A note on the polarity of Armillariella mellea

Veikko Hintikka

Finnish Forest Research Institute, Unioninkatu 40 A, SF-00170 Helsinki 17, Finland

Abstract. The external appearance of 120 single-spore isolates from a basidiocarp of *Armillariella mellea* was characterized by abundant white raised aerial mycelium, and differed markedly from that of the majority of a number of other isolates made from rhizomorphs and basidiocarps collected in nature. These had dark crustose areas and were generally without aerial mycelium. Both types of mycelia lacked clamp connections. Their appearance did not change during two years' cultivation. Crustose mycelia were obtained by pairing single-spore isolates. The pairing reaction followed a tetrapolar pattern.

Both types of mycelia had only one nucleus in the terminal cells of the hyphae. In older parts of the mycelium, especially in rhizomorphs, the number of nuclei per cell often exceeded 10. In basidiocarps collected in nature, distinctly dicaryotic and clamped hyphae were met with, especially at the bases of basidia.

Various explanations are suggested for the monocaryotic condition of the hyphal tips. As hyphal tip isolations from crustose mycelia obtained by pairing single-spore isolates, regularly gave only crustose mycelia, it is possible that the nuclei in the tips of the hyphae area diploid.

Introduction

Armillariella mellea (Vahl) Karst. is known as a variable species both in the field (Romagnesi 1970) and in culture (Gibson 1961, Raabe 1966). Its life cycle is considered to be homothallic (Kniep 1911), asexual or homomictic (Burnett 1956). Single-spore isolates have been found to be very variable in external characters (MacLean 1950, Raabe 1966), but apparently no evidence of their interaction or polarity has been reported (Raper 1966). In connection with an investigation of the pathogenicity of different strains of *Armillariella* in Finland, the following observations of evident polarity among single-spore isolates of this species were made.

Material and methods

One hundred and twenty single-spore isolates of *Armillariella mellea* were obtained from a single basidiocarp growing in a *Corylus* thicket on mull soil near a stump in Karkalinniemi nature reserve, Karjalohja parish, southwestern Finland. A spore deposit was made immediately after the collection, on October 10, 1969, on a sterile petri dish, and stored at +5° C. In April 1970, spores were spread thinly on the surface of 1 % Difco malt extract agar, and allowed to germinate for about 35 hr. A glass capillary was used under a microscope to detach a piece of agar with a single germinated spore, which was then blown out of the capillary tube onto an agar slant. The isolates obtained in this way were kept on Hagem or 1 % malt extract agar slants in test tubes, and stored at +5° C with transfers approximately twice a year.

The strains were crossed by inoculating two

strains at a distance of about 1 cm in 9-cm petri dishes on 1 % Difco malt extract agar, and by keeping them in the dark at room temperature. The results were recorded 1—1½ month after inoculation.

Results

Cultural characters of isolates made from basidiocarps, rhizomorphs and wood colonized by Armillariella. Seventy-five isolates were obtained from basidiocarps and rhizomorphs occurring in nature as well as from wood colonized by this fungus, from different parts of Finland. The cultural characters of these isolates were rather heterogeneous, but in general the aerial mycelium was totally lacking and in older parts of the mycelium dark brown crustose or laquerlike areas developed. When they were cultivated on malt extract agar, certain strains lacked crustose areas, but the aerial mycelium was as a rule scanty. The amount of rhizomorphs was very variable; they were lacking in some strains and in some others they were very abundant. Luminiscence was distinct in young well-aerated cultures. The appearance of the cultures agrees fairly well with the description of NOBLES (1948). The characters were stable when they were kept on Hagem agar for two years at +5° C.

Cultural characters of single-spore isolates. Figs. 1 and 2 depict typical single-spore isolates. The aerial mycelium is profuse, young mycelia almost forming a hemisphere, and the color is pale yellowish-brown to whitish. Usually no brown crustose areas are developed in the mycelium or there are only small patches or lines covered by the aerial mycelium. When the single-spore isolations were made, occasional (a few per cent of the total number) crustose isolates were obtained, but they were disregarded, as it was not always possible to be completely certain that only one spore was present. Rhizomorphs were formed in many of the isolates, as in the previous type, although they were perhaps slightly fewer. Clamps were absent in both types of mycelia. Young cultures had a strong luminiscence. These mycelia were also cultivated on Hagem agar at +5° C for two years without any appreciable changes in external appearance. So far neither mycelium type has produced basidiocarps in our laboratory. Similar mycelia have been reported for *Armillariella* by NOBLES (1948) and RAABE (1966).

Crossing experiments. When single-spore isolates from the same basidiocarp were crossed by growing them together in the same petri dish, in certain combinations the external appearance of the combined mycelium changed drastically (Fig. 1.). The aerial mycelium disappeared, except for a few red-brown tufts, and large crustose areas developed. There were also numerous rhizomorphs, and when their tips grew out of the substrate, they produced similar crusts. This change in the characters of the mycelium was constant in certain combinations. Table 1 shows the results of ca. 2000 crosses. The plus sign (+) denotes the production of crustose mycelium, the minus sign (—) the absence of change in the external appearance of the joint mycelium. It is evident that the reaction follows the normal tetrapolar pattern.

The appearance of the new crustose mycelium varied within rather wide limits, and the brown crustose areas were often quite small. However, when the same strain was crossed with numerous other strains, the change to crustose mycelium was easily seen.

In certain crosses only one of the strains produced crustose mycelium (Fig. 2), and when these crosses were repeated, it was always the same strain which became crustose. In addition, some unexplained interactions occurred in certain combinations. When, for example, strains 60 and 63 were grown together, the rhizomorphs, which in both strains were straight, became densely branched and strongly curved in the mixed culture (Fig. 3). This reaction, although constant, was seen in only a few crosses, and did not follow the tetrapolar pattern.

The number of nuclei per cell in different types of mycelia. A few preliminary observations were made on the number of nuclei per cell in different types of mycelia. Hyphae were fixed in abs. alcohol- acetic acid- lactic acid 6:1:1 or in Farmer's fluid and colored with GIEMSA's stain mainly according to

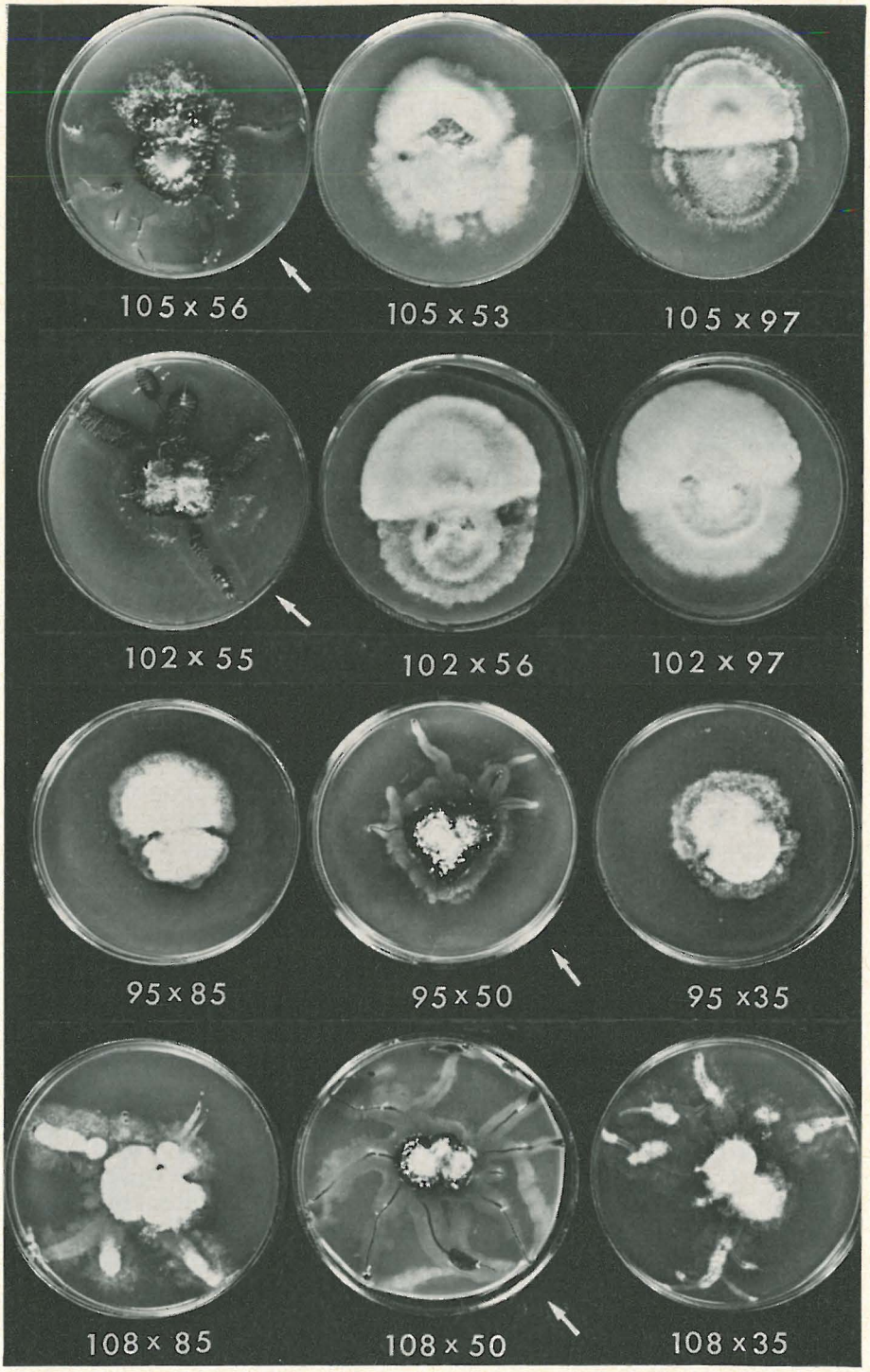

Fig. 1. Interaction of different monosporous isolates of *Armillariella mellea*. In certain combinations (arrow) the apperance of the joint mycelium regularly becomes dark brown and crustose, with red-brown mycelial tufts, in others the mycelium remains whitish with profuse aerial hyphae, as in the original monosporous mycelium. Crustose mycelia are formed on the average in every fourth cross, in accordance with the tetrapolar pattern.

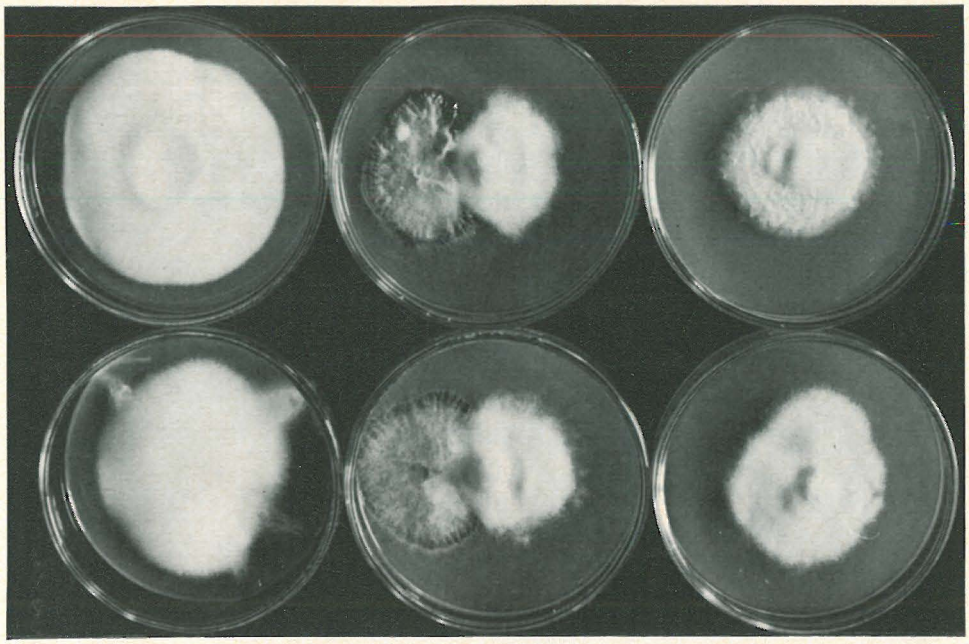

Fig. 2. Interaction of strains 24 and 21. Left: strain 24 grown alone, centre: 24 and 21 together, right: 21 alone. In both cases the appearance of strain 24 became crustose in mixed culture.

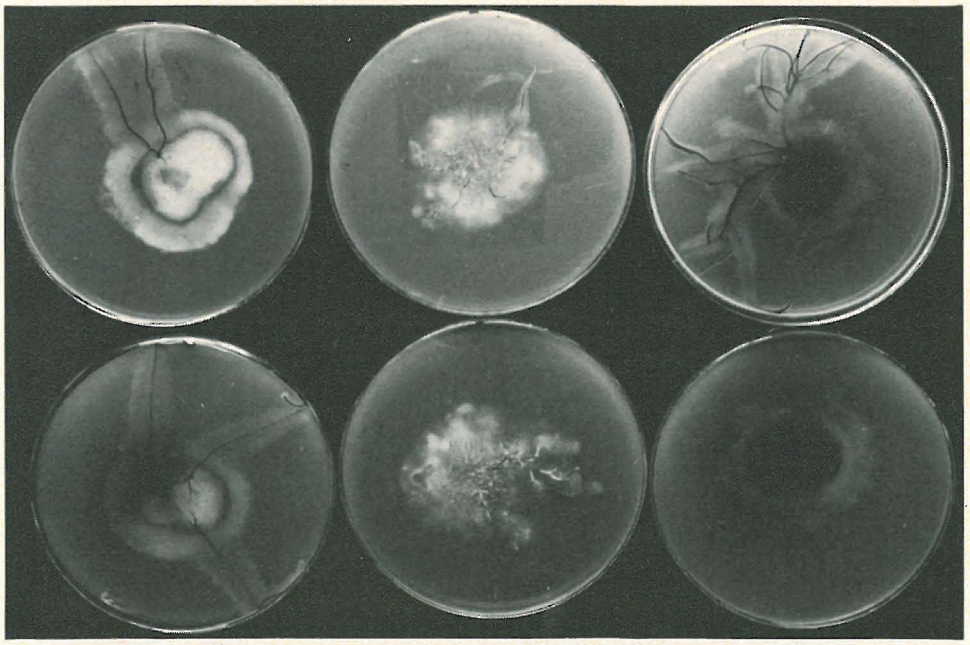

Fig. 3. Interaction of strains 63 (left) and 60 (right). The rhizomorphs of each strain were straight ad sparsely branched when it was grown alone, but densely branched in mixed cultures (centre).

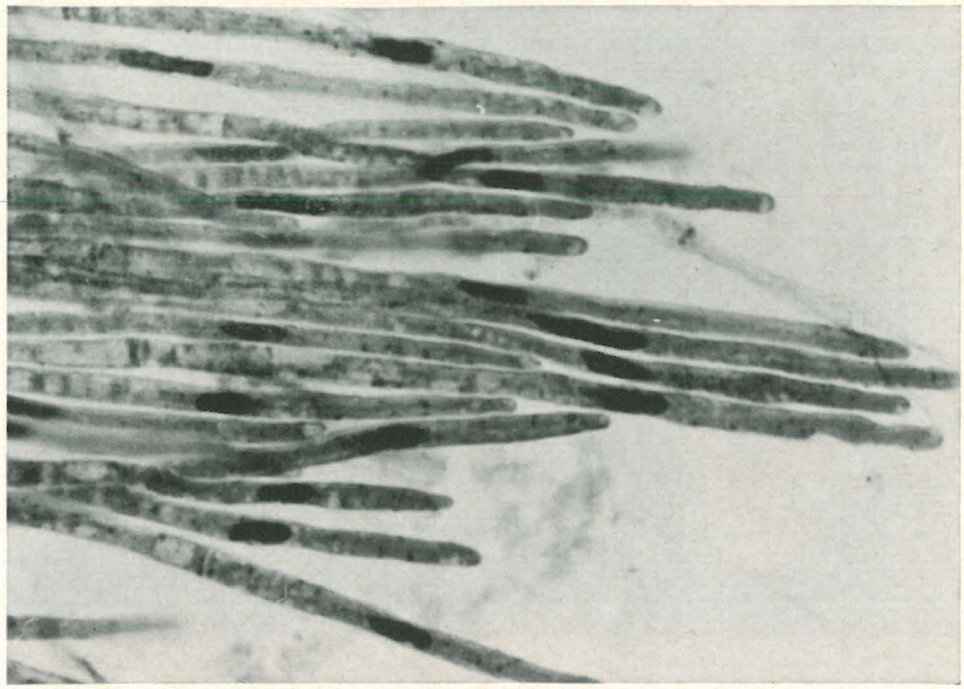

Fig. 4. Uninucleate hyphal tip cells of a crustose mycelium of *Armillariella mellea*.

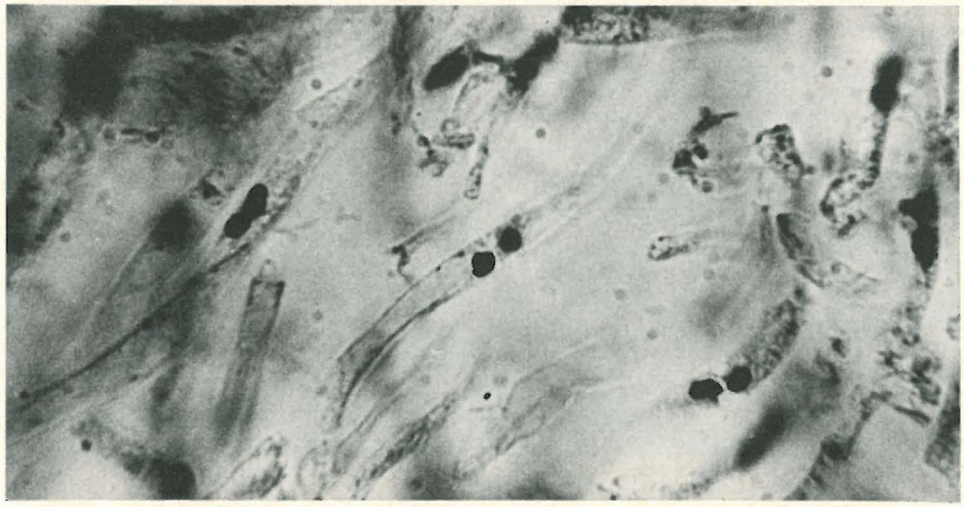

Fig. 5. Dicaryotic hyphae from subhymenium of *Armillariella mellea*.

WARD & CIURUSEK (1962). Different types of mycelia proved to be similar in regard to the number of nuclei per cell. Growing tips at the margin of the mycelium had always only one nucleus (Fig. 4). Older cells, 10—20 cells inwards, showed an increased amount of nuclei, up to 10—15, especially in the enlarged cells of the rhizomorphs. The same holds true for the large cells of the basidiocarp, where numerous nuclei per cell were met with. In addition, basidiocarps regularly had hyphae, which, in contrast to the vegetative mycelium, had clamp connections, especially at the base of the basidia, and their cells regularly had two paired nuclei (Fig. 5). The presence of clamp connections in basidiocarps has earlier been mentioned by SINGER (1962).

Discussion

The difference in external appearance between single-spore isolates and dicaryotic mycelia of the same species is well known and has been noted in different groups of Hymenomycetes and Gasteromycetes. However, the uninuclear condition of the evidently paired mycelium makes the nuclear cycle in *Armillariella* rather problematic. Preliminary observations suggest the following sequence in this species: 1) a uninucleate spore forms 2) uninucleate, unclamped hyphae, with abundant white aerial mycelium and without crustose areas. Older cells are multinucleate and rhizomorphs are present. Two of these mycelia unite into 3) uninucleate, clampless mycelium without aerial hyphae and with brown crustose areas. Older cells are multinucleate. This mycelium develops basidiocarps, which have 4) binucleate hyphae with clamp connections, and which produce basidia and spores.

The uninucleate condition of the cells at the tips of the hyphae of *Armillariella* has been demonstrated several times since the observations of KNIEP in 1911 (BERLINER & DUFF 1965, MOTTA 1969). For this reason, and in view of the lack of clamp connections, the species has been regarded as homothallic or homomictic (BURNETT 1956), or imperfectly known (RAPER 1966). Unfortunately the mycelia have not so far produced any basidiocarps in our laboratory, and none of the present observations can definitely be connected with the formation of basidiocarps. The results reported here evidently point to some kind of interaction between monosporous mycelia, because, without pairing the normal crustose mycelia met with in nature could not be obtained from single-spore isolates in the laboratory.

Certain external influences, high carbon dioxide concentrations for instance, cause the normal crustose mycelium to change into floccose aerial mycelium, but the present differences seem to be constant.

The following points deserve consideration in attempts to explain the aberrant mononuclear state of *Armillariella*.

a) Nuclei can migrate, and there are strains of *Schizophyllum* where the terminal cell is uninucleate, and the other nucleus of the dicaryon is located in an older part of the hypha (RAPER 1966). In order to check whether this is possibly the case in *Armillariella* isolations from hyphal tips were made from crosses 12×17 (crustose) and 11×45 (floccose), by inoculating them on malt extract agar. About three weeks after the inoculation, isolates of single tips of marginal hyphae, consisting of several cells, were made by the same method as the single-spore isolations. From the former cross, 65 mycelia were obtained, all of which were crustose. From the latter, 78 were obtained, most of which were totally devoid of crustose areas, and a few of which had small crustose patches, though none were as typically crustose as the former mycelia (Fig. 6). Although single cells were not isolated, this result suggests genetic differences between the growing hyphal tips.

b) The clear tetrapolar pattern displayed by the crossings makes it rather unlikely that plasmatic factors are involved.

c) The paired mycelium may be diploid. There are mutants in both *Schizophyllum* (PARAG & NACHMAN 1966) and *Coprinus lagopus* (CASSELTON 1965; see also KOLTIN, STAMBERG & LEMKE 1972), in which the vegetative mycelium is uninucleate and diploid, although it seems that such forms are not met with in nature. Among basidio-

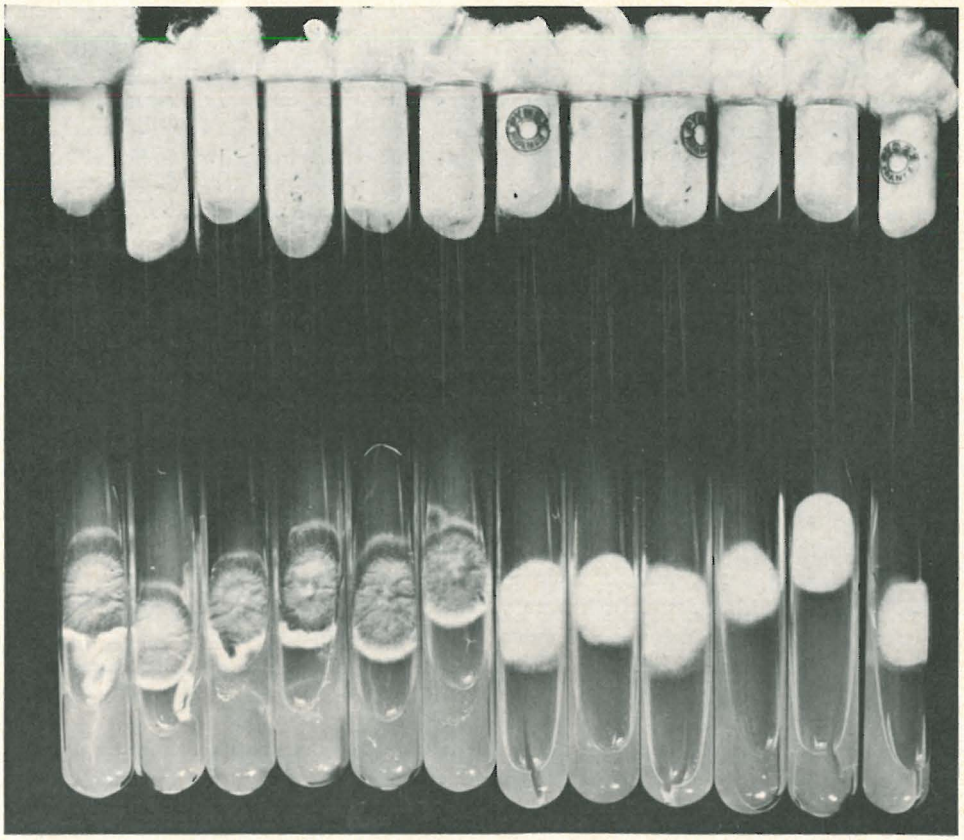

Fig. 6. Isolations from hyphal tips of a cross producing crustose mycelium (left) and a cross where the mycelium continued to resemble that of single-spore isolates (right).

mycetes, *Hemileia* is reported to have a short diploid vegetative phase (RAJENDREN 1967). Unfortunately attempts to determine the amount of DNA by the Feulgen staining method were not successful.

It may be noted that there are some similar species which are regarded as asexual and which resemble *Armillariella* in having clamp connections only at the bases of the basidia.

More comprehensive investigations concerning the nuclear cycle in *Armillariella* are being carried out at our laboratory by Mr. KARI KORHONEN.

The present experiments indicate that, despite of the uninucleate condition of the hyphal tips, there may be some kind of exchange of genetic factors between single spore isolates.

Acknowledgements. This study was carried out at the Finnish Forest Research Institute and financed by a grant from the Finnish National Research Council for Agriculture and Forestry. The author is indebted to Mr. KARI KORHONEN, M. Sc. and to Miss RITVA KLEMETTI for their skilful assistance.

REFERENCES

BURNETT, J. H., 1956: The mating systems of fungi I. — New Phyt. 55, 50—90.

CASSELTON, L. A., 1965: The production and behaviour of diploids of Coprinus lagopus. — Gen. Res. 6, 190—198.

GIBSON, I. A., 1961: A note on variation between isolates of Armillaria mellea (Vahl ex Fr.) Kummer. — Trans. Brit. Mycol. Soc. 44, 123—128.

KNIEP, H., 1911: Über das Auftreten von Basidien im einkernigen Mycel von Armillaria mellea. — Zeitschr. Bot. 3, 529—553.

KOLTIN, V., J. STAMBERG, & P. A. LEMKE, 1972: Genetic structure and evolution of the incompatibility factors in higher fungi. — Bact. Rev. 36, 156—171.

MACLEAN, N. A., 1950: Variation in monospore cultures of Armillaria mellea. — Phytopathol. 40, 968 (Abstract).

MOTTA, J. J., 1969: Cytology and morphogenesis in the rhizomorph of Armillaria mellea. — Am. J. Bot. 56, 610—619.

NOBLES, M. K., 1948: Studies in forest pathology. VI. — Can. Journ. of Research C 26, 281—431.

PARAG, Y. & B. NACHMAN, 1966: Diploidy in the tetrapolar heterothallic basidiomycete Schizophyllum commune. — Heredity 21, 151—154.

RAABE, R. D., 1966: Variation of Armillaria mellea in culture. — Phytopath. 56, 1241—1244.

RAJENDREN, R. B., 1967: A new type of nuclear life cycle in Hemileia vastatrix. — Mycologia 59, 279—285.

RAPER, J. R., 1966: Genetics of sexuality in higher fungi. New York. 283 pp.

ROMAGNESI, H., 1970: Observations sur les Armillariella (I). — Bull. trim. Soc. Myc. Fr. 86, 257—266.

SINGER, R., 1962: The Agaricales in modern taxonomy. — Weinheim. 915 pp.

WARD, E. W. B. & K. W. CIURUSEK, 1962: Somatic mitosis in Neurospora crassa. — Am. J. Bot. 49, 393—399.

	53	30	35	12	55	40	81	11	56	20	25	17	97	10	50	3
2	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
5	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
17	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
20	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
26	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
29	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
31	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
34	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
37	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
42	+	(+)	+	+	–	–	–	–	–	+	–	–	–	–	–	–
45	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
56	+	(+)	+	+	–	–	–	–	–	–	–	–	–	–	–	–
61	+	(+)	+	+	–	–	–	–	–	–	–	–	–	–	–	–
67	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
72	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
85	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
90	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
98	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
109	+	(+)	+	+	–	–	–	–	–	–	–	–	–	–	–	–
110	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
118	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
120	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
1	–	–	–	–	+	+	+	+	–	–	–	–	–	–	(+)	–
3	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
4	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
8	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
10	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
36	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
39	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
43	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
44	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
46	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
47	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
50	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
57	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
59	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
68	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
69	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
73	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
74	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
94	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
97	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
99	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
100	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
102	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
104	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
106	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
113	–	–	–	–	+	(+)	+	+	–	–	–	–	–	–	–	–
114	–	–	–	–	+	(+)	+	+	–	–	–	–	–	–	–	–
115	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
117	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
122	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
123	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
126	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
130	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	+	+	+	–	–	–	(+)	–
9	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–
12	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	–
15	–	–	–	–	–	–	–	–	+	+		+	–	–	–	–
16	–	–	–	–	–	–	–	–	+	+		+	–	–	–	–
18	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
21	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
24	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
27	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
30	–	–	–	–	–	–	–	–	+		+	+	–	–	–	–
32	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
35	–	–	–	–	–	–	–	–	+		+	+	–	–	–	–
45	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
49	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
51	–	–	–	–	–	–	–	–	+		+	+	–	–	–	–
52	–	–	–	–	–	–	–	–	+		+	+	–	–	–	–
53	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
58	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
60	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
71	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
79	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
83	–	–	–	–	–	–	–	–	+	+	+		+	–	–	–
84	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
86	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
87	–	–	–	–	–	–	–	–	+	+	+		–	–	–	–
91	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
92	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
93	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
101	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
103	–	–	–	–	–	–	–	–	+	+	+	+	–	–	(+)	–
105	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
112	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
116	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
119	–	–	–	–	–	–	–	–	+	+		+	–	–	–	–
128	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–
7	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
11	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
13	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
14	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	
22	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
23	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
25	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
28	–	–	–	–	–	–	–	–	–	–	–		+	+	+	+
33	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
38	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
40	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
41	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
54	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
55	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
62	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
63	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
64	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
65	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
66	–	–	–	–	–	–	–	–	–	–	–	–	+		+	+
70	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
76	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
77	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
78	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
80	–	–	–	–	–	–	–	–	–	–	–	–	+	(+)	+	+
81	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
82	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
88	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
89	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
95	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
96	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
107	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
108	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
111	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
121	–	–	–	–	–	–	–	–	–	–	–	–	+	(+)	+	+
125	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
127	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+
129	–	–	–	–	–	–	–	–	–	–	–	–	+	+	(+)	+
131	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+

Table 1. Crosses with single-spore isolates from the same basidiocarp. + denotes formation of a crustose mycelium, — denotes absence of change in the external appearance of both mycelia, and (+) indistinct reaction.