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Genetics of Speciation in the Basidiomycetous Genus *Polyporus*

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Summary. The wood rotting basidiomycete *Polyporus* (subgenus *Leucopori* Quel.) was chosen in order to investigate by genetic parameters the validity of the classical species concept based on typological characters.

Species deliminations in this genus are derived from morphological characters and depend mainly on the size of hymenial pores. They were compared with those assigned from mating relations between 26 races of different geographic origin.

All races could unequivocally be grouped into three separate entities corresponding with the typological species *P. arcularius*, *P. brumalis* and *P. ciliatus* on the basis of the following results:

1. As expected, the basic breeding system in *Polyporus* is the tetrapolar mechanism of homogenic incompatibility controlled by multiple alleles of the mating type factors *A* and *B*.

2. All intraspecific combinations were fertile. A conspicuous barrage formed in those crosses where dikaryotization and fruiting were impaired. This barrage is characterized by a clear zone, about 1-2 mm wide, free of aerial hyphae and reduced hyphal density in the growth medium. The delay observed in the onset of the sexual cycle was caused by retarded fusion of hyphae and blocked exchange of nuclei despite a compatible combination of the mating types.

3. Using two races of *P. ciliatus* as an example, it was revealed that barrage formation is started by the specific interaction of three independent genes (bi^+/bi = barrage initiation, bfI_1/bfI_2 and $bfII_1/bfII_2$ = barrage formation) in a way characteristic for systems of heterogenic incompatibility: barrage formation requires the presence of the allele bi^+ in at least one mating partner additional to a heterogeneity in both *bf*-genes.

4. Interspecific combinations were sterile. There is no hyphal fusion between the mating partners and because of the mutual repulsion a sharp line formed in the area of contact that was designated as border line. Its formation is independent of mating type or nuclear status of the confronted mycelia.

The good correspondence of the species limits derived from morphological and genetic data indicates the applicability and validity of both the typological and the biological species concept. The latter, however, proved superior in compensating the variability of morphological characters, at least in higher fungi.

The bearing of our results and other known control mechanisms of the sexual cycle on the definition of the category 'species' are integrated in a proposed modification of the biological species concept: populations (races) belong to different species when the failure to interbreed and to produce viable offspring is not caused by genetic parameters operating in completion of the sexual cycle.

Key words: Genetics of speciation – Speciation in *Polyporus*

Introduction

The species concept in biology is based mainly on morphological criteria (e.g. Wettstein 1901) but there have been attempts recently to use physiological characters (e.g. bacteria: Buchanan and Gibbons 1974; yeasts: Lodder 1970). From an evolutionary point of view, the most appropriate species definition stresses the unlimited exchange of genetic material: 'A species consists of one or more populations, the individuals of which can interbreed, but which can not exchange genes with members belonging to other species' (King 1974). However, this species concept based on genetic parameters is rarely used by taxonomists.

There are a number of reasons why this may be so: 1. a lack of knowledge of the factors controlling the life cycle and sexuality; 2. the long generation time of many spe-

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cies, making genetic analyses rather time consuming, and 3. interfertility studies often give confusing results due to crossing barriers (e.g. incompatibility: Esser 1961; Esser and Blaich 1973).

Many of these problems do not apply to the fungi, especially to basidiomycetes where a large number of interfertility studies have been reported (Adams and Roth 1967; Duncan and MacDonald 1967; Edwards and Kennedy 1973; Kemp 1975; Macrae 1967). None of these investigations, however, were interpreted in terms of species delimination or speciation. We have therefore chosen the genus *Polyporus* Fr. (subgenus *Leucopori* Quel.), a common group of wood rotting basidiomycetes, to compare the 'classic' typological species limits, which in this genus are based mostly on fruit body morphology, e.g. the size of the hymenial pores (Kreisel 1963), with those derived from the genetic analysis of fertility relationships.

In *Polyporus* fruit bodies are produced on monokaryotic as well as on dikaryotic mycelia (Stahl and Esser 1976) and the same genes (fi^+,fb^+) determine the ability of both types of mycelia to produce these fruit bodies (Esser and Meinhardt 1977). The formation of a dikaryon is controlled by the tetrapolar mechanism of homogenic incompatibility requiring a combination of monokaryons with different A and B factors according to the general formula $A_xB_x \times A_yB_y$ or abbreviated $A \neq B \neq$ (see Esser and Kuenen 1967; Raper 1966).

With minor exceptions, our results have confirmed the classification proposed on morphological characters. Fur-

Table 1. Compilation of the *Polyporus* races used showing species classification, origin (including substrate and date of isolation when known), mating types and ability to fruit in monokaryons. Mating type designation followed the sequence of races.

Species	Race Nr.	Substrate	Date	Mating type 1.		2.		Monokaryotic fruiting
	1[a]	Fagus	05.63	*A*1	*B*1	*A*2	*B*2	+
	2		11.71	*A*2	*B*3	*A*4	*B*4	+
	3	Betula	30.10.74	*A*2	*B*6	*A*6	*B*5	+
	4[a]	Fagus	24.06.67	*A*7	*B*7	*A*8	*B*8	+
ciliatus	5[b]	hardwood	16.09.58	*A*7	*B*7	*A*8	*B*8	+
	6[b]		17.05.61	*A*9	*B*9	*A*10	*B*10	+
	7	Fagus	10.05.73	*A*11	*B*11	*A*12	*B*12	+
	8[b]		17.05.55	*A*13	*B*13	*A*14	*B*14	+
	25[a]			*A*15	*B*15	*A*16	*B*16	+
	9			*A*1	*B*1	*A*2	*B*2	+
	10			*A*3	*B*3	*A*4	*B*4	+
	11	Fagus	30.10.74	*A*5	*B*5	*A*6	*B*6	–
	12	Fagus	8.11.74	*A*7	*B*7	*A*8	*B*8	+
	13	Fagus	24.11.74	*A*9	*B*8	*A*10	*B*10	+
	14	Fagus	29.04.75	*A*8	*B*12	*A*11	*B*10	–
	15	Fagus	14.03.67	*A*5	*B*13	*A*14	*B*14	–
brumalis	16	Quercus	12.12.68	*A*15	*B*15	*A*16	*B*12	+
	17	on log	31.10.55	*A*17	*B*17	*A*18	*B*18	–
	18			*A*19	*B*4	*A*8	*B*12	–
	19			*A*11	*B*8	*A*22	*B*22	+
	20			*A*23	*B*23	*A*24	*B*24	+
	21			*A*25	*B*25	*A*26	*B*26	+
	22		8.04.69	*A*19	*B*23	*A*26	*B*3	+
	23			*A*17	*B*24	*A*30	*B*25	+
arcularius	26			*A*1	*B*1	*A*2	*B*2	–
brumalis?	27			*A*1	*B*1	*A*2	*B*2	–

[a] received as *P. ciliatus* f. *lepideus* or *P. lepideus* (25)

[b] received as *P. brumalis*; redetermined as *P. ciliatus*

Origin of strains: 1: Inst. Pflanzenpathol., Univ. Göttingen (Germany); 2, 3, 9-14: Kalwes forest, Ruhr-Univ. Bochum (Germany); 4, 15, 16: Bundesanstalt Land- und Forstwiss. Hann. -Münden (Germany); 6-8: Schiller Univ. Weimar (Germany); 5, 17: Can. Dept. Fish. For., Ottawa (Canada); 18: Amer. Type Culture Collection, Rockville, Md. (USA) (ATCC 9.385); 19: Centraalbureau voor Schimmelcultures, Baarn (Netherlands) (CBS 470.72); 20: Dept. Environ., Princess Risborough (England); 21: For. Res. Inst., Dehra Dun (India); 22, 23, 25, 26: Univ. Claude Bernard, Lyon (France) (AD 717, AD 719, AD 720, AD 726); 27: For. Prod. Lab., Melbourne (Australia) (174a)

thermore it was possible to overcome the confusion caused by various crossing barriers in recognizing the so called barrage formation as a mechanism of heterogenic incompatibility occurring between races and to distinguish this from the border line formed between species.

Material and Methods

The origin and some characteristics of 26 races used are summarized in Table 1.

Culture conditions, media, isolation of basidiospores, induction and selection of auxotrophic mutants, genetic analyses and statistical methods were those described by Stahl and Esser (1976) with the following modifications: fruit body induction in *Polyporus brumalis* and *P. arcularius* was only possible on unfiltered corn meal agar (60 g corn meal boiled in 1 l H_2O for 15 min; before autoclaving, 20 g agar is added) at temperatures between 10 and 15° C (*P. brumalis*) or 25° C (*P. arcularius*), respectively. All experiments using auxotrophic mutants were performed on minimal medium as described by Kitamoto and Kasai (1968); nutritional deficiencies were compensated for by the addition of 10^{-3} mol/l of the metabolite required.

The velocity of nuclear migration was measured indirectly by observing the progression of clamp formation in a pre existing monokaryon according to the method of Buller (1931).

The generation of protoplasts was achieved with extracellular hydrolytic enzymes from the culture filtrate of *Trichoderma harzianum* (de Vries and Wessels 1972). Reversion and fusion experiments were done according to the method of Anne and Peberdy (1975)

Results

I Morphological and Genetical Characterization

To obtain monokaryons with defined mating type from each race, fruit bodies had to be induced under laboratory conditions. The influence of temperature on fruiting proved to be species specific: races of *P. brumalis* did not fruit above 15° C, *P. ciliatus* required ca. 22° C and the only isolate of *P. arcularius* required even higher temperatures of ca. 25° C.

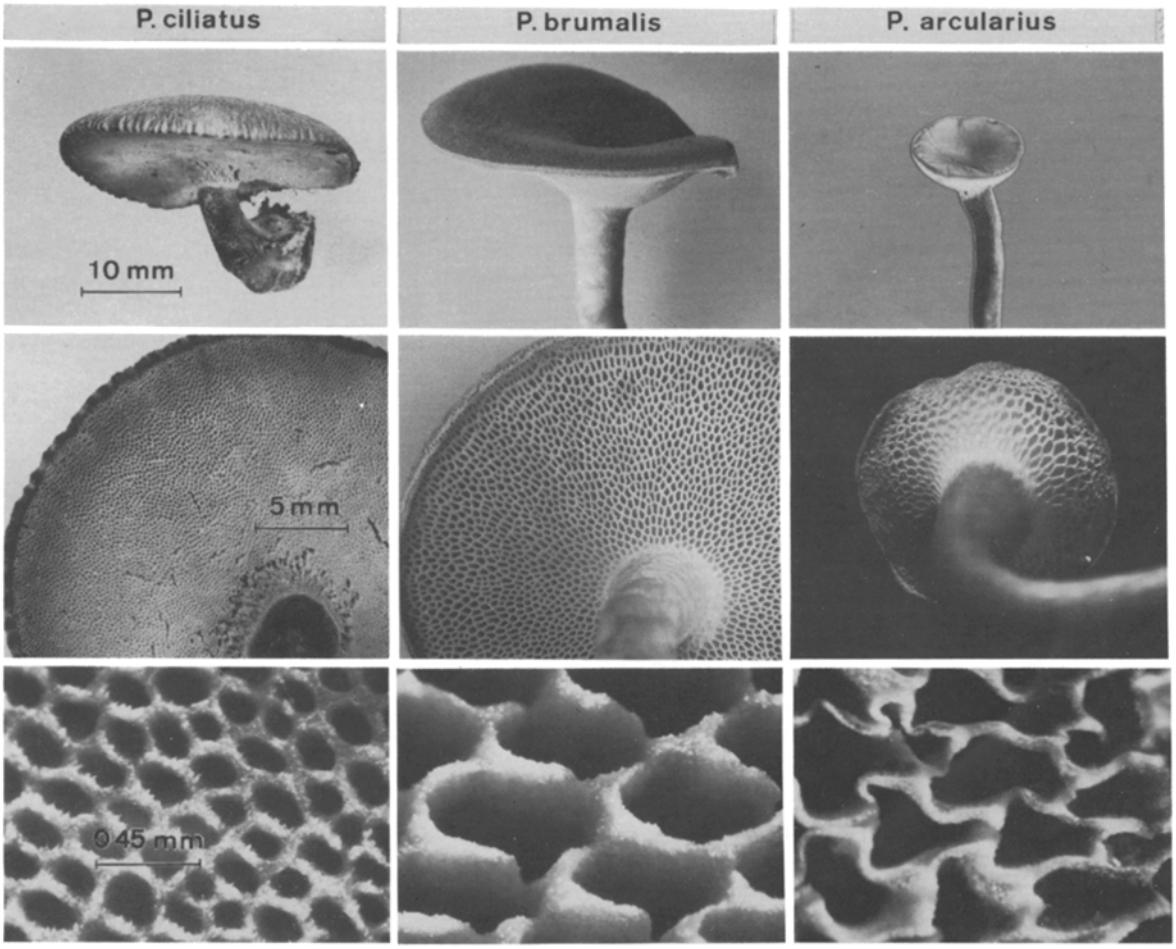


Fig. 1. Morphology of fruit bodies from three species of *Polyporus*. Top: shape of fruit bodies; middle: view of the hymenophore; bottom: enlarged view of the hymenophores showing pore size (From Esser and Hoffmann 1977)

Variations in culture conditions caused gross morphological changes in fruit bodies of all species, as described by Plunkett (1956, 1958, 1961) or Schwantes and Gessner (1974). The time for fruiting varied individually from 8-25 days (*P. ciliatus*) and from 3-12 weeks (*P. brumalis*), respectively; low fruiting seemed to be correlated with the absence of monokaryotic fruiting (Table 1), an indication that the *fi*$^+$-locus (Esser and Meinhardt 1977) was involved in promoting dikaryotic fruiting.

Under optimal conditions the fruit bodies showed the typical shape which serves as a basis for classification in this genus (Fig. 1). The differences in size and shape of the hymenial pores could be used as reliable diagnostic characters (Jahn 1963, 1969; Kreisel 1963).

Monokaryons of each race could be assigned to four mating types as expected from the work of Vandendries (1936a, b) and David and Romagnesi (1972). Using the Buller phenomenon (Raper 1966), the mating type configuration of each dikaryon was determined. Together with the results of allele-specificity determinations (intraspecies combinations) these data are integrated in Table 1.

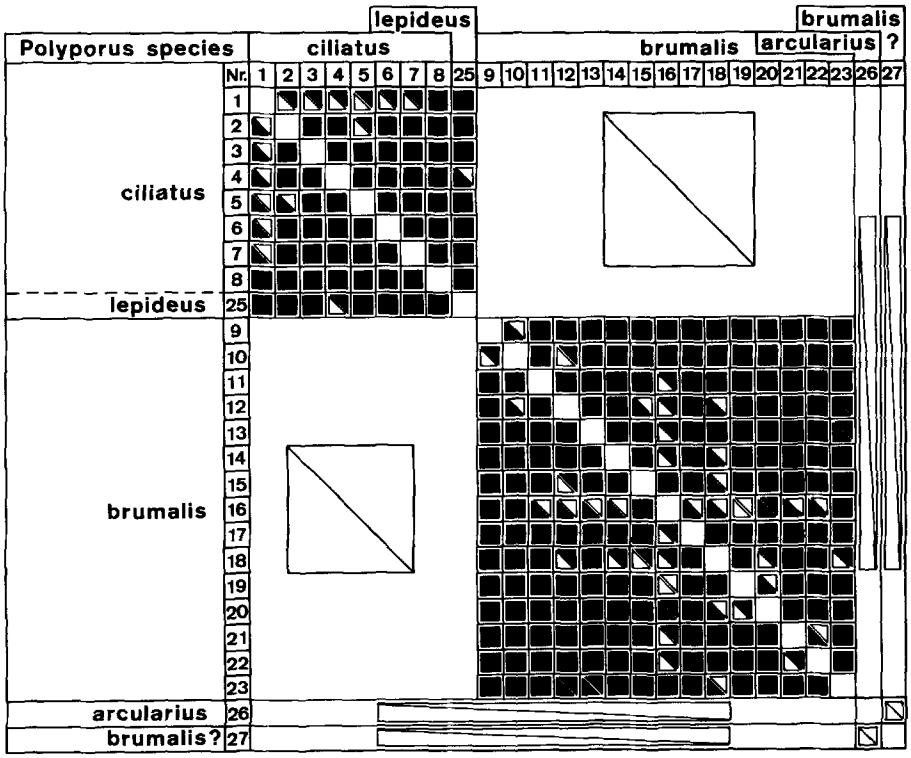
In conclusion, the 26 dikaryotic races of the genus *Polyporus* could be assigned to three morphologically distinct groups on the basis of fruit body characters (Fig. 1) and the conditions necessary for fruiting induction. Dikaryon formation in each race is controlled by the tetrapolar mechanism of homogenic incompatibility.

II Mating Relations

Compatible monokaryons of all races (representing the mating types in parental combinations) were crossed in all possible combinations. The results are summarized in Figure 2 and can be interpreted as follows:

The mating relations indicate the presence of four interfertility groups characterized by the ability to intercross in every intragroup combination and no indication of di- (or hetero-) karyon formation between members of different groups. With the exception of race 27, these groups correspond exactly with those derived from morphological criteria; each can thus be considered as a true biological species.

Three cases of misclassification could be detected by our mating experiments as indicated in Table 1: races, originally received as *P. brumalis* had to be transferred to *P. ciliatus* on the basis of compatibility and a comparison of hymenia.



■ Normal contact, clamp connections and fruit bodies formed when A≠B≠

▩ Barrage formation

▩ Barrage formation with unilateral nuclear migration (only in the dark part)

▨ Border line, neither clamp connections nor dikaryotic fruit bodies formed in all combinations (from Esser and Hoffmann 1977)

Fig. 2. Mating relations in intra- and interspecies combinations of monokaryons from 26 races of different species of *Polyporus*

1 Intraspecific Combinations

As mentioned, all intraspecific combinations were fertile, i.e. dikaryon formation is controlled mainly (exceptions see section III) by the individual mating type combination. In *Polyporus brumalis* (15 races), a total of 23 *A* and 21 *B* factors were identified and in *P. ciliatus* (9 races), there were 14 *A* and 16 *B* factors (Table 1). The lack of fertility between species led to the compilation of parallel series of factors.

Using the formula of Whitehouse (Raper 1966), an estimate of the total number of alleles occurring in nature was possible: approximately 40-50 *A* and 40-70 *B* specifities. These values, though based on only a small number of individuals, are lower than those reported for other well known tetrapolar basidiomycetes (Raper 1966). Perhaps this is correlated with the lack of recombinable subunits of the compatibility factors in *Polyporus* (Stahl 1975).

Several crosses in the two species *P. brumalis* and *P. ciliatus* were distinguished by the appearance of a clear zone devoid of aerial hyphae in the area of contact resembling a barrage (Vandendries 1932). This phenomenon was most pronounced in combinations with the three races 1, 16 and 18 (Fig. 2), but was also detected in other combinations independent of the mating type configuration. In most cases it was accompanied by a unilateral retardation of fruiting, indicating the operation of an incompatibility system superimposed on the homogenic system controlled by the mating type factors.

In the literature, especially in publications dealing with higher basidiomycetes, the term barrage is used for all kinds of mycelial interactions showing some sort of separation (Vandendries 1932; Brodie 1936; Traquair and Kennedy 1974; Adams and Roth 1967). Even interactions between sister monokaryons of a single cross ($A \neq B \neq$ combinations in *Schizophyllum*, Raper 1966) were termed barrage. In order to avoid further confusion, it is proposed that this designation should be exclusively confined to phenomena of heterogenic incompatibility.

2 Interspecific Combinations

All interspecific combinations were sterile and did not in any way influence the monokaryotic fruiting of either partner. However, these were characterized by a sharp separation of the two mycelia in the area of contact. This was termed 'border line' and can clearly be distinguished from barrage formation and normal contact in intraspecific matings (Fig. 3).

This phenomenon is certainly not unique to the genus *Polyporus*. Reports of peculiar 'contact-' or 'demarcation-lines' in interspecies combinations of *Pleurotus* (Anderson et al. 1973; Bresinsky et al. 1977) and *Auricularia* (Duncan and MacDonald 1967) point to its general occurrence and therewith its taxonomic value.

In order to investigate the possibility of bypassing this interspecific sterility and to gain an insight in to its mechanism, forced heterokaryosis between auxotrophic mutants of *P. brumalis* and *P. ciliatus* was attempted by either mixing cultures on minimal medium (using either monokaryons or dimon-matings) or by trying to induce protoplast fusion. In no case studied, however, was there any evidence for the formation of a prototrophic heterokaryon, thus stressing the total genetic isolation of each species.

III Fertilization Barriers

As already shown in Figures 2 and 3, two morphologically and functionally different forms of fertility barriers exist in *Polyporus*: barrage formation, reducing fertility in certain intraspecific crosses, and a border line connected with intersterility between species. A genetic analysis of the underlying mechanisms was therefore possible only in the first case.

1 Barrage Formation

a. Morphological aspects. The results of microscopic studies of the events occurring during barrage formation are shown in Figure 4: the barrage presents itself as a broad and clear zone of contact between the two incompatible mycelia; the density of hyphal growth is strongly reduced (Figs. 4a, b, e); pigment production in this area was never observed.

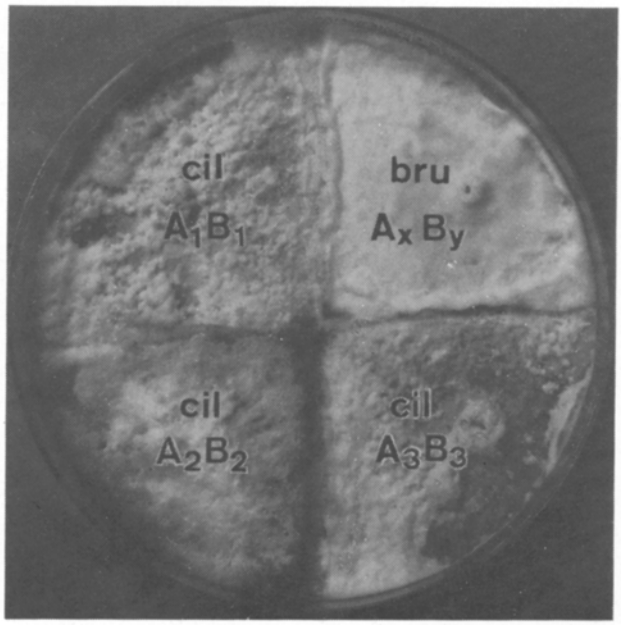


Fig. 3. Intra- and interspecies interactions between monokaryons of *Polyporus* species. Petri dish co-inoculated with *P. ciliatus* (cil) and *P. brumalis* (bru) showing normal contact (left) barrage (bottom) and border line (top and right)

In comparison to the normal compatible combinations (Fig. 4c, d), barrage forming crosses are characterized by the following events:

1. Fusion of hyphae is retarded by 5-7 days, regardless of the combinations of mating type factors.

Normally dikaryon formation between compatible monokaryons takes place within a few hours of hyphal contact (Fig. 4c). When a barrage is formed, however, anastomoses and clamp connections can be seen only after 7-9 days of growth (Figs. 4h, i). The hyphae frequently grow past each other avoiding anastomosis (Fig. 4h).

2. Nuclear migration is confined mainly to one monokaryon as indicated in Figure 2. Its direction is dependent on the strain used, e.g. always unilateral in races 1 and 18.

Spreading of the dikaryotic state into this region occurs by growth of dikaryotic hyphae formed in the contact zone, as demonstrated by comparison of velocities (2.4 mm/h nuclear migration – unhindered; 0.22 mm/h hyphal extension rate; 0.27 mm/h spreading of dikaryon – hindered side).

As a result of effects 1. and 2. there is a delay in fruiting time: 7-9 days on the 'unhindered' side and 21-23 days on the other. After this time, the culture medium is often too exhausted to produce normal shaped fruit bodies and they remain rudimentary.

3. Two weeks after their first contact, a gradual lysis of hyphae can be seen in the barrage (Figs. 4g, j).

Since there is no immediate lytic reaction, as in the incompatibility reaction in *Podospora anserina* (Blaich and Esser 1971; Esser 1959a, b), it is not possible at present to correlate hyphal lysis and barrage formation in *Polyporus*.

b. Genetic analysis. Preliminary experiments had confirmed that incompatibility and barrage formation in *Polyporus* are controlled by nuclear genes. Because of their rapid fruiting, races 1 and 4 of *P. ciliatus* were chosen for a detailed analysis.

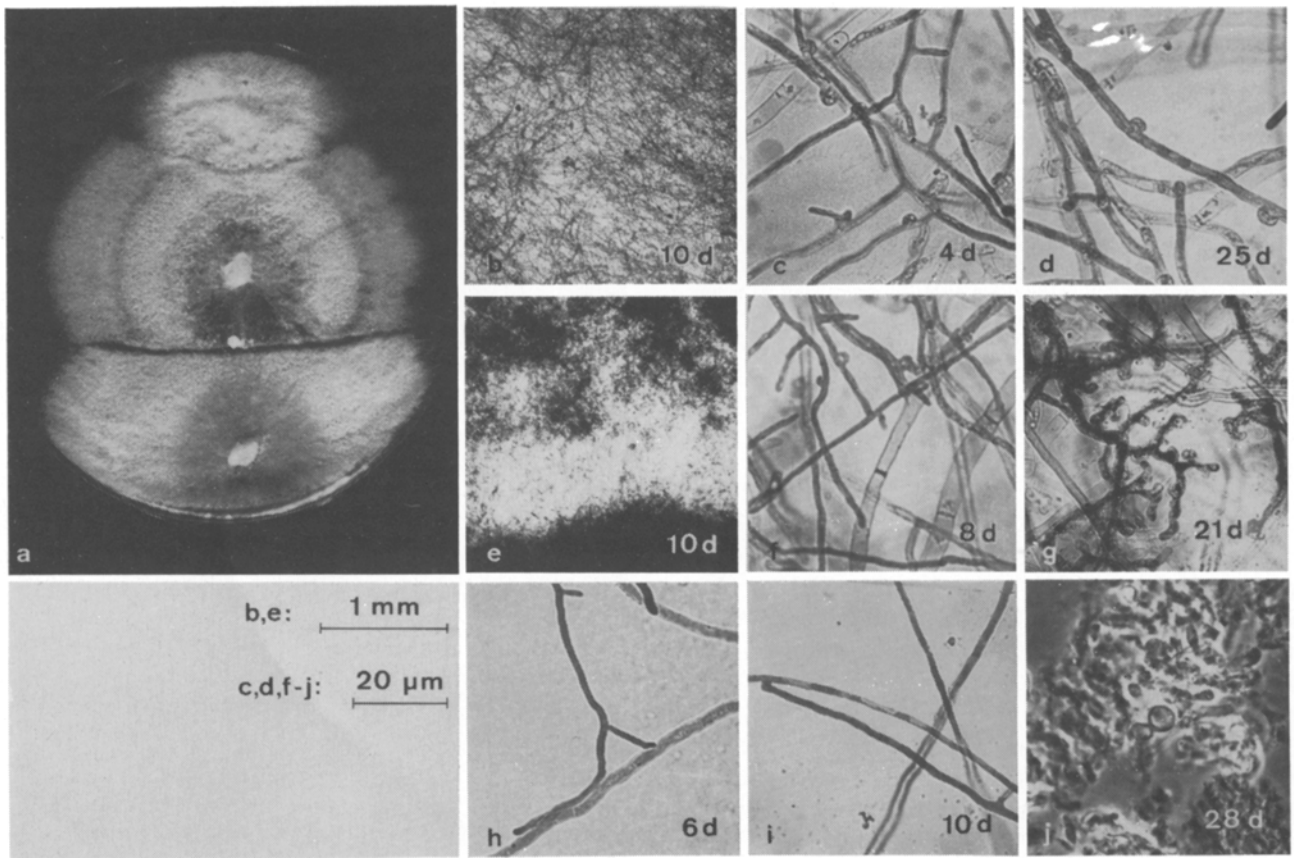


Fig. 4. Barrage formation in intraspecies combinations between monokaryons of *P. ciliatus* compared with normal contact. (a) Survey, showing petri dish inoculated with three monokaryons; top: normal contact; bottom: barrage formation; (b-d) microscopical view of normal contact; (e-j) stages of hyphal contact between barrage forming monokaryons. Age of mycelia is given in days; for further information see text. (From Esser and Hoffmann 1977)

This required isogenization of the strains by means of repeated inbreeding: the first combination did not yield segregation patterns interpretable in simple mechanisms. This indicated the presence of additional factors, already known in *Podospora* (Esser 1965) or *Aspergillus* (Caten 1971), influencing the incompatibility reaction. Their influence might be one possible explanation for a reaction type ('uncertain reaction' in Figs. 5 and 6), which could neither be classified as barrage or normal contact. Partial activation of the incompatibility mechanism (action of the *bf*-genes alone, see below) may also lead to this phenomenon.

The first unequivocal segregation pattern was obtained in the third generation. It is shown in Figure 5 and was used to build up a working hypothesis to account for the mechanism of incompatibility.

A minimum of two genes is operating in barrage formation: one is acting in heteroallelic combination, as can be seen by the symmetric distribution of the main 'barrage negative' groups (upper left and lower right quadrant in Fig. 5); the other evokes the asymmetric distribution of barrage in the upper right quadrant of Fig. 5, indicating the inequality of its two alleles. At least one of the two monokaryons must carry this 'active' allele for the expression of a barrage.

The similarity of this mechanism to the control of monokaryotic fruiting (Stahl and Esser 1976) led us to adopt the following symbols for the incompatibility alleles in *Polyporus:* bi^+/bi (barrage initiation) and bf_1/bf_2 (barrage formation). For the realization of a barrage, the minimum required is the presence of bi^+ in one partner and a heterogeneity of both partners at the *bf* locus.

The hypothesis might then be presented as:
bi^+ + heteroallelic combination of *bf* = barrage.

Four different combinations of the two genes *bi* and *bf* are possible:

1. $bi\ bf_1 \times bi\ bf_2$ (normal contact)
2. $bi^+bf_1 \times bi\ bf_1$ („ „)
3. $bi^+bf_1 \times bi\ bf_2$ (barrage)
4. $bi^+bf_1 \times bi^+bf_2$ („)

Progeny from each of these combinations were analyzed to test our hypothesis further. All but the fourth type of combination reacted as expected from our previous results: normal contact between all monokaryons in cross 1 – with the occasional appearance of the unclear type of reaction ('uncertain reaction') – and cross 2. In cross 3 segregation identical to that presented in Figure 5. The pattern obtained in cross 4 is shown in Figure 6.

Instead of the monofactorial segregation bf_1/bf_2, the action of an independent second gene is clearly visible from the 'tetrapolar' distribution of barrage and normal contact. Its functional identity – barrage formation in heteroallelic combinations – allowed this gene to be classified as a second *bf*-locus. Thus our hypothesis needs to be extended.

bi^+ + heteroallelic combination of both *bf*-genes = barrage.

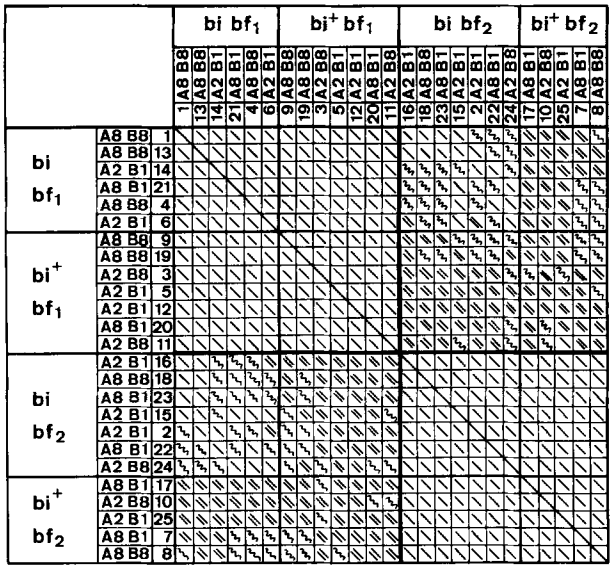


Fig. 5. Genetic analysis of barrage formation between races 1 and 4 of *P. ciliatus.* Segregation pattern and derived genotypes of 25 monokaryotic progeny of the third generation

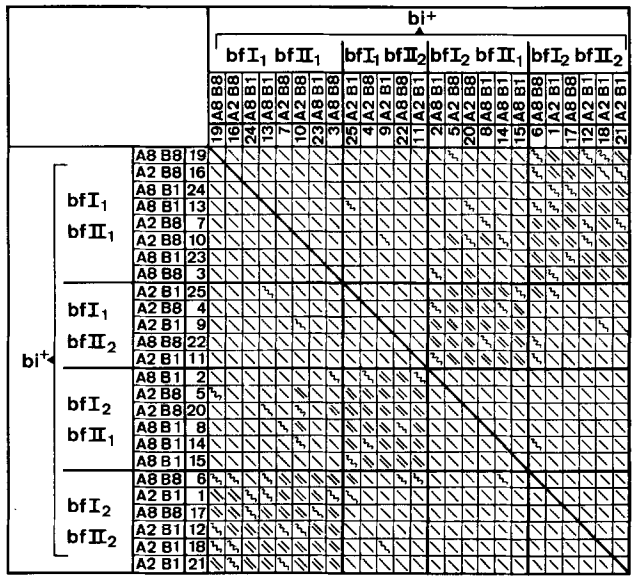


Fig. 6. Genetic analysis of barrage formation between races 1 and 4 of *P. ciliatus.* Specification of the *bf* genotypes from progeny of the hypothetical combination $bi^+\ bf_1 \times bi^+\ bf_2$ derived from the results of Figure 5. All monokaryons are characterized by their mating type and number of isolation. Symbols as in Figure 5. For further explanations see text

In a series of further experiments it was found that other races of both species *P. brumalis* and *P. ciliatus* follow an identical genetic pattern of barrage formation as found in the races 1 and 4 of *P. ciliatus*. Therefore, barrage formation within the genus *Polyporus* is controlled by at least three genes, the alleles of which act according to a mechanism of heterogenic incompatibility.

The identification of the genotypes necessary for barrage formation and incompatibility allowed two more analyses:

1. the determination of the genotype of each race.

The extensively studied races 1 and 4 of *P. ciliatus* have the following combination of genes:

race 1: $A1B1\ bi^+\ bfI_1\ bfII_2$ plus $A2B2\ bi^+\ bfI_2\ bfII_2$
race 4: $A7B7\ bi\ \ bfI_1\ bfII_1$ plus $A8B8\ bi\ \ bfI_2\ bfII_1$

In all other 'active' barrage formers (e.g. race 18 in Fig. 2), the allele bi^+ was demonstrated. First evidence suggests that it might be the cause of lack of nuclear migration.

2. The analysis of linkage relationships between the three barrage genes.

These data are summarized in Table 2. The kind of genetic analysis undertaken (single strand analysis: Esser and Kuenen 1967) made a statistical verification necessary. The mating type factors were included in this study for they are obligatory heteroallelic in each fertile crossing.

From the segregation values obtained in the F_1 and the recombination frequency calculated from these, there is no evidence for linkage between any of the five genes studied. In this context it should be mentioned that despite the vast number of publications on intraspecific antagonism in basidiomycetes (Adams and Roth 1967; Duncan and MacDonald 1967; Esser and Blaich 1973; Kemp 1970; Macrae 1967; Rayner and Todd 1977) there has been no genetic analysis of the underlying mechanism. Our results in two species of the genus *Polyporus* fill this gap and give additional evidence for the universal distribution of systems of heterogenic incompatibility (Esser and Blaich 1973) and its action as a mechanism of genetic isolation and species formation (Esser 1965).

2. Border Line Formation

The formation of a line of increased hyphal density (Fig. 3) which has been termed border line and is often associated with pigment production was characteristic for all combinations of monokaryons (and dikaryons) of different species. It can thus be used in the diagnosis of species delimination in *Polyporus* (Fig. 2).

Microscopic observation did not reveal any morphological peculiarities in this zone. Rare hyphal fusions could be detected, most of them originating from hyphae of the same monokaryon. As reported for some species of *Ganoderma* (Cabral 1951), interspecific fusion could not be fully excluded. As indicated in part II.2, however, the failure to achieve interspecific complementation of nutritional deficiencies does not support this interpretation.

Discussion

In order to prove the applicability and validity of the biological species concept, the basidiomycetous genus *Polyporus* was chosen because its life cycle is easily accessible under laboratory conditions and its taxonomic position is rather clearly defined. An extended study of the mating relations of 26 races belonging to three species allows the following conclusions which may be used for classification purposes in this genus:

1. In intraspecific combinations the basic tetrapolar mechanism of homogenic incompatibility requiring different *A* and *B* factors for the formation of clamp connec-

Table 2. Compilation of data from the investigation of linkage relations between the genes *bi, bfI, bfII* and the mating type factors A and B (germination frequency of spores ⩾ 92%)

Genes or factors	Number of viable spores	Segregation of F_1-genotypes				Statistical verification of F_1-segregation df = 3		Frequency of recombination (ρ) confidence limits	
		P_1	P_2	R_1	R_2	χ^2	p-value	= 1.96σ	
bi/A	126	33	36	29	28	1.300	0.8 – 0.7	0.547	0.060
bi/B	126	31	29	36	30	0.921	0.9 – 0.8	0.476	0.068
bfI/A	129	39	35	28	27	0.590	0.9	0.573	0.058
bfI/B	129	36	27	30	36	1.888	0.6	0.488	0.063
bfII/A	50	13	15	13	9	1.600	0.7 – 0.6	0.560	0.094
bfII/B	50	12	13	15	10	1.280	0.8 – 0.7	0.500	0.100
bi/bfI	103	31	29	21	22	2.765	0.5 – 0.4	0.582	0.063
bi/bfII	25	6	4	7	8	1.408	0.7	0.400	0.155
bfI/bfII	50	10	12	16	12	1.600	0.6	0.440	0.106

tions may be overlapped by a mechanism of heterogenic incompatibility which is expressed macroscopically as barrage formation.

2. In interspecific combinations there is complete sterility indicated by the formation of a border line.

I Classification of the Genus Polyporus

Traditionally, *Polyporus* species are diagnosed by minor morphological differences (Kreisel 1963; Donk 1969; Jahn 1969) which, from a genetic point of view, might represent the expression of simple mutational steps as are known for other differences in fungi (Esser and Kuenen 1967). The almost total convergence of typological and genetical species limits derived from successful intraspecies matings and unsuccessful hybridization experiments in interspecies combinations was therefore somewhat unexpected.

Considering that the absolute limits of a species will never be delimited by any method (Nelson 1963) both concepts have led, in the case of *Polyporus*, to usable propositions. The genetical, however, has an advantage with respect to the great variability of polyporaceous fungi as shown by Martin and Gilbertson (1976).

This variability is probably the cause of three mis-identifications of *P. ciliatus* (Table 1) which were obtained as *P. brumalis* and also of the proposed separation of *P. ciliatus* f. *lepideus* as a new species (Jahn, pers. commun.). The unequivocal results of the mating experiments presented in this paper and also some data of David and Romagnesi (1972) speak against such classifications.

In addition to the morphological species characters of size and shape of the hymenial pores used by Kreisel (1963), our results provide three new parameters:

1. The formation of a border line in every interspecies mating (Fig. 2 and 3).

2. The species specific temperature requirements for fruiting.

3. The interspecies fertility, even in the case of barrage formation where fruiting is unilateral and delayed.

II Proposition of a Modified Biological Species Concept

A species concept has not only to define the content of the biological category 'species', but also to consider the mechanisms by which the integrity of this category is maintained and what may influence the experimental analysis of the species in other ways. In *Polyporus*, which may be taken as a typical example of many basidiomycetes, this includes a knowledge and consideration of all parameters (such as homogenic and heterogenic incompatibility, genes for fruit body induction) which interfere with the basic premise of the species concept: the ability to exchange genetic material between conspecific individuals.

Conscious of these problems, we therefore propose a modification of the classic biological species concept: Populations (races) belong to different species when the failure to interbreed and to produce viable offspring is not caused by genetic parameters operating in completion of the sexual cycle.

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