Ryuso TANAKA* & Isuzu KOGA*: **Karyological studies on *Lentinus edodes*, a basidiomycete**

田中隆荘*・古賀いすづ*: シイタケに関する核学的研究

Introduction *Lentinus edodes* (Berk.) Sing is utilized as an important edible mushroom. Although several breeding experiments have been carried out (Komatsu and Kimura, 1964a, b, 1968), there is no cytological report on this species as yet. In the present investigation the process of somatic nuclear division in the secondary mycelia and its chromosome number are dealt with.

Materials and Methods *Lentinus edodes* (Berk.) Sing, strain No. 1303, used in the present investigation was obtained from the Laboratory of Meiji Seika Co., Ltd. in Itsukaichi-cho, Hiroshima Prefecture, Japan. According to the members of the Laboratory, this strain is highly stable in hereditary behavior and is a good producer of fruit-bodies. The present authors would like to express their gratitude to the members of the Laboratory of Meiji Seika for the kind supply of this strain.

The secondary mycelia of *L. edodes* were cultured on an agar medium consisting of malt extract at 25°C for 3 to 7 days, then fixed and stained by the following HCl-Giemsa staining method (Aist, 1969). Good growing secondary mycelia were fixed in a mixture of absolute ethanol and glacial acetic acid (3:1) at 5°C for more than 1 hour, then immersed in 35% ethanol for 10 minutes and washed sufficiently with distilled water. After immersing in 1N HCl at room temperature for 1 to 2 minutes, they were hydrolyzed in 1N HCl at 60°C for 6 minutes. The hydrolyzed mycelia were washed sufficiently with distilled water and immersed in 1/15 mole phosphate buffer (pH 7.0) for about 10 minutes. The materials were stained in the following staining solution for 0.5 to 1 hour. The staining solution was made by diluting Giemsa solution (Wako Pure Chemical Industries, Ltd.) at the ratio of 3 drops to 1 ml phosphate buffer (pH 7.0) and then filtering the solution.

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A small segment of the stained material was put on a slide glass and mounted with the staining solution. After putting a cover glass over the material, it was squashed and observed.

Chromosome number was studied in both the somatic nuclear division of the mycelia and the meiotic nuclear division during basidiospore formation in the gills of the fruit-body. The gills were fixed in a mixture of 70% ethanol and glacial acetic acid (3 : 1) and stained by the HCl-Giemsa staining method.

The nuclei which were stained by the HCl-Giemsa staining method were morphologically compared with those stained by Feulgen's staining. In the Feulgen's staining, the materials were fixed in the same way as the HCl-Giemsa staining method. They were hydrolyzed in 1N HCl at 60°C for 5 minutes, reacted in Schiff's reagent for more than 3 hours, rinsed several times in tap water. The materials were squashed in 45% acetic acid solution on a slide glass.

Observations The nuclei in both the mycelial cells and the basidia were clearly stained by the HCl-Giemsa staining showing the structural characteristics of the chromatin and chromosomes. The results obtained from the Feulgen's staining were the same except slightly lighter.

1. The somatic nuclear division in secondary mycelial cells (Figs. 1A–G, 2A–C).

In all of the mycelial cells at interphase two nuclei were observed. The two nuclei were observed to be ellipsoidal and of the same size, about 2.0 μ × 2.5 μ. The interphase nuclei often possessed one to two darkly stained condensed chromatic bodies in addition to many lightly stained chromatic granules. The nucleoli, the unstainable round bodies, were observed in most interphase nuclei. In many interphase nuclei the darkly stained condensed chromatic bodies were observed attached to the nucleoli (Fig. 1A).

In the nuclei at prophase, chromosomes were observed as lightly stained threads (Fig. 1B). In each of the chromosomes several darkly stained heterochromatic regions were observed.

Chromosomes at metaphase were counted to be n=8 (Figs. 1C, 2A). The metaphase chromosomes were observed to be rod-shape and about 0.4 μ to 0.6 μ in size. Constriction were not distinct. One to two of the small chromosomes were stained lightly. One to two of the large chromosomes

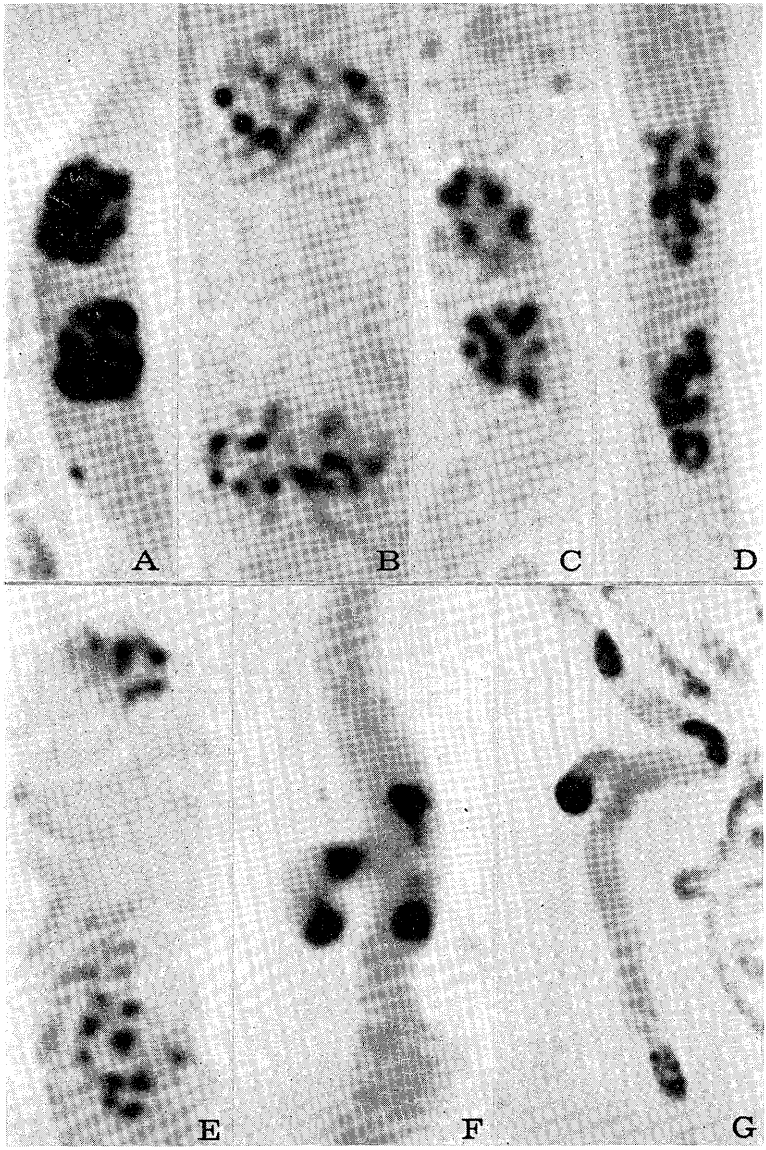

Fig. 1. Somatic nuclear division in the secondary mycelial cells of *Lentinus edodes*. A, interphase just entering the mitotic stage. B. prophase. C and D, metaphase, showing variation in condensation among chromosomes of a complement. Eight chromosomes can be counted in one of the nuclei (lower side) of C. E, anaphase, showing 16 sister chromosomes in one of the nuclei (lower side). F, early telophase, showing a clamp and some laggards. G, late telophase, showing condensation of the nucleus in the clamp. A-F, ×5300, G, ×2650.

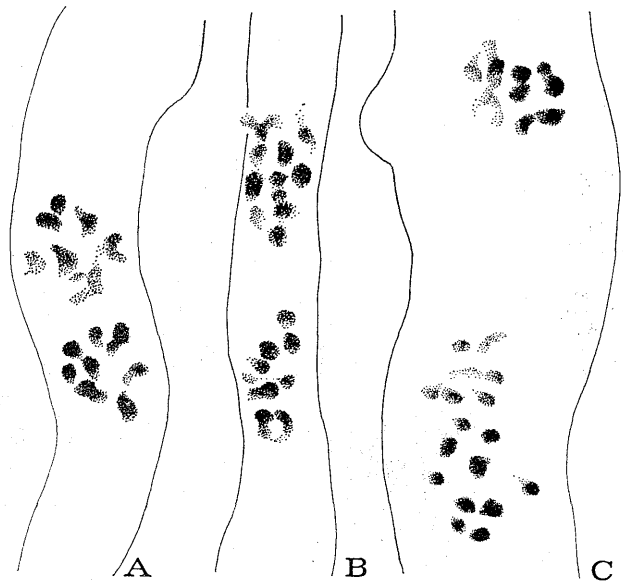

Fig. 2. Text-figures of Fig. 1C, D and E, respectively. ×5300.

were found to stain darker than those compared with the other chromosomes of the complement. It was often observed that at metaphase all of the eight chromosomes were arranged in groups of two. One to two chromosomes were observed separating into sister chromosomes precociously at late metaphase (Figs. 1D, 2B). At anaphase one to two small chromosomes were observed showing lagging movement towards the poles (Figs. 1E, 2C).

Each of the mycelial cells at the stages from metaphase to anaphase formed a clamp. It was observed that the nucleus, situated at the apical side of the mycelial cell, usually moved into the clamp. At early telophase one of the daughter nuclei which was formed in the clamp moved back into the mycelial cell. During the movement of the daughter nuclei, several, usually two to four, lagged chromosomes were observed (Fig. 1F). The daughter nucleus at telophase finally seemed to include the lagged chromosomes, because there was no laggard at late telophase (Fig. 1G). The telophase nuclei showed strong condensation and were of ring-shape. Among the four daughter nuclei the nucleus which moved into the hind mycelial

cell through the clamp showed a delay in the diffusion of chromatin as compared with the other three nuclei in the daughter mycelial cells (Fig. 1G).

It was observed that the two nuclei in a mycelial cell showed a slight difference in the morphological features of chromatin during somatic nuclear division. In most mycelial cells the nucleus, located at the proximal side of the mycelial cell, was observed to be slightly precocious as compared with the nucleus at the apical side.

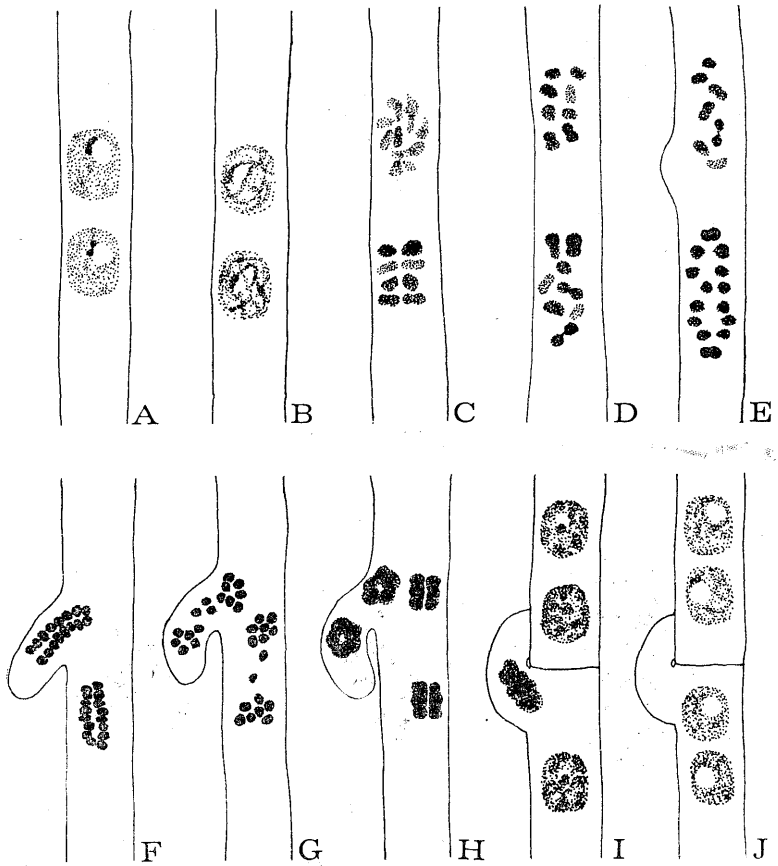

Fig. 3. Half-schematic representation of the process of somatic nuclear division in the secondary mycelial cells of *Lentinus edodes*. A, interphase. B, prophase. C and D, metaphase. E, F and G, anaphase. H and I, telophase. J, early interphase.

The results of the observations on the somatic nuclear division of mycelial cells are shown half-schematically in Fig. 3.

2. The meiotic nuclear division in basidia (Fig. 4A–F).

Eight bivalent chromosomes were observed at meiotic diakinesis and

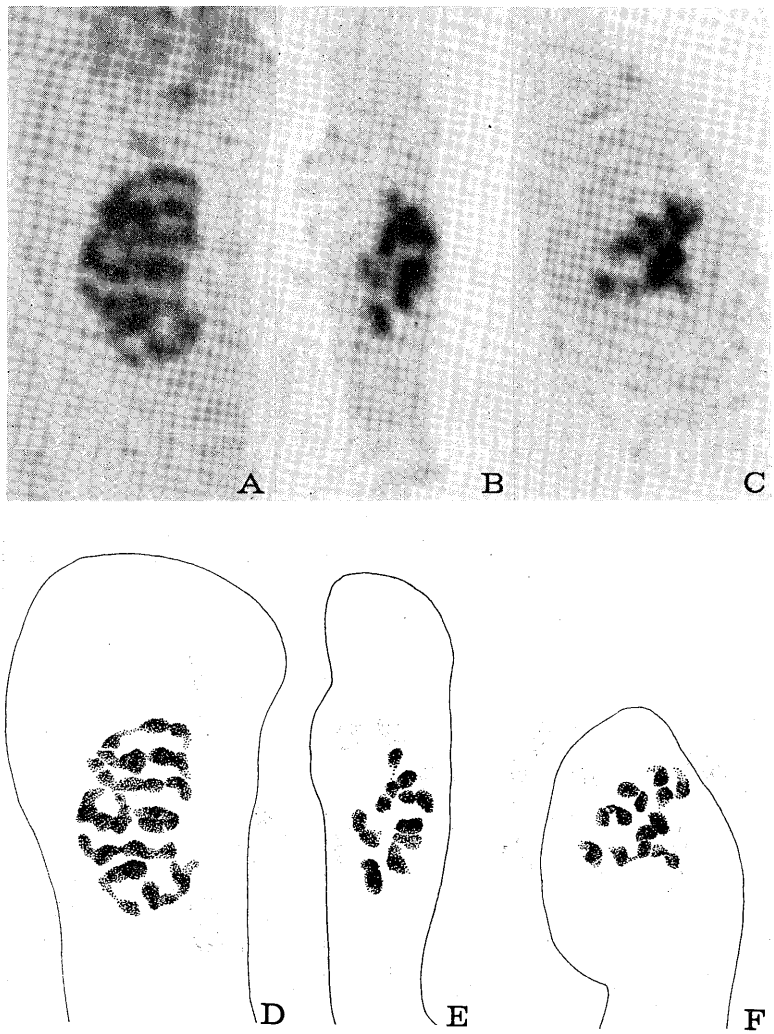

Fig. 4. Meiotic chromosomes in the basidia of *Lentinus edodes*. A, diakinesis. B and C, metaphase I. D, E and F, text-figures of A, B and C, respectively. ×5300.

metaphase I in the basidia (Fig. 4A–F). The bivalent chromosomes were observed to be almost equal in size about 0.4 μ to 0.6 μ in length. The bivalent chromosomes showed a slight variation in the degree of condensation at metaphase I and in the movement of chromosomes at anaphase I (Fig. 4B, C, E and F). In most basidia at anaphase I one to two precocious movement and one to two delayed laggards were observed.

Summary

1. The chromosome number of *Letinus edodes* (Berk.) Sing was found to be n=8 in the somatic division of secondary mycelia and in the meiotic division of basidia.

2. The metaphase chromosomes were observed to be rod-shape and about 0.4 μ to 0.6 μ in length. The eight chromosomes at metaphase showed variation in the stainability, *i.e.*, it comprised of one to two lightly stained chromosomes and one to two darkly stained chromosomes.

3. In both the mycelia and basidia chromosomes were found showing one to two precocious movement at late metaphase and one to two lagging at late anaphase.

4. The daughter nucleus which moves into the proximal daughter mycelial cell through the clamp showed high condensation during the movement. This nucleus was found to enter into the diffused state later than the other three nuclei at interphase.

Literature cited

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Lentinus edodes (Berk.) Sing シイタケの二次菌糸の核分裂，担子器の減数分裂

を観察し，次のことを確かめた。染色体数は n=8 である。中期染色体は，桿状で，長さ約 0.4 μ～0.6 μ，染色不揃いで，淡染する中位大のもの 1～2 個，濃染する大形のもの 1～2 個を含んでいる。後期染色体は，縦裂と分配に関して，染色体間で遅速がある。嘴状突起を通って基部娘細胞に移動する娘核は，染色質が凝縮状態である。この娘核は，他の 3 娘核に比べて，染色質の分散が遅れる。

□内田　享 監修：**谷津・内田　動物分類名辞典**　B5 pp. 1411 18,000 円，1972，2 月，東京，中山書店。谷津直秀先生の「動物分類表」といえば，動物のいろいろなことを知るのに便利であるのはもちろんだが，谷津先生の創意になる各群の記事が，たいへんに楽しいので有名な本であった。初版が大正 3 年（1914）で，戦後昭和 27 年（1952）に第 7 版が出たから，大変いきの長い本でもある。それを内田 享氏の監修のもとに，40 人をこえる各部類の専門家が，夫々の得意のところを分担し，なるべく谷津先生の解説を残す一方，分類を最新のものにし，併せて多くの増補を行って，題名と出版所を変えて出版されたのが本書である。内容がたいへん増加され，従って定価も大分高くなったけれども，盛り込まれた内容を考えたら安いものといえよう。

各ページを左右に割り，左側に属までの分類表を，右側にこれに対応する解説や，主な代表種の名と，それに関する興味のある，あるいは重要なデータを書き添えてあり，この中に谷津先生時代のものも含まれている。この分類表が本書の生命であって，苦心の跡がみられる編集で，多勢の書き振りの違う専門家を，制御されるのに大変なことであったろうとお察しした。これだけ一貫して整理されたものは，手近かにないから便利この上もなく，座右に置いて大いに重宝している。イリオモテヤマネコ，化石のマチカネワニなどの分類上の位置もわかるなど，新らしいデータが十分に入っている。

しかしなんといっても部門によって，人間に対する関係の仕方が違うので，鳥類など，英独仏伊西の呼び名までいれたが，種名は多くは省いて属のレベルで説明をつけたのに対して，二枚貝のところは各属毎に必ず一種名とその和名だけは欠かさずに挙げるといった様式であったり，原生動物のところは属名と文献を列記したアカデミックな色彩が強く，ミドリムシやラッパムシの和名もでて来ないのは，少々統一が悪く不便であると思われた。それに植物と違って群の名は漢字を使ってあるのが多く，たとえば異紐虫類，根口水母類，捩神経類など，いわゆる重箱読み的のもの，海蕾，叉棘，吻蛭，鼈竜の各類などは，専門家以外には手がでない。これらはルビをつけて頂きたいし，やさしい漢字の海百合類なども，存外読みが必要なのではないか。それと各類や各属のおおよその大きさとひろがりを添える工夫をしてほしかった。どうも文句をつけすぎるが，便利この上なしの良書故に，隴を得て蜀を望むの思いを諒とされたい。

（前川文夫）