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# Nuclear selection in oidium formation from dikaryotic mycelia of *Flammulina velutipes*

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**The effect of nuclear dominance in monokaryotic oidium formation from dikaryotic mycelia in a tetrapolar basidiomycete, *Flammulina velutipes*, was examined. A total of 46 monokaryotic stocks were used to produce 194 hybrid dikaryotic stocks by crossing. The proportion of homokaryons among the oidium isolates from dikaryotic mycelia was over 95%. The staining of nuclei of oidia with propidium iodide showed that over 90% of oidia were monokaryotic and suggested that these oidia had single haploid nuclei at the G1 stage. The monokaryotic oidium isolates from hybrid dikaryons were backcrossed to parental monokaryotic stocks. Although most of the monokaryotic oidium isolates (except for those from 17 hybrid dikaryons from a total of 194 test stocks) showed nuclear types similar to only one of the parental stocks, the process seems to produce essentially the split nuclear type composition. Therefore, the monokaryotization in oidium formation from dikaryotic mycelia essentially involves the process of nuclear selection. The two separate results of hierarchies of relative dominance among two nuclei of the parental dikaryons in the monokaryotic oidium formation by grouping with incompatibility factor compositions were determined. Only a few discrepancies were found in the hierarchies between the two specific nuclear compositions of hybrid dikaryons.**

Key Words——basidiomycete; *Flammulina velutipes;* monokaryotization; nuclear selection; oidium formation.

Most edible mushrooms belong to basidiomycetous fungi. Their cells of mycelia and fruit-bodies are heterokaryons composed of two haploid nuclei with different mating types in one cell unit. Due to the coexistence of two kinds of nuclei in one cell unit, these organisms may follow somewhat different inheritance rules from typical diploid genetics. Besides, little is known about the manner of the conjugate nuclear division that occurs in heterokaryotic cells.

There are two kinds of monokaryotization in basidiomycetous mushrooms. The first case is the formation of monokaryotic hyphal cells at the margin of the dikaryotic mycelial colony, as reported for *Pholiota nameko* (T. Ito) S. Ito & Imai in Imai (Arita,1964, 1979; Masuda et al., 1995). Mycelium monokaryotization was also reported in *Collybia velutipes* by Ashan (1952) and Aschan-Aberg (1960). Further, the mycelium monokaryotizations are assumed to be the ones that conjugate nuclear division. They ended in failure in the terminal dikaryotic mycelia. We propose that a cascade process is involved in the dikaryotic cell division, in which the first dividing nucleus may act as the leading nucleus and the other as the following nucleus in conjugate nuclear division (Masuda et al., 1995). The second kind of monokaryotization is the formation of monokaryotic oidia from dikaryotic mycelia in such mushrooms as *Coprinus cinereus* (Schaeff.:Fr.) S. F. Gray (Rao and Niederpruem, 1969), *Favolus arcularius* (Fr.) Ames. (Kitamoto, unpublished data), *Flammulina velutipes* (Curt.:Fr.) Sing. (Brodie, 1936), *Hypsizygus marmoreus* (Peck) Biglow (Yamanaka, 1995), and *P. nameko* (Arita, 1979; Cao et al., 1999a). In *F. velutipes*, most of the oidia produced from dikaryotic mycelium have only one nucleus in hyphal cells (Brodie, 1936; Takemaru, 1954).

In a previous paper, we demonstrated that nuclear selection was involved in the process of mycelium monokaryotization from dikaryotic mycelia in a bipolar basidiomycete, *P. nameko* (Masuda et al., 1995). Most of the monokaryotic mycelial isolates from the margin of dikaryotic colony had nuclear types similar to only one of the parental monokaryotic stocks. Therefore, a relative dominance is active in the selection of one of the two nuclei of the cells in monokaryotization. We also demonstrated similar results of nuclear selection in oidium monokaryotization from dikaryotic mycelia of the same fungus (Cao et al., 1999b).

In the present study, we examined the nuclear selection in monokaryotic oidium formation from dikaryotic mycelia of a tetrapolar basidiomycete, *F. velutipes*.

## Materials and Methods

**Organisms** Monokaryotic line stocks obtained by single spore isolation from parental wild and cultivated dikaryot-

Table 1. Monokaryotic stocks and tester strains of *Flammulina velutipes*.

| Monokaryotic stocks | | Monokaryotic stocks | | Monokaryotic stocks | |
|---|---|---|---|---|---|
| Stock No. | Mating type | Stock No. | Mating type | Stock No. | Mating type |
| J-52 | A1B1 | J-39 | A2B2 | WA-2* | A3B3 |
| J-53 | A1B1 | J-117 | A2B2 | | |
| J-130 | A1B1 | J-138 | A2B2 | WB-2* | A5B5 |
| J-134 | A1B1 | J-151 | A2B2 | WB-6* | A5B5 |
| R-21 | A1B1 | J-180 | A2B2 | | |
| R-107 | A1B1 | R-7 | A2B2 | WD-8* | A7B7 |
| R-143 | A1B1 | R-10 | A2B2 | WE-27* | A9B9 |
| R-144 | A1B1 | R-100 | A2B2 | | |
| R-153 | A1B1 | R-117 | A2B2 | S-1* | A2B1 |
| R-204 | A1B1 | R-129 | A2B2 | S-2* | A1B1 |
| R-207 | A1B1 | R-146 | A2B2 | S-5* | A2B2 |
| | | | | S-8* | A1B2 |
| A-79 | A1B2 | A-17 | A2B1 | | |
| A-89 | A1B2 | B-12 | A2B1 | | |
| A-90 | A1B2 | B-18 | A2B1 | | |
| A-91 | A1B2 | B-21 | A2B1 | | |
| A-93 | A1B2 | B-19 | A2B1 | | |
| B-41 | A1B2 | T-6 | A2B1 | | |
| B-44 | A1B2 | T-7 | A2B1 | | |
| | | T-9 | A2B1 | | |

The monokaryotic stocks marked with "*" were also used as tester stocks.

ic strains of *F. velutipes* were used. The method applied for single spore isolation was described earlier (Masuda et al., 1995). The incompatibility factors of the monokaryotic stocks were determined by crossing them against the tester stocks of known incompatibility factors. The factors of the stocks used for the present experiments were assigned temporary numbers in which the incompatibility factors of monokaryons, S-1, S-2, S-5, and S-8, from "Nakano" cultivated strains were assigned as A2B1, A1B1, A2B2 and A1B2, respectively. The wild strains WA2: A3B3, WB27: A5B5, WD8: A7B7, and WE-27: A9B9 were also used as the tester stocks (Table 1).

**Preparation of the hybrid dikaryons** The dikaryotic strains used for oidium production were prepared by crossing them with two compatible monokaryotic stocks. A total of 46 monokaryotic stocks were used to produce over 200 hybrid dikaryons by crossing. The crossing was carried out by inoculating two parental monokaryotic stocks on the center of a PDA plate, 4 mm apart. After incubating for about 10 d at 25°C, the colony on the PDA plate was inspected for the formation of clamp connections under microscope as evidence for dikaryotic mycelium formation. The hybrid dikaryons thus confirmed were isolated onto a PDA slant, which was then incubated for 2 wk at 25°C to allow mycelial growth before preservation at 5°C.

**Preparation of oidium isolates** The method for isolation of oidia was as follows: A small piece of a mycelial fragment was excised from a dikaryotic stock slant and inoculated onto 15 ml of PDA medium in 50-ml Erlenmeyer flasks. The cultures were incubated at 25°C for 2 wk in the dark to allow oidium formation on an aerial hypha of the mycelium colony. For preparing oidium suspension, 4 ml of sterilized water was poured into the flask, and the colony surface was scratched to fragmentize aerial mycelia and to release oidia from the aerial hyphae. Mycelium fragment suspension with oidia was transferred into Corning centrifuge tubes and agitated vigorously for 1 min with a vortex mixer. The suspension was then filtered to remove mycelium fragments with a 3G2 glass filter (Iwaki Glass Co. Ltd.), and the filtrate was centrifuged at 2,500 rpm for 10 min. The oidium sediment was suspended in a small volume of water, and then diluted with water to make $10^2$–$10^3$ oidia/ml. The oidium concentration was determined by counting the number of oidia with a hemocytometer (Kayagaki Erika Kogyo Co., Ltd.). The oidium suspension (0.1 ml) was taken into 2 ml of melted warm PDA soft agar, mixed thoroughly, and poured on a PDA plate to prepare a bi-layer agar plate. The medium composition was described earlier (Masuda et al., 1995). Oidial cultures were incubated at 25°C in the dark. The oidium cells usually germinated after 3 d, and formed mycelium colonies. When the colony exceeded 2 mm in diam, it was excised from the agar plate and planted onto PDA slants to prepare the oidium isolate stocks. The isolate was checked under microscope for clamp connections on hyphae to classify the stock as monokaryotic or dikaryotic.

**Determination of nuclear types of oidium isolates** Nuclear type determination of monokaryotic oidia was carried out after preparing the isolate stocks, and was performed by using the incompatibility factors as nucleus markers. The monokaryotic oidium isolates were crossed with the tester strains of the same incompatibility factor compositions as each of the two parental strains. The presence or absence of clamp connections indicated that the oidium nuclear type was different from or the same as that of the tester strain.

**Determination of nuclear DNA content** 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, then washed with ice-cooled distilled water. They were centrifuged at 3,000 rpm 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 $\mu$g of propidium iodide and 0.5 mg of RNase A (Sigma Chemical Co.) dissolved in NS buffer (10.0 mM Tris, 0.25 M sucrose, 7.0 m$M\beta$-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) that included a fluorescent light measuring apparatus (Olympus, model OSP-1). The DNA content of the mycelia in the S-5 monokaryotic stock was assigned as a reference to determine the rela-

Table 2. Nuclear state of oidial isolate stocks from hybrid dikaryotic stocks of *Flammulina velutipes*.

| Stock No. of hybrid | Parental monokaryons | Number of mono./total* | Percentage of monokaryons |
|---|---|---|---|
| | (A1B1)×(A2B2) | | |
| JJ1 | J-52 ×J-117 | 14/20 | 70 |
| JJ2 | J-52 ×J-138 | 20/20 | 100 |
| JJ3 | J-53 ×J-117 | 20/20 | 100 |
| JJ4 | J-134×J-117 | 19/20 | 95 |
| JR1 | J-52 ×R-7 | 20/20 | 100 |
| JR2 | J-52 ×R-10 | 17/20 | 85 |
| JR3 | J-52 ×R-117 | 19/20 | 95 |
| JR4 | J-52 ×R-129 | 20/20 | 100 |
| JR5 | J-52 ×R-146 | 20/20 | 100 |
| JR6 | J-53 ×R-146 | 20/20 | 100 |
| JR7 | J-130×R-117 | 20/20 | 100 |
| JR8 | J-130×R-129 | 18/20 | 90 |
| JR9 | J-130×R-146 | 18/20 | 90 |
| JR10 | J-134×R-117 | 20/20 | 100 |
| RJ1 | R-21 ×J-117 | 19/20 | 95 |
| RJ2 | R-21 ×J-180 | 20/20 | 100 |
| RJ3 | R-107×J-117 | 20/20 | 100 |
| RJ4 | R-107×J-138 | 20/20 | 100 |
| RJ5 | R-107×J-151 | 20/20 | 100 |
| RJ6 | R-143×J-117 | 20/20 | 100 |
| RJ7 | R-144×J-117 | 20/20 | 100 |
| RJ8 | R-144×J-138 | 19/20 | 95 |
| RJ9 | R-144×J-151 | 18/20 | 90 |
| RJ10 | R-144×J-180 | 19/20 | 95 |
| RJ11 | R-153×J-117 | 17/20 | 85 |
| RJ12 | R-153×J-180 | 20/20 | 100 |
| RJ13 | R-207×J-151 | 20/20 | 100 |
| RJ14 | R-204×J-117 | 19/20 | 95 |
| RJ15 | R-207×J-39 | 18/20 | 90 |
| RR1 | R-21 ×R-100 | 20/20 | 100 |
| RR2 | R-144×R-100 | 19/20 | 95 |
| RR3 | R-153×R-117 | 20/20 | 100 |
| Mean value | | | 95.8 |
| | (A1B2)×(A2B1) | | |
| AA1 | A-79×A-17 | 20/20 | 100 |
| AB1 | A-89×B-19 | 18/20 | 90 |
| AB2 | A-89×B-21 | 20/20 | 100 |
| AB3 | A-90×B-18 | 19/20 | 95 |
| AB4 | A-90×B-19 | 18/20 | 90 |
| AB5 | A-90×B-21 | 20/20 | 100 |
| AB6 | A-91×B-18 | 12/12 | 100 |
| AB7 | A-91×B-19 | 17/20 | 85 |
| AB8 | A-93×B-18 | 12/14 | 86 |
| AB9 | A-93×B-19 | 18/20 | 90 |
| AB10 | A-93×B-21 | 20/20 | 100 |
| BA1 | B-41×A-17 | 20/20 | 100 |
| Mean value | | | 95.3 |

tive value among the various samples.

## Results and Discussion

**Nuclear state of the oidium isolates derived from hybrid dikaryons** Oidium monokaryotization appears to be a common phenomenon in oidium producing basidiomycetous mushrooms (cf. Brodie, 1936; Takemaru, 1954: Arita, 1979). Recently, Cao et al. (1999a) demonstrated, in the study of oidium monokaryotization in *P. nameko*, that most of the oidia from dikaryotic mycelia were monokaryotic. Further, the mother hyphae destined for oidium formation were multinucleate, and the oidium cells were produced by repeated partitioning to form single nucleus oidia (cf. Cao et al., 1999a). In the present study, we examined the nuclear state of oidium isolates from different cultivated dikaryotic strains that were crossed with parental monokaryons in the combinations of A1B1×A2B2 and A1B2×A2B1 in *F. velutipes*. The results are shown in Table 2.

The proportion of homokaryons (most of which were monokaryons) among the oidium isolates from dikaryotic mycelia was observed to be over 95% for both A1B1×A2B2 hybrids and A1B2×A2B1 hybrids. Therefore, the involvement of nuclear selection in monokaryotic oidium formation from the hybrid dikaryons is expected. The rates of monokaryotic oidium formation from the hybrids between two different mating combinations gave almost the same average values, suggesting that the characteristic of hybrid strains for monokaryotic oidium formation may not be linked with the genes of incompatibility factors. The nuclear staining of oidia with propidium iodide as described below demonstrated that over 90%

Table 3. DNA contents of mycelia and oidia produced from various stocks of *Flammulina velutipes*.

| | Nuclear state of hyphae | DNA contents[a)] | Number of test samples |
|---|---|---|---|
| Mycelium | | | |
| S-5 | Monokaryon | 1.00 (Reference[a)]) | 4 |
| NX-4(*P. nameko*) | Monokaryon | 0.92±0.20 | 7 |
| Oidia at G1 stage | | | |
| S-2 | Monokaryon | 1.10±0.21 | 20 |
| S-5 | Monokaryon | 1.14±0.22 | 12 |
| S-8 | Monokaryon | 1.14±0.35 | 5 |
| S-1×S-8 | Dikaryon | 1.04±0.11 | 8 |
| S-2×S-5 | Dikaryon | 1.12±0.20 | 17 |
| Oidia at G2 stage | | | |
| S-5 | Monokaryon | 2.31±0.31 | 4 |
| S-8 | Monokaryon | 1.68±0.35 | 3 |
| S-1×S-8 | Dikaryon | 2.12±0.23 | 6 |

[a)]The DNA content of S-5 monokaryotic mycelia was used as the reference. The DNA content of other samples is expressed relative to the reference.

Table 4. Analysis of nuclear types of the monokaryotized oidium isolates from various dikaryotic mycelium in *Flammulina velutipes*.

| Hybrid dikaryon | Predominant nuclear type of oidium isolates[a] | | |
|---|---|---|---|
| (A1B1×A2B2) | | | |
| J-52 ×J-117 | J-52 | (A1B1) | 18/20 |
| J-52 ×J-138 | J-138 | (A2B2) | 16/20 |
| J-53 ×J-117 | J-53 | (A1B1) | 17/20 |
| J-134×J-117 | J-117 | (A2B2) | 19/20 |
| J-52 ×R-7 | R-7 | (A2B2) | 20/20 |
| J-52 ×R-10 | J-52 | (A1B1) | 14/20 |
| J-52 ×R-117 | R-117 | (A2B2) | 16/20 |
| J-52 ×R-129 | — | | 10/20 |
| J-52 ×R-146 | R-146 | (A2B2) | 20/20 |
| J-53 ×R-146 | J-53 | (A1B1) | 20/20 |
| J-130×R-117 | J-130 | (A1B1) | 20/20 |
| J-130×R-129 | R-129 | (A2B2) | 11/20 |
| J-130×R-146 | J-130 | (A1B1) | 13/20 |
| J-134×R-117 | R-117 | (A2B2) | 20/20 |
| R-21 ×J-117 | J-117 | (A2B2) | 18/20 |
| R-21 ×J-180 | J-180 | (A2B2) | 13/20 |
| R-107×J-117 | J-117 | (A2B2) | 20/20 |
| R-107×J-138 | J-138 | (A2B2) | 18/20 |
| R-107×J-151 | J-151 | (A2B2) | 18/20 |
| R-143×J-117 | J-117 | (A2B2) | 20/20 |
| R-144×J-117 | J-117 | (A2B2) | 20/20 |
| R-144×J-138 | J-138 | (A2B2) | 16/20 |
| R-144×J-151 | R-144 | (A1B1) | 16/20 |
| R-144×J-180 | J-180 | (A2B2) | 19/20 |
| R-153×J-117 | J-117 | (A2B2) | 13/20 |
| R-153×J-180 | R-153 | (A1B1) | 20/20 |
| R-207×J-151 | J-151 | (A2B2) | 18/20 |
| R-204×J-117 | J-117 | (A2B2) | 18/20 |
| R-207×J-39 | J-39 | (A2B2) | 15/20 |
| R-21 ×R-100 | R-100 | (A2B2) | 17/20 |
| R-144×R-100 | R-144 | (A1B1) | 15/20 |
| R-153×R-117 | R-117 | (A2B2) | 20/20 |
| (A1B1×A3B3) | | | |
| J-53 ×WA-2 | WA-2 | (A3B3) | 10/10 |
| J-130×WA-2 | J-130 | (A1B1) | 7/10 |
| J-134×WA-2 | WA-2 | (A3B3) | 10/10 |
| R-21 ×WA-2 | WA-2 | (A3B3) | 10/10 |
| R-143×WA-2 | WA-2 | (A3B3) | 10/10 |
| R-144×WA-2 | WA-2 | (A3B3) | 8/10 |
| R-153×WA-2 | WA-2 | (A3B3) | 10/10 |
| R-204×WA-2 | WA-2 | (A3B3) | 7/10 |
| R-207×WA-2 | WA-2 | (A3B3) | 10/10 |
| (A1B1×A5B5) | | | |
| J-53 ×WB-2 | WB-2 | (A5B5) | 10/10 |
| J-130×WB-2 | WB-2 | (A5B5) | 9/10 |
| J-134×WB-2 | WB-2 | (A5B5) | 9/9 |
| R-21 ×WB-2 | WB-2 | (A5B5) | 7/10 |
| R-143×WB-2 | WB-2 | (A5B5) | 9/10 |
| R-144×WB-2 | WB-2 | (A5B5) | 7/10 |
| R-153×WB-2 | WB-2 | (A5B5) | 8/8 |
| R-204×WB-2 | WB-2 | (A5B5) | 7/9 |
| (A2B2×A3B3) | | | |
| J-138×WA-2 | WA-2 | (A3B3) | 9/10 |
| J-151×WA-2 | WA-2 | (A3B3) | 10/10 |
| R-7 ×WA-2 | R-7 | (A2B2) | 8/10 |
| R-100×WA-2 | R-100 | (A2B2) | 8/10 |
| R-117×WA-2 | WA-2 | (A3B3) | 8/10 |
| R-146×WA-2 | WA-2 | (A3B3) | 10/10 |
| (A2B2×A5B5) | | | |
| J-138×WB-2 | — | | 5/10 |
| J-151×WB-2 | — | | 5/10 |
| R-7 ×WB-2 | WB-2 | (A5B5) | 8/10 |
| R-100×WB-2 | R-100 | (A2B2) | 8/10 |
| R-117×WB-2 | WB-2 | (A5B5) | 5/9 |
| R-146×WB-2 | WB-2 | (A5B5) | 7/9 |
| J-138×WB-6 | WB-6 | (A5B5) | 7/10 |
| J-151×WB-6 | WB-6 | (A5B5) | 6/10 |
| R-7 ×WB-6 | R-7 | (A2B2) | 5/9 |
| R-100×WB-6 | WB-6 | (A5B5) | 5/9 |
| R-117×WB-6 | WB-6 | (A5B5) | 6/10 |
| R-146×WB-6 | WB-6 | (A5B5) | 10/10 |
| (A2B2×A7B7) | | | |
| J-138×WD-8 | J-138 | (A2B2) | 5/5 |
| J-151×WD-8 | J-151 | (A2B2) | 7/10 |
| R-7 ×WD-8 | WD-8 | (A7B7) | 5/9 |
| R-100×WD-8 | R-100 | (A2B2) | 9/10 |
| R-117×WD-8 | — | | 5/10 |
| R-146×WD-8 | R-146 | (A2B2) | 10/10 |
| (A2B2×A9B9) | | | |
| J-138×WE-27 | J-138 | (A2B2) | 10/10 |
| J-151×WE-27 | J-151 | (A2B2) | 6/10 |
| R-7 ×WE-27 | R-7 | (A2B2) | 10/10 |
| R-100×WE-27 | R-100 | (A2B2) | 8/10 |
| R-117×WE-27 | R-117 | (A2B2) | 6/10 |
| R-146×WE-27 | R-146 | (A2B2) | 10/10 |
| (A1B2×A2B1) | | | |
| A-79 ×A-16 | A-16 | (A2B1) | 16/20 |
| A-79 ×A-17 | A-17 | (A2B1) | 11/20 |
| A-79 ×B-12 | B-12 | (A2B1) | 15/20 |
| A-89 ×B-18 | B-18 | (A2B1) | 13/20 |
| A-89 ×B-19 | B-19 | (A2B1) | 20/20 |
| A-89 ×B-21 | B-21 | (A2B1) | 20/20 |
| A-90 ×B-18 | B-18 | (A2B1) | 20/20 |
| A-90 ×B-19 | B-19 | (A2B1) | 19/20 |
| A-90 ×B-21 | B-21 | (A2B1) | 19/20 |
| A-91 ×B-18 | B-18 | (A2B1) | 14/20 |
| A-91 ×B-19 | B-19 | (A2B1) | 20/20 |
| A-91 ×B-21 | B-21 | (A2B1) | 19/20 |
| A-93 ×B-18 | B-18 | (A2B1) | 12/20 |
| A-93 ×B-19 | B-19 | (A2B1) | 20/20 |
| A-93 ×B-21 | B-21 | (A2B1) | 20/20 |
| A-79 ×T-6 | A-79 | (A1B2) | 11/20 |
| A-79 ×T-7 | T-7 | (A2B1) | 20/20 |
| A-93 ×WB-2 | WB-2 | (A5B5) | 10/10 |
| B-44 ×WB-2 | WB-2 | (A5B5) | 8/10 |
| A-79 ×WB-6 | WB-6 | (A5B5) | 7/10 |
| A-89 ×WB-6 | WB-6 | (A5B5) | 9/10 |
| A-91 ×WB-6 | — | | 5/10 |
| A-93 ×WB-6 | WB-6 | (A5B5) | 10/10 |
| B-44 ×WB-6 | WB-6 | (A5B5) | 9/10 |
| (A1B2xA7B7) | | | |
| A-79 ×WD-8 | — | | 5/10 |
| A-89 ×WD-8 | WD-8 | (A7B7) | 7/10 |
| A-91 ×WD-8 | A-91 | (A1B2) | 7/9 |
| A-93 ×WD-8 | A-93 | (A1B2) | 6/10 |
| B-44 ×WD-8 | — | | 5/10 |
| (A1B2×A9B9) | | | |
| A-79 ×WE-27 | A-79 | (A1B2) | 9/10 |
| A-89 ×WE-27 | — | | 5/5 |
| A-91 ×WE-27 | A-91 | (A1B2) | 9/10 |
| A-93 ×WE-27 | — | | 5/5 |
| B-44 ×WE-27 | B-44 | (A1B2) | 9/10 |
| (A2B1×A3B3) | | | |
| A-17 ×WA-2 | A-17 | (A2B1) | 10/10 |
| B-12 ×WA-2 | B-12 | (A2B1) | 10/10 |
| B-18 ×WA-2 | WA-2 | (A3B3) | 10/10 |
| B-21 ×WA-2 | WA-2 | (A3B3) | 7/10 |
| T-6 ×WA-2 | WA-2 | (A3B3) | 10/10 |
| T-7 ×WA-2 | WA-2 | (A3B3) | 10/10 |
| T-9 ×WA-2 | WA-2 | (A3B3) | 10/10 |
| (A2B1×A5B5) | | | |
| A-17 ×WB-2 | WB-2 | (A5B5) | 9/10 |
| B-12 ×WB-2 | WB-2 | (A5B5) | 10/10 |
| B-18 ×WB-2 | WB-2 | (A5B5) | 7/9 |
| B-21 ×WB-2 | WB-2 | (A5B5) | 9/10 |
| T-6 ×WB-2 | WB-2 | (A5B5) | 10/10 |
| T-7 ×WB-2 | WB-2 | (A5B5) | 6/10 |
| T-9 ×WB-2 | WB-2 | (A5B5) | 10/10 |
| A-17 ×WB-6 | A-17 | (A2B1) | 6/10 |
| B-12 ×WB-6 | — | | 5/10 |
| B-18 ×WB-6 | — | | 5/10 |
| B-21 ×WB-6 | WB-6 | (A5B5) | 9/10 |
| T-6 ×WB-6 | T-6 | (A2B1) | 6/10 |
| T-7 ×WB-6 | WB-6 | (A5B5) | 10/10 |
| T-9 ×WB-6 | WB-6 | (A5B5) | 6/10 |
| (A2B1×A7B7) | | | |
| A-17 ×WD-8 | A-17 | (A2B1) | 7/8 |
| B-12 ×WD-8 | B-12 | (A2B1) | 6/10 |
| B-17 ×WD-8 | B-18 | (A2B1) | 6/9 |
| B-21 ×WD-8 | — | | 5/10 |
| T-6 ×WD-8 | T-6 | (A2B1) | 5/8 |
| T-7 ×WD-8 | T-7 | (A2B1) | 6/10 |
| T-9 ×WD-8 | T-9 | (A2B1) | 8/10 |
| (A2B1×A9B9) | | | |
| A-17 ×WE-27 | A-17 | (A2B1) | 7/10 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R-207×WB-2 | WB-2 | (A5B5) | 10/10 | A-89 ×T-6 | T-6 | (A2B1) | 20/20 | B-12 ×WE-27 | B-12 | (A2B1) | 6/10 |
| J-53 ×WB-6 | J-53 | (A1B1) | 6/10 | A-90 ×T-6 | T-6 | (A2B1) | 19/20 | B-18 ×WE-27 | B-12 | (A9B9) | 6/10 |
| J-130×WB-6 | WB-6 | (A5B5) | 6/10 | A-93 ×T-6 | T-6 | (A2B1) | 20/20 | B-21 ×WE-27 | B-21 | (A9B9) | 9/10 |
| J-134×WB-6 | — | | 5/10 | B-41 ×A-17 | A-17 | (A2B1) | 20/20 | T-6 ×WE-27 | T-6 | (A2B1) | 8/10 |
| R-21 ×WB-6 | WB-6 | (A5B5) | 7/8 | B-41 ×B-19 | B-19 | (A2B1) | 20/20 | T-7 ×WE-27 | T-7 | (A2B1) | 9/10 |
| R-143×WB-6 | WB-6 | (A5B5) | 8/10 | B-41 ×B-21 | B-21 | (A2B1) | 20/20 | T-9 ×WE-27 | T-9 | (A2B1) | 8/10 |
| R-144×WB-6 | WB-6 | (A5B5) | 8/10 | B-44 ×B-18 | B-18 | (A2B1) | 20/20 | | | | |
| R-153×WB-6 | WB-6 | (A5B5) | 7/9 | B-44 ×B-19 | B-19 | (A2B1) | 20/20 | | | | |
| R-204×WB-6 | — | | 5/10 | B-44 ×B-21 | B-21 | (A2B1) | 20/20 | | | | |
| R-207×WB-6 | WB-6 | (A5B5) | 6/9 | B-46 ×B-18 | B-18 | (A2B1) | 20/20 | | | | |
| (A1B1×A7B7) | | | | B-46 ×B-19 | B-19 | (A2B1) | 20/20 | | | | |
| J-53 ×WD-8 | J-53 | (A1B1) | 10/10 | B-46 ×B-21 | B-21 | (A2B1) | 20/20 | | | | |
| J-130×WD-8 | — | | 5/10 | B-41 ×T-9 | T-9 | (A2B1) | 20/20 | | | | |
| R-21 ×WD-8 | — | | 5/10 | B-44 ×T-9 | T-9 | (A2B1) | 20/20 | | | | |
| R-144×WD-8 | — | | 5/10 | B-46 ×T-9 | T-9 | (A2B1) | 20/20 | | | | |
| (A1B1×A9B9) | | | | (A1B2×A3B3) | | | | | | | |
| J-53 ×WE-27 | J-53 | (A1B1) | 7/10 | A-79 ×WA-2 | A-79 | (A1B2) | 10/10 | | | | |
| J-130×WE-27 | J-130 | (A1B1) | 8/10 | A-89 ×WA-2 | WA-2 | (A3B3) | 9/10 | | | | |
| J-134×WE-27 | J-134 | (A1B1) | 7/10 | A-91 ×WA-2 | WA-2 | (A3B3) | 10/10 | | | | |
| R-21 ×WE-27 | R-21 | (A1B1) | 8/10 | A-93 ×WA-2 | WA-2 | (A3B3) | 10/10 | | | | |
| R-143×WE-27 | R-143 | (A1B1) | 6/10 | B-44 ×WA-2 | WA-2 | (A3B3) | 10/10 | | | | |
| R-144×WE-27 | R-144 | (A1B1) | 7/10 | (A1B2×A5B5) | | | | | | | |
| R-153×WE-27 | R-153 | (A1B1) | 7/10 | A-79 ×WB-2 | WB-2 | (A5B5) | 10/10 | | | | |
| R-204×WE-27 | R-204 | (A1B1) | 7/10 | A-89 ×WB-2 | WB-2 | (A5B5) | 10/10 | | | | |
| R-207×WE-27 | R-207 | (A1B1) | 9/10 | A-91 ×WB-2 | WB-2 | (A5B5) | 9/10 | | | | |

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

of oidia were monokaryotic, i.e., oidium cells had only one nucleus.

**Nuclear phase of oidia** Cao et al. (1999a) reported that the oidia from dikaryotic mycelia in *P. nameko* contained various numbers of nuclei per cell, and most had only one nucleus per cell. Furthermore, the majority of the oidia were haploid with the DNA content of G1 phase. A similar histochemical experiment was conducted to determine the nuclear phase in oidia of *F. velutipes* by determining the relative DNA contents of oidia. The results are shown in Table 3.

The propidium iodide staining for nuclear DNA in monokaryotic oidium cells showed that most oidia (over 95%) had DNA values corresponding to that of the reference, suggesting that these oidia had single haploid nuclei at the G1 stage. However, the remaining 5% had twice the reference value, and it is suggested that those nuclei showing doubled DNA content were at the G2 stage. Comparison of the average DNA value (1.11) of monokaryons of *F. velutipes* with that of *P. nameko* (0.94) suggested that the nuclear DNA size of the former species was significantly 1.18 times higher than that of the latter species (cf. Cao et al., 1999a).

**Nuclear selection in the monokaryotic oidium formation from various hybrid dikaryons** The involvement of nuclear selection in mycelium and oidium monokaryotization had been demonstrated by using several mated products in a bi-polar basidiomycete, *P. nameko* (Arita, 1964; Masuda et al., 1995, Cao et al., 1999b). In order to confirm whether nuclear selection was involved in monokaryotic oidium formation in a tetrapolar basidiomycete, *F. velutipes*, we attempted to determine which parental nucleus predominated in the monokaryotic oidia from 194 dikaryotic hybrid stocks. Twenty (or ten) monokaryotic oidium isolates from each hybrid dikaryon were back-crossed to the corresponding parental incompatibility factor tester stocks to determine their nuclear type. The results are shown in Table 4.

Except for those from 17 hybrid dikaryons in a total of 194 test stocks, all of the monokaryotic oidium isolates showed nuclear types similar to only one of the parental stocks. We conclude that the monokaryotic oidium formation from dikaryotic mycelium of *F. velutipes* also involves nuclear selection. Although one nuclear type was recovered at high rates in the oidia from most hybrid dikaryons, the process seems to produce essentially the split nuclear type composition. A similar result has been reported in the monokaryotic oidium formation in a bipolar basidiomycete, *P. nameko* (Cao et al., 1999b). On the other hand, mycelium monokaryotization may occur primarily in *P. nameko* rather than exclusive nuclear selection from two parental nuclear types (Masuda et al., 1995).

**Hierarchy of nuclear selection in monokaryotic oidium formation from various hybrid dikaryons** Table 4. also suggests that the selection of one of the two nuclei in the dikaryotic cells in monokaryotic oidium formation followed a relative dominance rule. In the case of R-143× J-117 hybrids, only the J-117 nucleus was found in the monokaryotic oidia, but the same nucleus was recessive

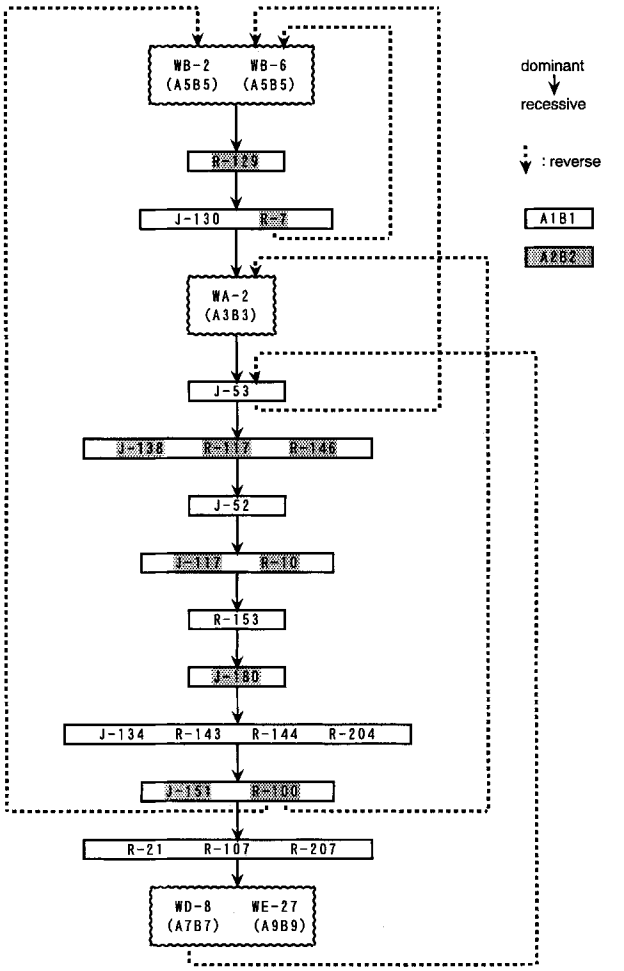


Fig. 1a. Hierarchy of nuclear selection in monokaryotic oidium formation from various hybrid dikaryons in matings between monokaryons having A1B1 or A2B2 incompatibility factors and with tester strains in *Flammulina velutipes*.

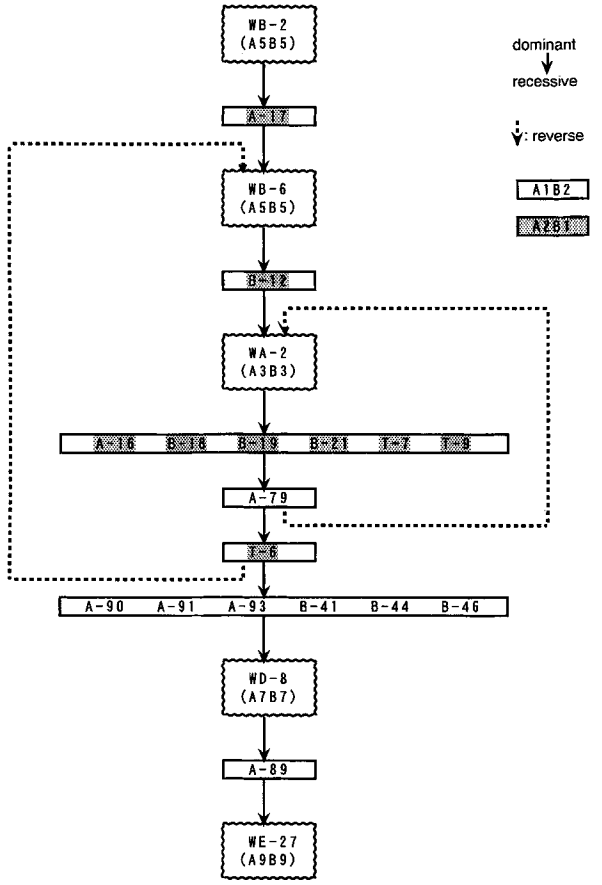


Fig. 1b. Hierarchy of nuclear selection in monokaryotic oidium formation from various hybrid dikaryons in matings between monokaryons having A1B2 or A2B1 incompatibility factors and with tester strains in *Flammulina velutipes*.

with respect to the J-52 nucleus in the monokaryotic oidium formation from the J-52×J-117 hybrid. The separate results of hierarchies of relative dominance among two nuclei of the parental dikaryons in the monokaryotic oidium formation were determined among the same incompatibility factor composition groups of hybrid dikaryons (Figs. 1a, 1b). Only a few discrepancies were found between the two specific nuclear compositions of hybrid dikaryons. Therefore, we conclude that a rule of relative dominance is active in the selection of the nucleus for monokaryotization in oidium formation from dikaryons in both bi- and tetra-polar basidiomycetous mushrooms (cf. Masuda et al., 1995; Cao et al., 1999b).

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