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Pleurotus ostreatus – Breeding Potential of a New Cultivated Mushroom

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Summary. Two isolates of *Pleurotus ostreatus* from North America and one from Germany are interbreedable. Under identical conditions at low temperatures, their fruiting bodies are hard to distinguish. However, shape and colour and several other characters vary with culture conditions. The American stocks fruit well at temperatures from 4 to 24°C, the German ones only below 15°C. Four types of hybrids between German and American *Pleurotus* were obtained: i) The whole fruiting process is temperature sensitive as in German *Pleurotus*. ii) It proceeds at 4-24°C as in American stocks. iii) Fruiting initiation is insensitive but sporophore development is sensitive to elevated temperatures. iv) Primordia formation and initial sporophore development depend on temperatures below 15°C, but pileus expansion and spore discharge continue above 20°. The involvement of separate genes for the single developmental steps and the use of temperature sensitivity for commercial varieties are discussed. One sporeless strain, "F42x11", with considerable fruiting bodies has been obtained. In intrastock di-mon-matings this character was dominant.

The cultivation of the two "traditional" mushrooms, *Lentinus edodes* in Japan, and *Agaricus bisporus* all over the world, has advanced rapidly in the last decade. The mushroom industry developed profitable methods of handling tons of wood, straw and other lignin- and cellulose-containing agricultural wastes, in order to prepare suitable substrates for fungi. This, with the pure culture techniques for spawn production in modern plants, paved the way for the cultivation of other lignin- and cellulose-consuming mushrooms. The oyster mushroom, *Pleurotus ostreatus* is one favorite. It is believed to be harmless to healthy trees (Busse 1920), though it may cause considerable damage under certain circumstances (Hepting 1935; Singer 1975; Toole 1959). It grows easily under artificial conditions and gives reasonable yields of readily developing sporophores.

The first attempts to grow *Pleurotus ostreatus* for human consumption were made during the first world war in Germany (Busse 1920; Falck 1917), but the idea of using wood debris, saw-dust or straw for food production (Falck 1917) has only spread recently: food shortage is no longer a local and temporary incident, but a global problem. The discussion of the culinary values of the fruiting bodies - ranging from merely "edible" to "agreeable", "good" and "delicious" - is inadequate. Food production from agricultural wastes becomes a necessity, and *Pleurotus* is a suitable subject. More highly prized mushrooms, for instance *Boletus edulis* or *Cantharellus cibarius*, are mycorrhizal fungi. There is little chance that they could be grown on artificial media, let alone waste products, in the near future. Thus it might be worthwhile to improve wild *Pleurotus ostreatus* towards a "crop plant".

This study is a first attempt to examine the breeding potential of the oyster mushroom.

Material and Methods

Isolates of *Pleurotus ostreatus*

Two isolates were from the USA: isolate "F", from Florida, was received in 1964 from S.S. Block (Block et al. 1958); isolate "M" was a multispore culture from a spore deposit kindly provided by Dr. A.H. Smith, Ann Arbor, Michigan, in 1971. The third, "G", was a culture from the plectenchyma of a fruit body collected in Westfalen, West Germany in 1964. From all three sources basidiocarps were derived and spore deposits collected. Monokaryons were isolated by microscopical selection of germlings from single spores. They are marked with "mon" and "F", "M" or "G", according to their origin, or with a number. Dikaryons are characterized by "di" or the number of their component monokaryons, for instance "F-di", "F42x11". In reciprocal or in di-mon-matings, the monokaryon that received the nuclei is written first.

Culture media*

a) Malt extract agar: 20 g "Maltzin trocken, hell" from Diamalt (München) was dissolved in 250 ml dist. water. The pH of the solution was raised 1,5 by titration with 1 n NaOH, then 1,4 g $CaCl_2 \cdot 2H_2O$ was added,

* Grade of chemicals "pro analysis" if article number is not given

well mixed and autoclaved 15 min at 121° C. After cooling to room temperature the extract was filtered. 75 ml filtrate, 425 ml dist. water and 7,6 g agar (Merck No. 1614) were mixed and sterilized 15 min at 121°C. 15 ml medium was poured into plastic petri dishes of 8,5 cm diameter. The final pH was 4,85-4,95.
b) Sterilized straw-peat substrate in 1 liter glasses according to Till (1962), but without cotton-seed meal.

Asparagine Solution

Na-phosphate buffer pH 6,5 (0,05m) with 2% asparagine monohydrate (Merck 1565) and 5% glucose monohydrate (Eger 1970).

Conditions for mycelial growth

Cultures in petri dishes were wrapped in polyethylene bags (0,05 mm thick); cultures in glasses were closed with glass lids and covered with sterilized foam-plastic (1 cm thick). All were kept in a dark room at 23-24°C and 75-80% rel. humidity.

Conditions for primordia formation

1) Warm conditions: 23-24°C, continuous light of 700 lux (Osram fluorescent lamps "L 65W/30") and 75-80% rel. humidity. As humidity was constant and high, cultures in petri dishes were taken out of the polyethylene bags. Only the glass lid had to be removed from cultures on Till-substrate, (but the foam-plastic kept on) and some water poured on the surface of the mycelium.
2) Cold conditions: 4-15°C. a) 50 litre refrigerators with continuous light of 700 lux (Osram "L32 W/30"). b) Windows with daylight maxima of 1000-3000 lux. The petri dishes were kept in "moist chambers" made of polyethylene bags (0,03 mm thick) with 4 holes of 5 mm diameter, punched 2 to 2,5 cm above the bottom. A stripe of antibacterial paper towel ("Hostess" from "Feldmühle") was placed in each, and the base covered with water. The top was closed with a rubber band.

Conditions for fruiting

Warm conditions: 20-24°C, 300-700 lux continuous light from fluorescent lamps, as above, or daylight with maxima up to 2000 lux. Cold conditions: as above. For development of normal sporophores, the lids had to be removed from the petri dishes and the foam-plastic from the 1 litre glasses. All cultures were individually kept in moist chambers as described above. The rooms for fruiting were separate from those for mycelial growth and primordia formation, in order to prevent contamination by spores.

Inoculation of cultures and matings

The surface of the Till-substrate in glasses was covered with one whole agar culture that had just penetrated the petri dish. Inoculation of agar plates took place only under light of wave lengths longer than 600 nm. 2 mm inocula were cut from the growing margin of a 5 to 6 day-old culture, that had been kept in continuous darkness at least between two successive transfers. For fertility tests, each inoculum was put exactly in the centre of the petri dish. For "normal matings" of two monokaryons, small pieces of mycelium in the logarithmic growth phase were placed side by side. In "reciprocal matings", the monokaryon that was intended to receive the nuclei was put in the centre of the plate and grown for 5 days, then the inoculum of the donor monokaryon was added to the margin of the colony. In matings between dikaryons and monokaryons (= di-mon-matings), the monokaryon was pregrown for 5 days and the dikaryon added to its margin.

Estimation of dikaryotization

Successful dikaryotization was determined microscopically 6 days after mating. Reciprocal and di-mon-matings were considered positive if clamp connections were detectable at the far edge opposite the second inoculum and additionally at several other places around the pregrown monikaryon (Fig. 1). This ensured, that dikaryotization occurred broadly (not only in a narrow sector) and ruled out mistakes caused by overgrowth of a slow growing monokaryon by single hyphae of a faster growing partner. From negative matings after 10 days one to several small inocula from the margin of the colony were subcultured on fresh plates in order to examine nuclear migration.

Fertility tests with new established dikaryons

Small inocula were cut from microscopically examined spots and placed in the centre of agar plates. After 6 days adaptation to continuous darkness, fresh inocula from the margin of the colony were transferred to the test plates. Temperature conditions for fruiting were chosen according to the needs of the component strains.

Induction of primordia

To induce primordia, the dark grown cultures had to be exposed to light and 1 ml of asparagine solution was added to the centre of each. As time of induction is critical for successful primordia formation (Eger

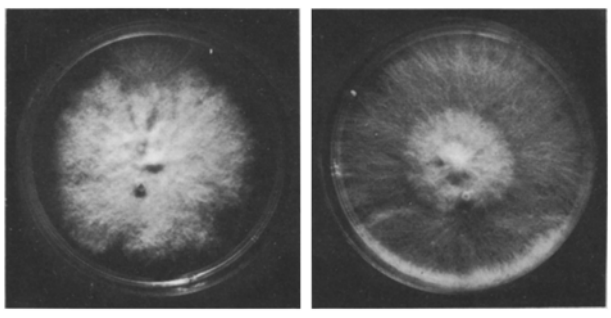

Fig. 1. Di-mon-matings between a "F"-monokaryon, in the centre of the plate, and the dikaryon "F42x11", that was added five days later to the margin of the pregrown colony. In the incompatible mating (left) only a single dikaryotic sector has evolved. In the hemicompatible mating (right) the dikaryon is established allround

1970; Eger 1970a; Eger et al. 1974), fast growing mycelia were treated when the hyphal tips were one to two mm from the edge of the plate, slow growing ones when the mycelium had just penetrated the whole medium.

Basidiospore production

This was examined microscopically in dry preparations of lamellae under cover glasses with the 25 objective or by spore prints. To prepare prints, cut sporophores or pilei were placed on white paper on the bottom of petri dishes and returned to their proper fruiting conditions.

Experiments and Results

Comparison of *Pleurotus ostreatus* from North America and Germany under culture conditions

The mycelia of the two American isolates, "F" and "M", and the German, "G", can not be distinguished morphologically. In "F" and "M", primordia can be induced over a wide range of temperatures (4-25° C) and light intensities (40-4000 lux daylight or continuous artifical light) (Eger 1974). Sporophores develop in the same temperature range, but the minimum of light required for pileus expansion is about 200 Lux. In "G", primordia and basidiocarps will not form at temperatures above 15°C. Young fruit bodies transferred from low temperatures to 20°C and above cease growing.

The colour of the sporophores depends on light and temperature. Around the lower limit of light intensity, the pigments are pale, no matter what the temperature is. "F" and "M" may form basidiocarps as white as mycelium at 200 lux and 24°C. At the same temperature and high light intensities (above 2000 lux), the pilei are more or less brownish, but in bright light and at low temperatures they are grey to greyish brown. The lower the temperature and the higher the light intensity, the darker is their colour. Thus there is little difference from "G", which varies from very pale brownish-grey at low light intensities, to grey or blue grey at higher ones, and dark grey to dark brown with daylight maxima up to 4000 lux. Under conditions of poor colour development only the surface of the cap is tinged. However, if the pileus is dark, the lamellae may be pigmented too, though much paler. The stipe remains white and sometimes is tomentose. The pigments seem to be generated predominantly in the early phase of sporophore development. They fade during pileus expansion. Young, pale basidiocarps which are transferred from low to high illumination do not gain much colour during their further development.

At low temperatures and high light intensities, the fruit bodies of all three isolates are stout. With decreasing light and rising temperature the stipes elongate, while the pilei are reduced in size and may become more or less funnel like. As "G" will not fruit above 20°C, it lacks the pale and slender (etiolated) basidiocarps obtained from the American isolates under elevated temperatures and low light intensities. If "G", "F" and "M" are grown at low temperatures side by side, they can hardly be distinguished from one another.

In heavy deposits, spores of "F" and "M" always become greyish or lilac-tinged after desiccation. Fresh spore prints are bright white. From "G" we could not obtain a single spore print thick enough to reveal such colour changes: at low temperatures spore deposition is slow and the sporophores dry out before a heavy print can be obtained; at elevated temperatures, however, spore production ceases.

Mating experiments

Mating types

Monokaryons from "F" and "G" could easily be grouped into four incompatibility classes, respectively. However, among germlings of "M"-spores, one of the four mating types was scarce (only one in 32!). Compatible matings in "F" and "M" can usually be cognized with the naked eye by faster growth and different appearance of the dikaryon (Eger 1974). In "G" the macroscopical characters of mono- and dikaryons are not so distinct. There was no reaction that would allow us to distinguish reliably common B-heterokaryons by morphological characters, as described for other isolates of *Pleurotus ostreatus* (Anderson et al. 1973; Eugenio and Anderson 1968; Terakawa 1960), or common A-heterokaryons (Raper 1966). Matings between "F" and "G"-, "M"- and "F"- or "G"- and "M"-monokaryons were 100% compatible. Consequently, A- and B- factors must be different in all three isolates.

Table 1. Success of reciprocal matings with monokaryons of American and German origin (2 replicas per mating) and fertility of resulting dikaryons

		"F"-monokaryons							"M"-monokaryons				
"G"-monokaryons		3	6	9	12	132	741	808	3	6	12	16	29
donor of	6	pf	**pF**	pF	**pF**	——	——	**p**-	——	**p**F	——	-	——
nuclei	8	**p**F	——	-	**p**F	——	**pF**	**p**-	——	**p**F	——	**pF**	——
	11	pF	**pF**	——	-	——	**pF**	**p**-	——	**p**F	——	-	——
	14	——	**pf**	pf	——	——	——	**p**-	**pF**	**pF**	——	——	——
recipient	6	pF	pF	**pF**	pF	p-	pF	pF	**pF**	**pF**	**p**F	**pF**	**pF**
of nuclei	8	**p**F	**p**F	p-	-	**pf**	**pF**	**p**F	**pF**	**pF**	**pF**	**p**F	**pF**
	11	**p**F	-	p-	**p**F	pF	-	pF	pF	**pF**	**p**F	**p**F	**pF**
	14	-	-	-	-	-	-	-	**pF**	**pF**	**pF**	**p**F	**pF**

—— = dikaryotization failed within 6 days
Fertility of established dikaryons within 40 days after induction (4 replicas each under cold and warm conditions respectively):
- = not tested
p = more or less primordia under cold conditions only
p = primordia under cold and warm conditions
F = normal fruiting bodies under cold conditions only
F = normal fruiting bodies under cold and warm conditions
f and **f** = abortive fruiting bodies only
"G6, 8, 11, 14" represent the four mating types

Matings between monokaryons of German and American origin

When "F"- and "G"- or "M"- and "G"-monokaryons were mated, dikaryons resulted within 6 days, as in matings of compatible monokaryons of the same origin. However, in several cases, dikaryotization proceeded only into the German mate, while the American maintained its monokaryotic morphology or did not grow at all. The restraint of "G"-nuclei by mycelium of "F"- and "M"-origin was especially obvious in reciprocal matings. While "F"- and "M"-nuclei reached the oposite edge of the pregrown "G"-monokaryon within 6 days in 100 % of cases, German nuclei failed to establish dikaryons within American hyphae in 45 % of pairings (22 in a total of 48, see long cross bars in Table 1).

Matings between monokaryons and dikaryons

In legitimate, compatible matings, where the three types of nuclei involved have quite different A and B factors, dikaryotization of the monokaryon should proceed regularly and rapidly (Raper 1966). Table 2 shows that this was true in pairings of "F-mon" with "M-di" and vice versa. It did not however apply to matings with one partner of German origin ("G-mon × F-di", "G-mon × M-di", "F-mon × G-di", "M-mon × G-di"): here dikaryotization took place within 6 days in only a fraction of matings. After 10 days a further fraction revealed small dikaryotic sectors, or migrated nuclei could be traced in subcultures. In the rest, no migration was detectable in either way. In legitimate, hemicompatible matings (f.i. "F3" or "F868 × F-di"; "M6" or "M25 × M-di"; "G14", "G2", "G11 × G-di" in table 2), dikaryotization was evident after 6 days in at least part of the replicas of each pairing, and occurred after 10 days in all of them. In illegitimate matings, single dikaryotic sectors might eventually occur after 7 to 10 days.

In di-mon-matings the growth of the participating mycelia is affected manifoldly. The dikaryotic inoculum may grow vigorously, feebly or become completely suppressed by the monokaryon. The established new dikaryon may exceed the growth rate of the dikaryotic inoculum or be extremely poor. However, these interactions show no relation to incompatibility factors.

Fruiting behaviour of hybrid dikaryons with German and American components

In the German isolates fruiting initiation, sporophore development and spore production are sensitive to temperatures above 15°C. At 20-24°C they are suppressed completely. In the American stocks however, fruiting will take place very well from 4-24°C. In all

Table 2. Success of di-mon-matings (2 to 5 replicas each)

monokaryons		dikaryons F	M	G
F	3	+	+	+
	868	+	+	+
	136	±	+	+
	12	±	+	±
	132	±	+	±
	417	±	+	±
	64	-	+	-
	547	-	+	-
	620	-	+	-
	741	-	+	-
M	6	+	+	±
	25	+	+	-
	16	+	±	-
	3	+	-	-
	12	+	-	-
	31	+	-	-
	11	±	-	-
	29	±	-	-
G	14	+	+	+
	8	+	+	-
	2	+	-	+
	11	+	-	+
	6	+	-	-
	5	-	-	-
	15	-	-	-
	29	-	-	-

Dikaryotization within 6 days:

+ = in all replicas
± = in part of the replicas
- = no dikaryotization at all

"F" an "M" from American, "G" from German origin

three isolates, under their individual optimal fruiting conditions, the frequency of cultures forming primordia is 100 %, and of those forming fruiting bodies 95-100 %, within 40 days.

In hybrid dikaryons obtained from reciprocal matings of German and American monokaryons, the response of fruiting to temperature was variable (Table 1). Below 15°C abundant primordia could be induced in all 60 hybrids tested. 45 of them (75 %) were similarly able to produce more or less primordia at temperatures above 20°C. In the remaining 15 (25 %), fruiting initiation was negatively affected by elevated temperatures. Not a single primordium had formed within 40 days of observation. In seven dikaryons (12 %) further development was blocked completely (for instance "F808 × G6, G8, G11, G14"). In 30 cases (50 %) sporophores were only formed below 15°C (see "G8" or "G11 × F3"). The remaining 23 (38 %) produced fruiting bodies under both cold and warm conditions. Several hybrids developed abortive sporophores ("F3 × G6", "F9 × G14"). In some hybrids, where sporophore production occurred only under cold conditions, pileus expansion and spore discharge continued when the cultures were transferred to 20°C or more. Sensitivity to higher temperatures was not due to plasmatic factors. Of hybrids with German plasma, 34 % would fruit at elevated temperatures; with American plasma, 32 % would do so. Temperature sensitivity is likely to be inherited by the nuclei, but the same nuclear combination in reciprocal crosses does not always bring about the same fruiting behaviour (see Table 1, "F808").

Hybrid dikaryons derived from di-mon-matings of "G-mon × F42x11" showed similar variability in temperature sensitivity. In one (4 %) fruiting initiation was blocked completely, in 5 (23 %) no primordia were formed at 20-24°C, but the remainder 16 (73 %) produced fruiting body initials under warm and cold conditions. In 11 (50 %) development did not proceed beyond the primordial stage. The remainder were capable of sporophore production (see Table 3). In seven (32 %) sporophore development was sensitive to elevated temperatures. The remainder (18 %) fruited in either case.

"Sporeless" and its behaviour in di-mon-matings

Ten years ago we obtained dikaryon "F42x11", which produces sporeless fruiting bodies. Since then we have subcultured it many times and tested it on different culture media. The sporeless character is stable and does not depend on nutritional factors. Fruiting occurs as regularly and readily as in the original "F"-isolate. The sporophores are considerable in appearance (Fig.2), but behave somewhat abnormally. With side illumination, the stipes show phototropic response and the pilei maintain their radial symmetry. The yields on Till-substratum are close to those of normal dikaryons. Sporeless varieties of *Pleurotus* would be very useful for the commercial production of this fungus (see Discussion), so we tried to combine this character with strains of different origin. As the component monokaryons of "F42x11" have been lost during the years, we used the buller phenomenon (Raper 1966) in di-mon-matings with monokaryons from "F", "M" and "G". Fruiting of newly established dikaryons was tested

Fig. 2. Fruiting bodies of the sporeless strain "F42x11" growing in a 1-litre glass jar

under warm and cold conditions. The results are summarized in Table 3. As would be expected (because illegitimate di-mon-matings could not be avoided), in pairings of "F-mon" with "F42x11" a rather low proportion was successful (6 out of 16). From the derived dikaryons, three produced abortive sporophores, the other three were like "F42x11" without spores. From 7 matings with "M-mon", five were successful. All the derived dikaryons developed fruiting bodies. One showed "F42x11" characteristics, the other four were normal. From 32 matings with "G-mon", 22 dikaryons were derived. Eleven of them produced sporophores, one, abortive ones. None had "F42x11"-like, sporeless fruiting bodies.

Discussion of Results

In the last decade a *Pleurotus* from Florida has spread among mushroom growers throughout the world because of its ease of cultivation and tolerance of higher temperatures. There has been much dispute among growers and scientists as to whether this *Pleurotus* is *Pleurotus ostreatus* (Fr.) Kummer. The senior author was the first to express doubts (Eger 1965). However, this question can only be clarified experimentally as the species concept in the genus *Pleurotus*, especially the section *Pleurotus*, is not yet clear (Singer 1975). We decided therefore to compare the *Pleurotus* from Florida with authentic *Pleurotus ostreatus* from Michigan and a specimen of *Pleurotus ostreatus* from Germany. The latter was collected before the first *Pleurotus* from Florida was introduced to Europe. It is necessary to emphasize this, as the Florida-*Pleurotus* has been grown commercially and propagated in a completely uncontrolled way throughout our country- and even sold to households for cultivation! It may have contaminated our native woods.

From both American sources, spore deposits have been obtained that turned greyish or lilac after desiccation in those portions of the prints that were heavy and had collected moisture from the sporophores. In the German isolate not a single spore print could be obtained that was heavy enough to reveal this character. The reason is that under fruiting conditions below 15°C spore deposition from cut pilei is slow. At higher temperatures (20°C and above) spore discharge ceases. Several taxonomists, including modern European ones, do not pay much attention to this colouration of spore prints (Kuehner and Romagnesi 1974; Moser 1967; Smith 1963, but some North American authors consider the *Pleurotus* that produces lilac-tinged spore deposits to be a separate species, *Pleurotus sapidus* Kalchbr. (Anderson et al. 1973; Groves 1962).

When grown under identical conditions at low temperature and high illumination, no morphologial differences were discernible between the three isolates. Characters such as colour of the pileus and the lamellae, shape of the pileus, more or less decurrent lamellae, length and thickness of the stipe and formation of a smooth or tomentose surface of the stipe

Table 3. Transmission of "sporeless" in di-mon-matings with "F42x11"

origin of monokaryons	no. of matings		replicas per mating	fruiting characteristics of new dikaryons			
	total	successful		no sporophores	normal sporophores	"F42x11" sporeless	abortive sporeless
F	16	6	3	0	0	3	3
M	7	5	5	0	4	1	0
G	32	22	2	11	10	0	1

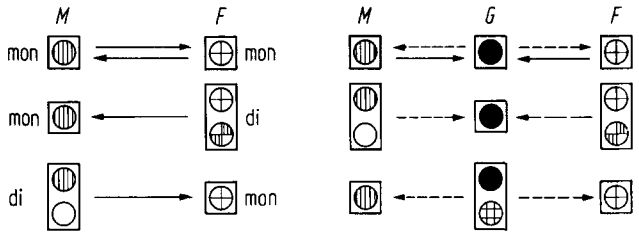

Fig.3. Restriction of dikaryotization in compatible matings between a German ("G") and two American ("F" and "M") stocks.
Explanations: ⟸⟹ free dikaryotization
⟸- - -⟹ dikaryotization more or less restricted in many cases

were variable according to culture conditions (see also Anderson et al. 1973; Zadražil and Schliemann 1975). At temperatures above 15°C, and up to 24°, the American stocks fruited well and were indistinguishable from one another. In contrast, the fruiting of German *Pleurotus* was sensitive to elevated temperature and inhibited completely above 20°C. Temperature sensitive mutants are known in plants, animals, fungi, bacteria and viruses (modern textbooks of genetics), therefore temperature sensitivity may not be taken as a character of taxonomic value.

Recently, and for the first time, a *Pleurotus* was discovered in the USA that produced a cream-coloured spore print but was otherwise indistinguishable from *Pleurotus sapidus* (Anderson et al. 1973). However, this isolate failed to interbreed with a great number of different isolates of *Pleurotus sapidus* and was therefore considered to be a separate species. This is a basis for discussion as, in Hymenomycetes, fusion of hyphae between two different mycelia is generally accepted as an expression of taxonomic relationship, and the establishment of a dikaryon with conjugated nuclear division as evidence that they belong to the same species (Boidin and des Pomeys 1961; Macrae 1967; Tu et al. 1969). Matings of monokaryons originating from German "G"- and American "F"- and "M"-isolates led to hybrid dikaryons with normal growth rates and appearance. A considerable number of them produced normal fruiting bodies with basidiospores that were able to germinate, no matter whether the component monokaryons were both American or mixed. This is good reason to regard the *Pleurotus* stocks of our study as a single species. However, it should not be overlooked that there are different degrees of compatibility between the two American isolates on the one hand, and the German and the American ones on the other. Figure 3 summarizes the results of matings with compatible A and B-factors. In pairings of two monokaryotic American strains, dikaryotization proceeded easily and rapidly in either direction. In di-mon-matings, "F"-nuclei may migrate somewhat more slowly within "M"-hyphae than "M"-nuclei in "F"-mycelium. In matings between monokaryons of American and German origin, dikaryotization often proceeded unidirectionally within the German partner only. In di-mon-matings it was frequently more or less restricted in either direction. Cultural conditions influence the buller phenomenon of nuclear migration (Snider and Raper 1958). In our experiments, substratum, moisture, temperature, light exclusion and age of inocula were identical. The results, therefore, actually reflect differences in nuclear and (or) plasmatic interactions. It has been demonstrated that nuclear migration and selection is not only favored by heterogeneity at the A- and B- loci, but also at other ones (Raper 1966). However, such a complicated process as conjugated division must require cooperation not only between the alleles at the sexual incompatibility loci, but presumably at other sites as well, and with plasmatic factors. Heterogeneity should therefore be limited. It is understandable that the system responsible for dikaryotization would operate in matings of relatives only. Triangular relationships in di-mon-matings may render the proper arrangements more difficult than in matings of two nuclei.

Dikaryons from different matings show great variability in fruiting behaviour. Some are sterile, and not a single primordium is initiated. Others produce primordia, but they do not develop further. In several dikaryons abnormal fruiting bodies are formed with only rudimentary pilei. In one dikaryon, "F42x11", pilei expand but yield no basidiospores. The majority fruit normally. In *Coprinus macrorhizus*, mutants have been obtained which block fruiting at corresponding stages (Takemaru and Kamada 1971). These mutants, "initiationless", "primordiumless", "maturationless", "elongationless", "expansionless", "sporeless", are dominant to the normal gene in a dikaryon. Thus, for normal fruiting, a double dose of each gene is necessary. It is very likely that, in

Pleurotus too, fruiting is controlled by a number of genes operating at different stages of development.

If we view the dikaryons with regard to temperature sensitivity, we discover that hybrids of sensitive German and insensitive American strains display a similar variability. In some, primordia formation is only inhibited at temperatures above 20°C. In others, initiation of fruiting is insensitive to elevated temperatures, but further development will only proceed below 15°C. Finally, in a third group, primordia formation and the initial development of fruiting body structure are temperature-sensitive, but pileus expansion and sporophore production will proceed at elevated temperatures. Thus, temperature sensitivity may not be due to a single gene controlling the total fruiting process, but to several genes operating at single developmental stages. Perhaps these genes are identical with those responsible for primordia formation, sporophore development, pileus expansion and spore production. We do not know if temperature sensitivity is dominant, as we have been working with strains of high heterogeneity. Several alleles may exist at the different loci. However, if temperature-sensitive genes actually exist for the single developmental steps, it should be possible to breed strains that will produce sporeless fruiting bodies under optimal commercial conditions, but basidiospores will only be produced in the cold. Such strains would be extremely valuable for further expansion of *Pleurotus* cultivation.

Pleurotus has gymnocarpous fruiting bodies and is already discharging spores when the first lamellae are generated. Thus tremendous amounts of spores may be released on a farm, especially under warm growing conditions. As *Pleurotus* spores may act as allergenes in the human respiratory tract (Zadražil 1974), they may not only cause illness to farm workers, but also to sensitive persons in the vicinity. Spores may also act as vehicles for viruses, as in the cultivated *Agaricus bisporus* (Dieleman-van Zaayen 1972). The moment a virus emerges in *Pleurotus ostreatus*, the whole *Pleurotus* industry will be heavily struck, without proper counter measures at hand. There are additional hazards for all kinds of native woods suffering from pollution or otherwise reduced in vitality. Under such conditions, *Pleurotus ostreatus* might become a serious wood destroyer (Hepting 1935; Toole 1959), especially the commercial strains which have been selected for vigorous growth on all kinds of "artificial" substrates and for resistance to disease and temperature.

Our dikaryon "F42x11" is the only sporeless *Pleurotus* strain available at present. As it does not satisfy enough commercial demands we tried to combine the "sporeless" nuclei from "F42x11" with nuclei from different origins, using the buller phenomenon. The results obtained with this method have to be weighed thoroughly. In hemicompatible di-mon-matings, nuclear selection operates in favour of the compatible A- and B-factors. In incompatible ones, both nuclei of the dikaryon may eventually migrate through the monokaryotic hyphae and establish the old dikaryon within the hyphal tips. This, however, happens only late and sporadically. In fully compatible di-mon-matings, reestablishment of the old dikaryon within the tips of the monokaryons similarly may occur (Raper 1966). With our critical and early estimation of the success of dikaryotization, joint nuclear migration in matings of "F-mon × F42x11" should be eliminated. We therefore believe that "sporeless" in three dikaryons, out of a total of six derived from these matings, reflect migration and dominance of the corresponding gene. In matings of "M-mon × F42x11", one of five dikaryons displayed the "F42x11" characters. We are not sure in this case if it is due to migration of only a single nucleus, because these matings were fully compatible. In matings of "G-mon × F42x11", not one of 22 derived dikaryons had "F42x11"-like sporophores. Thus, either "sporeless" was absent in the migrating nucleus or it was unable to dominate the allele of the unrelated partner.

Our investigations show that there is good potential for improving wild *Pleurotus ostreatus*. Isolates from American and European origin are interbreedable. Temperature sensitivity of the German strains will only dominate in a fraction of the hybrids from matings of German and American monokaryons. Temperature sensitivity does not necessarily concern the total fruiting process. The latter may be divided into several stages which may behave independently towards temperature. It would seem possible to breed strains that are temperature-sensitive only in relation to spore production. Sporeless strains may be obtained also by mutation. They will behave dominantly in intrastock matings.

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