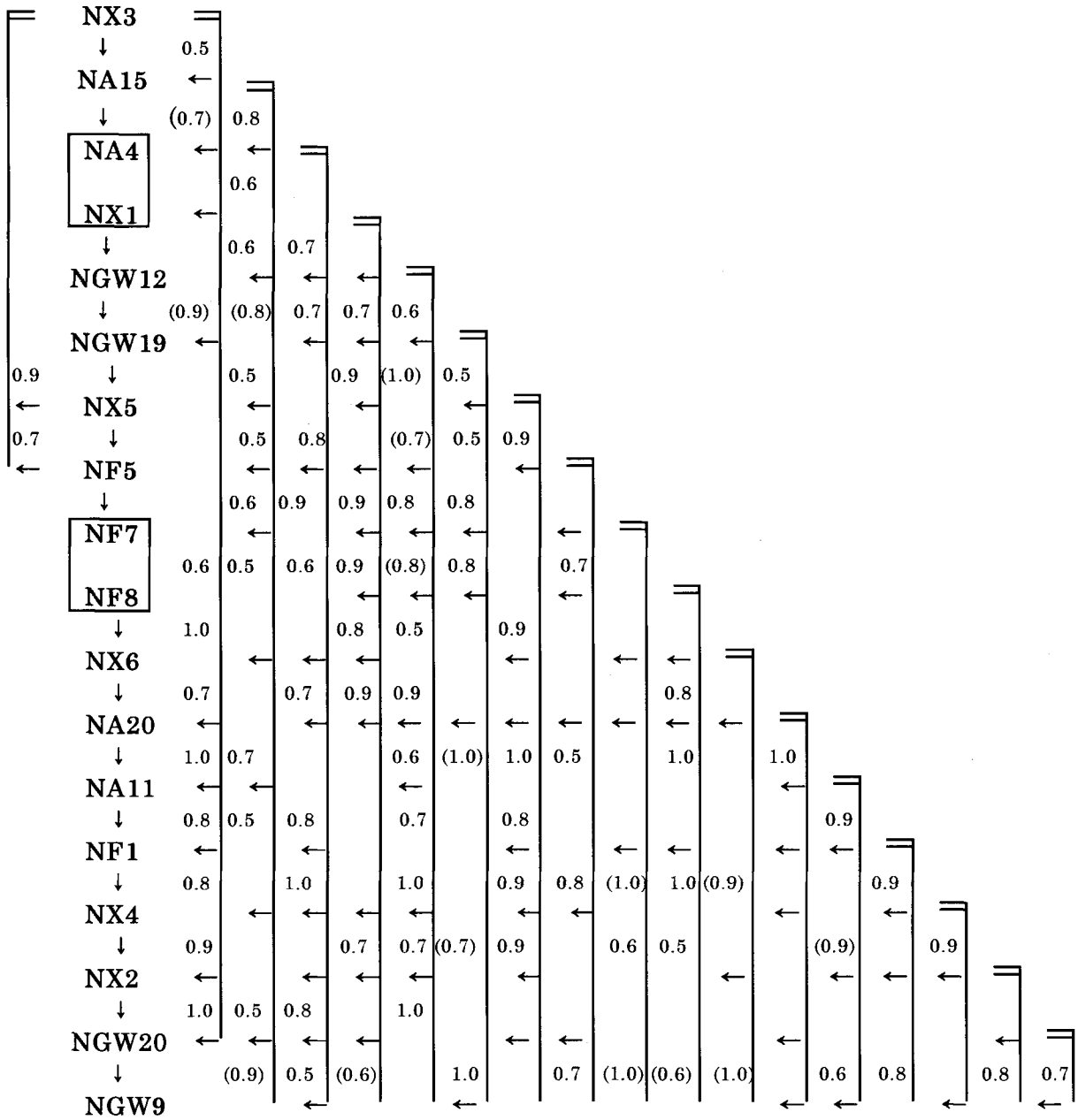

Fig. 1. Hierarchy of the nuclei of 18 parental monokaryotic stocks in the mycelium monokaryotization of their crossing products,* and relative dominance in the monokaryotic oidium formation from the corresponding hybrids in *Pholiota nameko*.
Arrows show the direction of dominant nucleus selection between the two nuclei of the dikaryotic hybrid mycelia in the mycelium monokaryotization. Boxed nuclear types are estimated to be equally dominant with the same incompatibility factor. Figures show the relative dominance nuclear types in the monokaryotic oidium isolates from dikaryotic hybrid mycelia. Figures in parentheses indicate that the relative dominance in mycelium monokaryotization is reversed in monokaryotic oidium formation from dikaryotic hybrid mycelia.
*See Masuda et al. (1995).

nuclear selection system may be applied in both types of monokaryotization in this mushroom.

This study was partly supported by the cooperative research program of the research center for pathogenic fungi and microbial toxicoses, chiba university ('97–06).

Literature cited

Arita, I. 1964. Dedikaryotization of dikaryotic mycelia in *Pholiota nameko*, Rept. Tottori Mycol. Inst. (Japan) **4**: 44–50. (In Japanese.)

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

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

Butler, G. M. 1972. Nuclear and non-nuclear factors influencing clamp connection formation in *Coprinus disseminatus*. Ann. Bot. (London) **36**: 263–279.

Cao, H., Tanaka, Y., Takeo, K. and Kitamoto, Y. 1999. Morphological and cytological aspects of oidium formation in a basidiomycete, *Pholiota nameko*. Mycoscience **40**: 95–101.

Furtado, J. S. 1966. Significance of the clamp connection in the Basidiomycetes. Persoonia **4**: 125–144.

Korhonen, K. and Hintikka, V. 1974. Cytological evidence for somatic diploization in dikaryotic cells of *Armillariella mellea*. Arch. Mikrobiol. **95**: 187–192.

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.

Nobles, M. 1937. The morphology and cytology of *Typhula trifolii* Rostr. Ann. Bot. (London) **1**: 67–98.

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

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

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