Studies on the Heterothallism of *Cortinellus Berkeleyana* Ito et Imai, an Economically Important Edible Mushroom in Japan.

By

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Contents.

I. Introduction.

In Japan, *Cortinellus Berkeleyana* Ito et Imai is one of the most important edible mushrooms. The cultivation of this fungus has been a very profitable branch of forest industry in this country. According to Ito and his collaborator[1] the fungus had been described for the first time by M. J. Berkeley in 1878 as *Agaricus (Armillaria) edodes* Berk. and then the following names were applied to this fungus by various authors.

Agaricus (Armillaria) edodes Berk. (Berkeley, 1878).
Collybia Schii-Take Siebold (Schroeter, 1886).
Armillaria edodes Berk. (Saccardo, 1887).
Lepiota Shiitake Tanaka (Tanaka, 1887).
? *Armillaria edodes* (Berk.) Sacc. (P. Hennings, 1897).
Cortinellus Shiitake (Schroet.) P. Henn. (P. Hennings, 1889, 1901; Shirai, 1900; Matsumura, 1904; Kawamura, 1914; Duggar, 1920; Kawagoe, 1924).
Shiitaker cortinellus Lloyd (Lloyd, 1924).
Cortinellus Berkeleyana Ito et Imai (Ito and Imai, 1925).

1) Ito, S. and Imai, S. (1925), On the taxonomy of Shiitake and Matsutake. Journal of the Society of Agric. & Forestry, Sapporo, 17: 74: 155—162. (Japanese.)

Table I.

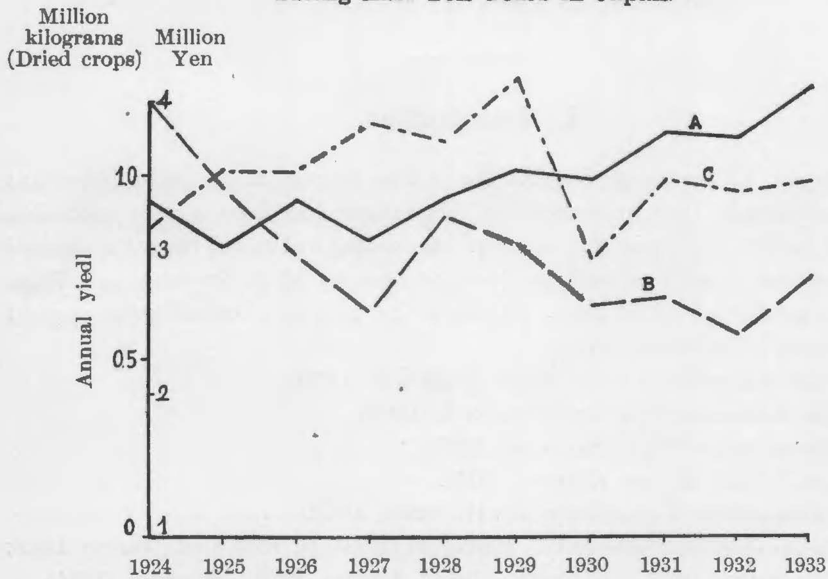
The Annual Yield of *Cortinellus Berkeleyana* Ito et Imai during Last Ten Years in Japan.

After the Tenth Statistical Report of the Japanese Department of Agriculture and Forestry. (1933)

	Yield in kilograms (As dried crops)	Value in Yen
1924	986,989.8	4,039,099
1925	852,270.4	3,411,027
1926	981,442.8	2,972,929
1927	871,354.8	2,587,710
1928	985,450.2	3,252,236
1929	1,043,279.4	3,046,204
1930	1,021,350.0	2,601,301
1931	1,154,506.8	2,686,525
1932	1,146,328.2	2,476,912
1933	1,290,911.4	2,897,598

Graph 1.

The Annual Yield of *Cortinellus Berkeleyana* Ito et Imai during Last Ten Years in Japan.



Remarks: A. The heavy line shows the annual yield of *Cortinellus Berkeleyana* as dried crops in million kilograms.

B. The broken line shows the annual yield of *Cortinellus Berkeleyana* in million Yen.

C. The chain shows the annual yield of *Armillaria Matsutake*, the most tasteful mushroom in Japan, in million Yen.

The Japanese name of this fungus, the "Shiitake", is the meaning of the mushroom growing on Pasania, the "Shii" being *Pasania Sieboldii* MAKINO, a kind of the oak ; and the "Take", mushroom. The fungus is artificially grown on cut stems of many species of the oak family, Fagaceae : *Pasania* spp., *Quercus* spp., *Castanea* spp. and *Carpinus* spp. The mushroom, usually used as dried crop, is not only most congenial to the taste of Japanese, but also highly nourishing food as it contains much ergosterol.

The production of this edible fungus in Japan increases steadily year after year as shown by the statistics given in Table I and Graph 1. In 1933 the annual yield as dried crops attains to 1,290,000 kilograms. The value of the crop to the producers varies from 2,500,000 Yen to 4,000,000 Yen annually. The fungus is produced chiefly in the prefectures Miyazaki, Ooita, Sizuoka, Kagosima, Kumamoto, etc. The annual yield in these more important localities are given in Table II.

Table II.

The Annual Yield of *Cortinellus Berkeleyana* Ito et Imai in the Chief Localities of the Production in Japan, in 1933.

After the Tenth Statistical Report of the Japanese Department of Agriculture and Forestry. (1933)

No.	Prefectures	Yield in kilograms (As dried crops)	Value in Yen
1.	Miyazaki	311,194.2	688,053
2.	Ooita	263,073.6	526,960
3.	Sizuoka	207,730.2	459,833
4.	Kagosima	98,752.2	285,742
5.	Kumamoto	84,147.6	215,170
6.	Miye	54,744.6	88,615
7.	Wakayama	34,945.8	80,481
8.	Ehime	45,748.2	74,079
9.	Kôti	40,887.0	68,335
10.	Aiti	23,846.4	62,772

Although the cultivation of *Cortinellus Berkeleyana* is one of the most important forest industry in some districts, cultural studies on this fungus have been little done so far in Japan. Therefore, the writers set up their cultural works of this economically important fungus. For the first step they dealt with the pure culture and the sexuality. As to the sexuality K. OIKAWA[1] has recently

1) OIKAWA, K. (1935), Siitake no Seikatu-si. Rigaku-Kai, 33 : 3 : 233–234. (Japanese.); and (1935), Sex in *Cortinellus Shiitake*. Bot. Magazine, 49 : 583 : 453–455. (Japanese with English summary.)

published his result of experiment. According to him *Cortinellus Shiitake* P. HENN. (*Cortinellus Berkeleyana* ITO et IMAI) is heterothallic and bipolar. Meanwhile the result of the present writers' study shows that this fungus is tetrapolar without irregularity. And the present paper deals with the tetrapolar mycelium of *Cortinellus Berkeleyana* and is a part of the paper "On the sexuality of *Cortinellus Berkeleyana* and its culture on saw-mill dust" read by the senior writer in the Forester-Meeting of the Ôsaka Local Bureau of Forestry, held in Hirosima on February 26, 1935.

The writers are obliged to Mr. K. OONAKADO and the other staffs of the Ôsaka Local Bureau of Forestry for their help during the course of this investigation.

II. Morphological Characteristics.

Prior to describing the results of the cultural work, brief morphological characteristics of this fungus are here given. The pileus is 4—10 cm. broad, commonly 5—7 cm., but occasionally considerably larger, at first conical to hemispherical, later expanding to plane, usually brown to dark brown, when dried, dark brown. At first it is provided with slightly whitish cottony scales, which are later detached off. The flesh is white, compact, somewhat flexible and durable to dryness. The stem is 2.5—5 cm. long 0.7—1.5 cm. thick, equal or slightly thicker towards the round base, straight or curved and somewhat excentric to the pileus, as the fungus grows on the side of standing trees. Even after the pileus has expanded, the stem is at first provided with cottony ring-like remains of the veil, which usually disappear later. The lower half of the stem is brown, and the upper portion or apex whitish. The gills or lamellae are white and sinuate to the stem. (See Plate I, Fig. 1 and Plate II, Fig. 2.)

The basidium is hyaline, cylindrical to spatulate, as shown in Plate V, Fig. 10 and 11, 22–30 μ long and 6–10 μ wide. It is provided with four slender pointed sterigmata which are 2—3 μ long 1 μ wide. The basidiospores are hyaline, globular, ellipsoid or ovate, pointed at the base (as shown in Plate V, Fig. 12). They are 4—7 μ (mean 5.92 μ) long 3–5 μ (average 4.04 μ) wide.

III. Source of the Cultures Studied.

The pure cultures of *Cortinellus Berkeleyana* ITO et IMAI were isolated for the first time on November 25, 1931, from a fruit-body sent by Mr. I. NOMURA, Tomi-mura, Akaiwa-gun, Okayama-ken, to Dr. M. KONDO, the director of the Institute. By the mating of the cultures, thus started, some fruit-bodies were secured in pure culture on saw-mill dust medium. From a fruit-body, thus obtained, a deposite of the basidiospores were shed on clear agar plate, a bit of the gills being cut from the pileus and attached to the cover of the plate. Under a microscope a single basidiospore, shed separately, was indicated and a monosporous culture was isolated. In this method a series of 27 monosporous cultures was obtained. The culture medium used in the isolation was 3% malt-extract agar. The hyaline basidiospores germinated in 24 hours. Some of the cultures thus obtained were not really single spore cultures, and discarded. The haploid cultures were kept in a condition of vigorous growth by making transfers once every month.

IV. Four Sexual Groups of *Cortinellus Berkeleyana* ITO et IMAI.

Twenty-three cultures of the haploid mycelium, secured as above, were paired on slant agar culture medium in test tubes in all possible combinations. After from seven to ten days culture at 24°C. a piece of agar block with the hyphae was taken from near the line of contact of the two haplophytes. And the diploidisation, or the process by which the haploid cell is converted into diploid cell, was examined. The most conspicuous characteristics of the diploid mycelium is the presence of the clamp-connections at the septa of the hyphae. Fig. 4 in Plate II shows the clamp-connection of *Cortinellus Berkeleyana* produced on the malt-extract agar. The presence of the clamp-connection was inspected microscopically, and the diploidisation of two haplophytes was determined. In the following table the result of the pairing are given in tabular from, a plus sign indicating the presence of the clamp-connections and a minus sign their absence.

Table III.

Heterothallism of *Cortinellus Berkeleyana* Ito et Imai, demonstrated by Pairing Monosporous Mycelium in all possible Combinations.

No. of Culture Strains		AB							ab					Ab						aB				
		2	6	8	10	13	18	25	7	12	15	19	21	1	3	14	17	20	23	4	5	9	11	24
AB	2	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
	8	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
	10	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
	13	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
	18	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
	25	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–

(Continued to the next page.)

Table III. (Continued.)

No. of Culture Strains		AB							ab					Ab						aB				
		2	6	8	10	13	18	25	7	12	15	19	21	1	3	14	17	20	23	4	5	9	11	24
ab	7	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	12	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	15	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	19	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	21	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ab	1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
	14	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
	17	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
	20	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
	23	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
aB	4	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−
	5	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−
	9	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−
	11	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−
	24	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−

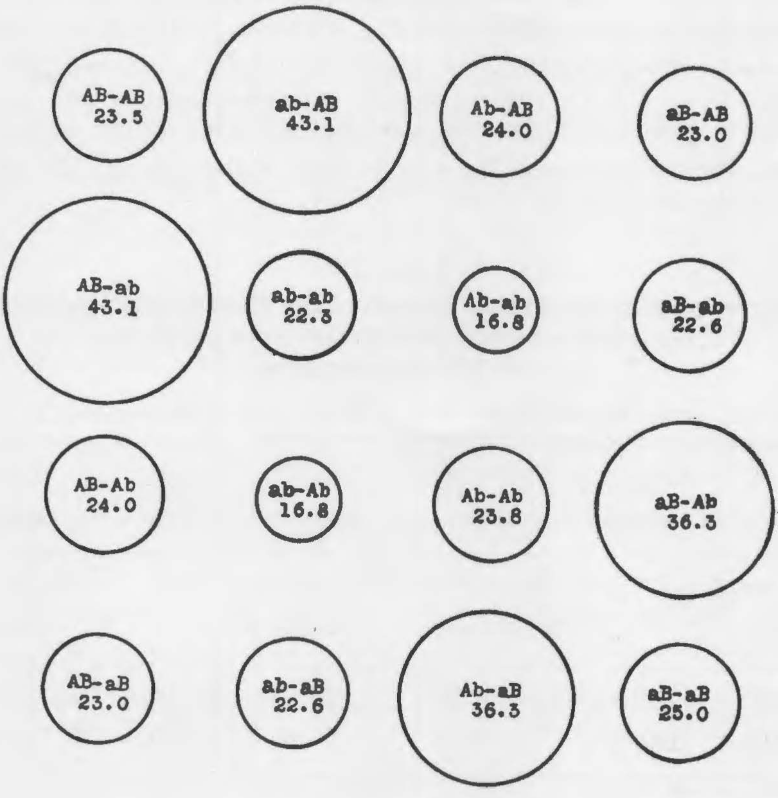
According to Table III, the clamp-connections took place only when the haploid mycelium of one of Nos. 2, 6, 8, 10, 13, 18 and 25 was paired with one of Nos. 7, 12, 15, 19 and 21. Therefore the former group of cultures, such as Nos. 2, 6, 8 etc. is of opposite sex to the latter group of Nos. 7, 12, 15 etc., and the symbols (AB) and (ab) were assigned respectively. On the otherhand the mycelia of Nos. 1, 3, 14, 17, 20 and 23 (a group assigned with the symbol Ab) mated only with those of Nos. 4, 5, 9, 11 and 24 (a group aB), and not with the others.

In the long series experiments there were no irregularities, the mycelia falling perfectly into four sexual groups: AB, ab, Ab and aB.

Graph 2.

Comparisons in the Growth of the Haploid and Diploid Mycelium of *Cortinellus Berkeleyana* Ito et Imai, Graphically demonstrated.

Showing average diameter of colonies after 10 days culture on potato-dextrose agar at 24°C.



V. Comparisons in the Hyphal Growth of the Haploid and the Diploid Mycelium.

As shown in some other species of Hymenomycetes, the hyphae of the diploid mycelium of *Cortinellus Berkeleyana* are some what different from those of the haploid mycelium. The haploid nature of the mycelium in this fungus is indicated not only by the simple septa or the absence of the clamp-connections of the hyphae but also by the comparatively wide-angled mode of branching of the leading radial hyphae. It is clearly shown in Fig. 5 in Plate III and Fig. 13 in Plate V. On the other hand the diploid nature of the mycelium is shown by the presence of the clamp-connections at the septa of the hyphae and the comparatively narrow-angled mode of branching of the leading hyphae, as shown in Fig. 6 in Plate III and Fig. 14 in Plate V. Further the writers compared the growth rate of the hyphae of the diploid and the haploid mycelium. The results of the comparisons are here given.

Comparisons in the hyphal growth on the malt-extract agar. A small, 2 mm. circular, bit of the agar medium with the hyphae of the haploid mycelium, AB, ab, Ab, or aB and the diploid mycelium consisting of AB×ab or Ab×aB was transfered on the center of a plate of 3% malt-extract agar medium. Three or four plates were used for each of the cultures studied. The plate cultures, thus prepared, were kept at 24°C. for 2 weeks. Then the diameter of the colonies was measured in rectangular two directions. The average value of these six or eight measurements are given in Table IV.

Table IV.

Comparisons in the Hyphal Growth of the Haploid and the Diploid Mycelium of *Cortinellus Berkeleyana* Ito et Imai on Malt-Extract Agar.

Average diameter of the colonies after 2 weeks culture at 24°C.

AB (6) 21.3 mm.	AB×ab (6×15) 40.5 mm.	ab (15) 24.5 mm.	Ab (20) 33.8 mm.	Ab×aB (20×24) 42.5 mm.	aB (24) 29.0 mm.
AB×ab (6×12) 49.0 mm.		AB×ab (15×18) 58.5 mm.	Ab×aB (20×4) 42.5 mm.		aB×Ab (24×17) 57.8 mm.
ab (12) 27.0 mm.	ab×AB (12×18) 48.8 mm.	AB (18) 34.5 mm.	aB (4) 24.0 mm.	aB×Ab (4×17) 48.3 mm.	Ab (17) 18.0 mm.

Table IV shows:

Average of the diameter of the colonies of the haploid mycelium
Ab and ab is 26.8 mm.

Average of the diameter of the colonies of the diploid mycelium
AB × ab is 49.2 mm. (1.84 times of the haploid mycelium).

Average of the diameter of the colonies of the haploid mycelium
Ab and aB is 26.2 mm.

Average of the diameter of the colonies of the diploid mycelium
Ab × aB is 47.8 mm. (1.82 times of the haploid mycelium).

The hyphal growth of the diploid mycelium of *Cortinellus Berkeleyana* is much better than that of the haploid mycelium. The similar experiments were repeated with many more strains. The results are given in Table V and VI.

Table V.

Comparisons in the Hyphal Growth of the Haploid and the Diploid Mycelium of *Cortinellus Berkeleyana* Ito et Imai on Malt-Extract Agar.

Average diameter of colonies after 10 days culture on malt-extract agar at 24°C.

		AB		ab		Ab		aB	
		6	18	12	15	17	20	4	24
AB	6	mm. 24.5	mm. 23.8	mm. 31.4	mm. 36.0	mm. 19.0	mm. 20.9	mm. 21.3	mm. 14.3
	18	23.8	23.8	38.0	40.5	16.3	17.6	18.2	15.4
ab	12	31.4	38.0	22.0	15.3	16.0	21.5	14.5	17.3
	15	36.0	40.5	15.3	16.3	15.0	14.1	21.7	15.1
Ab	17	19.0	16.3	16.0	15.0	14.0	17.7	28.3	27.2
	20	20.9	17.6	21.5	14.1	17.7	21.4	28.0	31.9
aB	4	21.3	18.2	14.5	21.7	28.3	28.0	19.5	22.4
	24	14.3	15.4	17.3	15.1	27.2	31.9	22.4	22.5

Table VI.

Summary of the Growth-Comparisons of the Haploid and the Diploid Mycelium of *Cortinellus Berkeleyana* Ito et Imai on Malt-Extract Agar.

Average diameter of colonies after 10 days at 24°C.

	AB	ab	Ab	aB
AB	mm. 24.0	mm. 36.5	mm. 18.5	mm. 17.3
ab	36.5	17.2	16.7	17.2
Ab	18.5	16.7	17.7	28.9
aB	17.3	17.2	28.9	21.7

Average diameter of colonies after 17 days at 24°C.

	AB	ab	Ab	aB
AB	mm. 56.2	mm. 80.0	mm. 49.9	mm. 40.1
ab	80.0	47.1	33.1	41.5
Ab	49.9	33.1	33.3	74.7
aB	40.1	41.5	74.7	48.5

According to the result given in the above tables, the average diameter of the colonies of the haploid mycelium AB and ab after 10 days culture on the malt-extract agar at 24°C. is 24.0 mm. and 17.2 mm. respectively. Meanwhile the diameter of the colonies of the diploid mycelium AB×ab is 36.5 mm.; and about 1.77 times of the mean diameter of the both haploid mycelium AB and ab. In the haploid mycelium Ab and aB the average diameter of the colony was 17.7 mm. and 21.7 mm. respectively, and in the diploid mycelium Ab×aB it was 28.9 mm., and about 1.47 times of the mean diameter of the haploid Ab and aB. The results after 17 days culture on the same culture medium show the similar relations.

Comparisons in the hyphal growth on the potato-dextrose agar. Similar experiments were carried out with the culture media of potato-dextrose agar. The results are given in Table VII and VIII.

Table VII.

Comparisons in the Hyphal Growth of the Haploid and the Diploid Mycelium of *Cortinellus Berkeleyana* Ito et Imai on Potato-Dextrose Agar.

Average diameter of colonies after 10 days culture at 24°C.

		AB		ab		Ab		aB	
		6	18	12	15	17	20	4	24
AB	6	mm. 24.5	mm. 21.0	mm. 36.5	mm. 43.0	mm. 17.5	mm. 24.0	mm. 24.5	mm. 21.0
	18	21.0	27.5	46.5	46.5	29.0	25.5	29.5	17.0
ab	12	36.5	46.5	24.0	21.5	24.0	22.0	15.0	20.0
	15	43.0	46.5	21.5	22.0	15.0	14.0	30.0	25.5
Ab	17	17.5	29.0	24.0	15.0	19.0	24.0	38.0	32.0
	20	24.0	25.5	22.0	14.0	24.0	28.0	37.5	37.5
aB	4	24.5	29.5	15.0	30.0	38.0	37.5	24.0	24.0
	24	21.0	17.0	20.0	25.5	32.0	37.5	24.0	28.0

Table VIII.

Summary of the Growth-Comparisons of the Haploid and the Diploid Mycelium of *Cortinellus Berkeleyana* Ito et Imai on Potato-Dextrose Agar.

Average diameter of colonies after 10 days culture at 24°C.

	AB	ab	Ab	aB
AB	mm. 23.5	mm. 43.1	mm. 24.0	mm. 23.0
ab	43.1	22.3	18.8	22.6
Ab	24.0	18.8	23.8	36.3
aB	23.0	22.6	36.3	25.0

Average diameter of colonies after 17 days culture at 24°C.

	AB	ab	Ab	aB
AB	mm. 33.6	mm. 60.1	mm. 27.8	mm. 30.9
ab	60.1	33.3	20.5	27.4
Ab	27.8	20.5	33.0	51.1
aB	30.9	27.4	51.1	31.1

According to the figures given in the above tables the average diameter of colonies of the haploid mycelium AB and ab after 10 days culture on the potato-dextrose agar was 23.5 mm. and 22.3 mm. respectively. The diameter of those of the diploid mycelium AB×ab was 43.1 mm., and about 1.88 times of the mean diameter of both of the haploid mycelium AB and ab. In the same time the mean of the colony-diameter of the diploid mycelium Ab×aB was 36.3 mm., when the haploid mycelium Ab and aB grew to 23.8 mm. and 25.0 mm. respectively; the former being 1.49 times of the average of the latters.

The growth after 17 days of the same cultures was 33.6 mm., 33.3 mm., 33.0 mm. and 31.1 mm. in the hyploid mycelia AB, ab, Ab and aB respectively, while it was 60.1 mm. and 51.1 mm. in the diploid mycelia AB×ab and Ab×aB respectively. The ratio in the growth rate of the diploid to the haploid mycelium was 1.8 in the former (AB and ab) and 1.6 in the latter (Ab and aB).

VI. Formation of the Fruit-Body from the Diploid Mycelium.

The haploid mycelium of *Cortinellus Berkeleyana* does not produce the fruit-body, whatever the conditions and the duration of the culture may be. On the contrary the diploid mycelium from the fruit-bodies, if the conditions were suitable for the formation. A few examples of the fruit-body formation are here given.

Every two of the monosporous cultures, were paired on February 13, 1934 and grown the saw-mill dust medium. The medium, used, consisted of two parts of dried saw-mill dust and about one part of one % water solution of cane sugar in volume. It was then filled in wide-mouthed bottles of about 500 cc. capacity, the bottles being plugged with cotton and sterilized. After about a month culture on this medium at 24°C. the content of the culture bottles was covered with the white mycelial growth. On March 19, 1934, they were taken to the celler, the temperature being 15°—17°C. About two months later the formation of the fruit-bodies was observed. Fig. 3 in Plate II shows one of these saw-mill dust cultures of the diploid mycelium AB×ab or No. 8 × No. 12, each of the haplophytes being isolated in November 1931. In the picture a fully matured fruit-body and many very young ones are seen.

Further, on October 2, 1934, every two of the monosporous cultures were paired in many combinations and grown on the saw-mill dust medium. After one month culture at 24°C. the white hyphae grew fully. Then the cultures were kept at 15°—18°C. and those of the diploid mycelium showed on January 20, 1935, some very young fruit-bodies. About ten days later a few of them grew to 4—6 mm. in size.

In short the diploid mycelium may produce the fruit-bodies in pure culture on the saw-mill dust medium for about three monthes at 15°—24°C.

VII. Summary.

1) The present paper deals with the sexuality of *Cortinellus Berkeleyana* ITO et IMAI, one of the most important edible mushrooms in Japan.

2) The fungus has been grown in single-spore culture on malt-extract agar, and mated.

3) The fungus is heterothallic and exhibits four sexual groups, without irregularity in its pairing reactions.

4) The diploid nature of the mycelium is indicated by the clamp-connections at the septa and the comparatively narrow-angled mode of branching of the leading radial hyphae, while the haploid nature being the contrary.

5) Further, the hyphae of the diploid mycelium grow much better than those of the haploid. In the growth rate the former is 1.5—1.8 times as large as the latter.

6) In pure culture the diplophite may produce the fruit-body after about three months on the culture medium of saw-mill dust at 15°—24°C.

Explanation of the Plates.

Plate I.

Fig. 1. The fruit-bodies of *Cortinellus Berkeleyana* ITO et IMAI, artificially grown on cut stems of *Quercus crispula* BLUME. (About one third of the natural size.)

Plate II.

Fig. 2. *Cortinellus Berkeleyana* ITO et IMAI, artificially grown on *Quercus crispula* BLUME. This picture shows the white gills, sinuate to stem or stipe, and the ring-like remains of the veil on a stem. The remaines are hardly seen when mature. (About 0.55 times of the natural size.)

Fig. 3. *Cortinellus Berkeleyana*, grown on the saw-mill dust medium in pure culture. A fully matured fruit-body and many very young ones, are shown on the side of the saw-mill dust column. The young ones are seen as black circular spots. (About 0.6 time of the natural size.)

Fig. 4. A clamp-connection of a hypha of the diploid mycelium of *Cortinellus Berkeleyana*, grown on the malt-extract agar medium at 24°C. (×2,200)

Plate III.

Fig. 5. Hyphae of the haploid mycelium of *Cortinellus Berkeleyana*, grown on the 3% malt-extract agar at 24°C. Showing the simple septa, or the absence of clamp-connections, and the comparatively wide-angled mode of branching of the leading radial hyphae. (×500)

Fig. 6. Hyphae of the diploid mycelium of *Cortinellus Berkeleyana*, grown on the 3% malt-extract agar at 24°C. Showing the clamp-connections at the septa and the comparatively narrow-angled mode of branching of the leading radial hyphae. (×500)

Fig. 7. Comparisons in size of the colonies of the haploid and the diploid mycelium of *Cortinellus Berkeleyana*, grown on the malt-extract agar at 24°C. for 2 weeks. The combinations of the mycelium are as follows:

AB (6)	AB×ab (6×15)	ab (15)
AB×ab (6×12)		ab×AB (15×18)
ab (12)	ab×AB (12×18)	AB (18)

Fig. 8. The same as above, the combinations being as follows:

AB (20)	AB×ab (20×24)	ab (24)
AB×ab (20×4)		ab×AB (24×17)
ab (4)	ab×AB (4×17)	AB (17)

Plate IV.

Fig. 9. Comparisons in size of the colonies of the haploid and the diploid mycelium of *Cortinellus Berkeleyana*, grown on the potato-dextrose agar at 24°C. for 17 days. The combinations of the haploid mycelium are given in the picture. Only the plates of the mycelium AB×ab (6×12) and Ab×aB (20×24) are of diplophytes; all the others are of haplophytes or mixed culture of haplophytes without hyphal fusions. The colonies of the diplophytes are much larger in size than those of the haplophytes.

Plate V.

Fig. 10. A part of the basidium layer of *Cortinellus Berkeleyana*. Showing the basidia. (×700)

Fig. 11. The basidia and the basidiospores of *Cortinellus Berkeleyana*. Showing the shape of the basidia, the sterigmata and the basidiospores. (×1,500)

Fig. 12. The basidiospores of *Cortinellus Berkeleyana*. (×1,500)

Fig. 13. Hyphae of the haploid mycelium of *Cortinellus Berkeleyana*, grown on the 3% malt-extract agar at 24°C. Showing the simple septa and the comparatively wide-angled mode of branching. (×150)

Fig. 14. Hyphae of the diploid mycelium of *Cortinellus Berkeleyana*, grown on the 3% malt-extract agar at 24°C. Showing the clamp-connections at the septa and the comparatively narrow-angled mode of branching. (×150)

PLATE I.

Fig. 1.

PLATE II.

Fig. 2.

Fig. 3.

Fig. 4.

PLATE III.

Fig. 5.

Fig. 6.

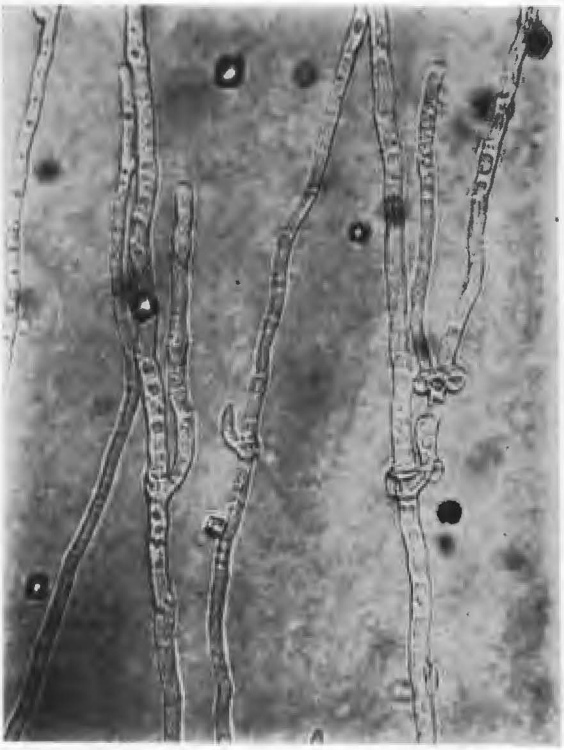


Fig. 7.

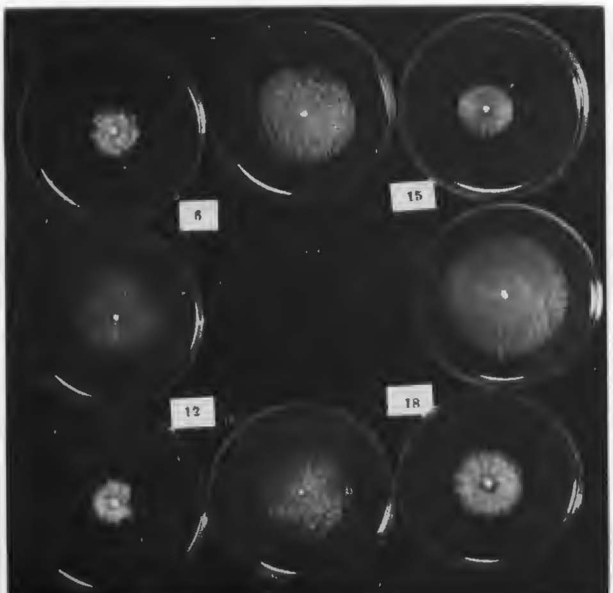


Fig. 8.

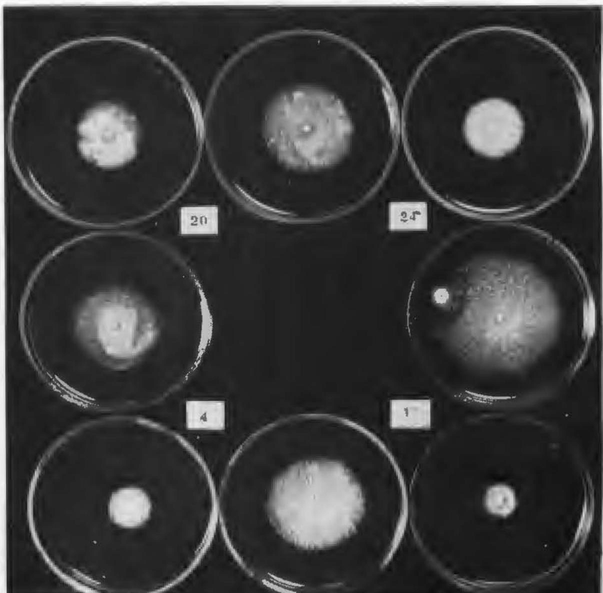


PLATE IV.

Fig. 9.

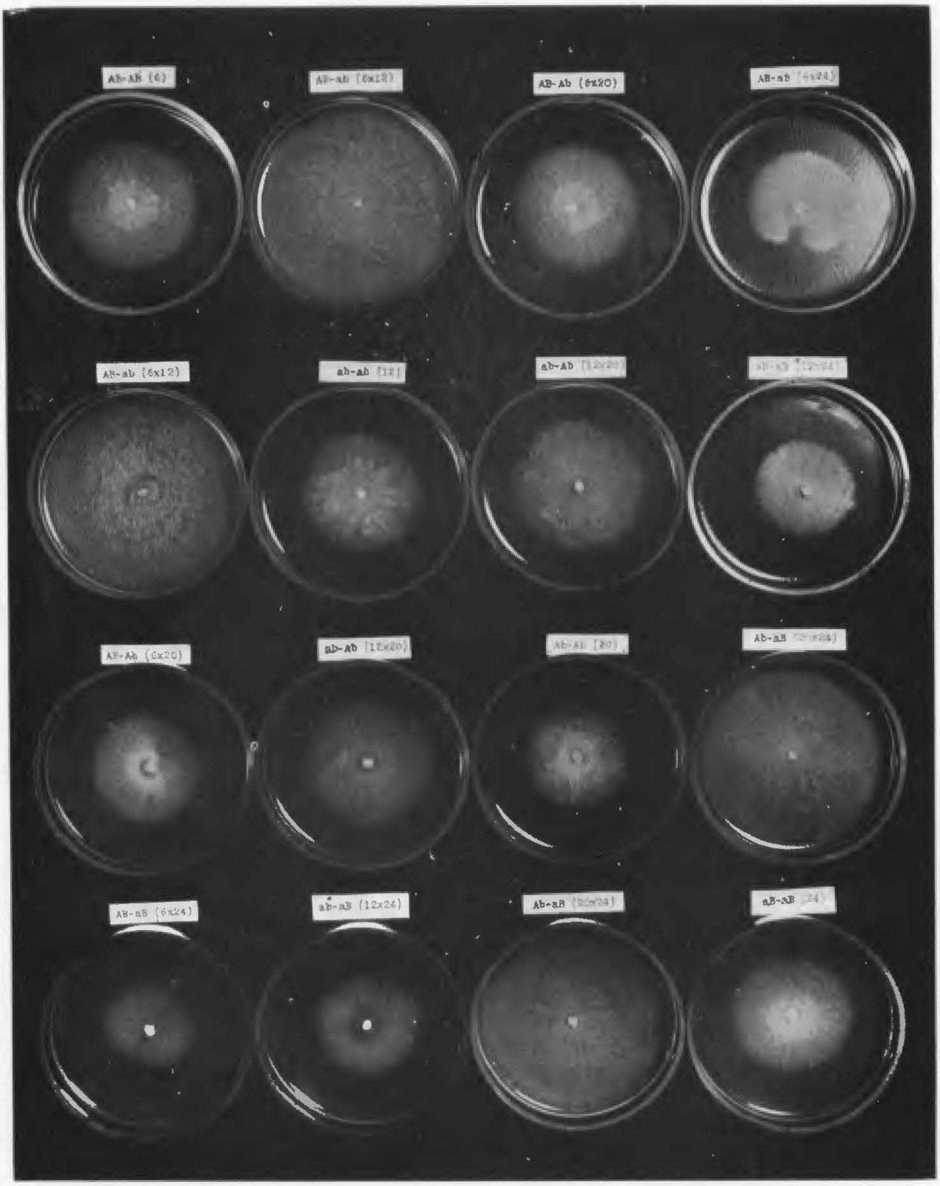
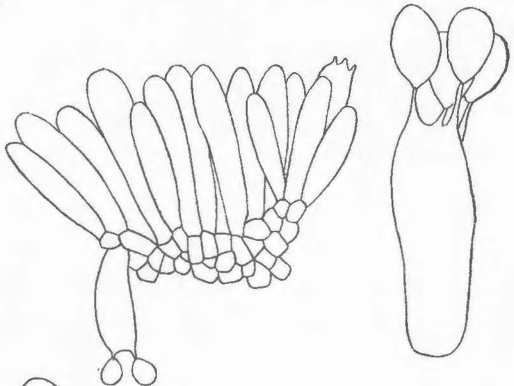


PLATE V.

Fig. 10.



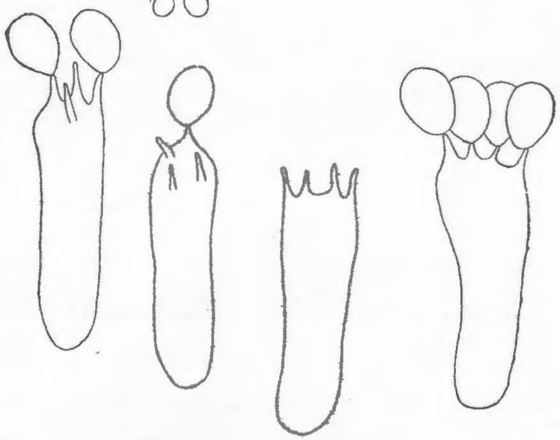


Fig. 11.

Fig. 12.

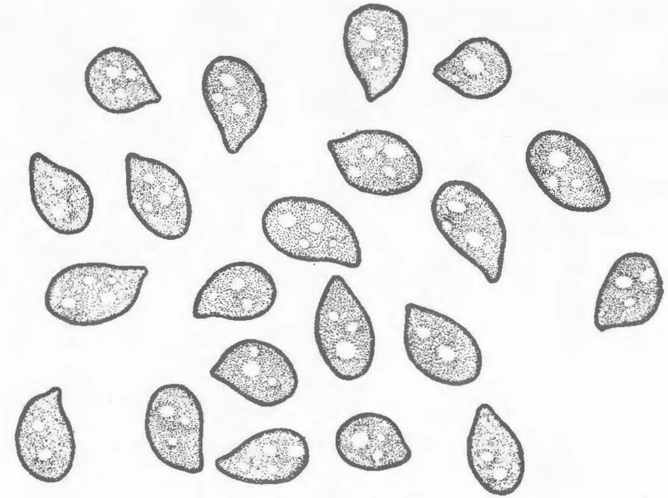


Fig. 13.

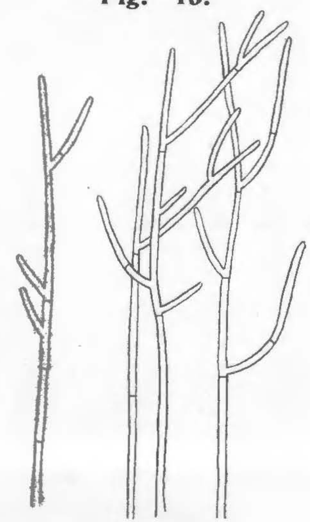


Fig. 14.