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Mating biology in *Peniophora cinerea* (Basidiomycetes)

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Mating tests were performed to analyze the genetic relationship between two intersterile sibling species in *Peniophora cinerea* (Fr.) Cooke in Europe. Two newly collected specimens from North Europe were found to be compatible with both sibling species, which strongly suggests a close genetic relationship and a sterility barrier of simple genetic origin. The two sibling species, which differ in their substrate selectivity, are accepted as subspecies. One subspecies is restricted to decorticated wood of *Fagus*, and occasionally the fruit bodies are associated with insect galls. Intersterility was also found in some combinations with two other specimens from Canada and Turkey, but no linkage was found with a particular substrate. Specimens from Taiwan were found to be partially compatible with specimens from Europe, Turkey, and Canada. Distinct differences between the subspecies were found in banding patterns from isoelectric focusing of buffer-soluble mycelial proteins. It is proposed that the kind of intersterility found here is intraspecific and should be looked upon as part of a propagation strategy.

Key words: speciation, evolution, Basidiomycetes, isoelectric focusing, insect gall, mating test.

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Les auteurs ont effectué des essais de croisement afin d'analyser la relation génétique de deux lignées du *Peniophora cinerea* (Fr.) Cooke de l'Europe et dont les descendants sont interstériles. Deux spécimens récemment récoltés dans le nord de l'Europe se sont avérés compatibles avec les descendants des deux lignées, ce qui suggère fortement l'existence d'une étroite parenté génétique et que la barrière de stérilité a une provenance génétique simple. Les auteurs acceptent les deux lignées, lesquelles diffèrent dans leur choix de substrat, comme des sous-espèces. Une première sous-espèce est restreinte au bois décortiqué de *Fagus*, et occasionnellement, les fructifications sont reliées à des gales d'insectes. On a également retrouvé l'interstérilité dans certaines combinaisons avec deux autres spécimens, du Canada et de la Turquie, mais dans ce cas il n'a pas été possible de faire le lien avec un substrat particulier. Les spécimens originaires de Taiwan se sont avérés partiellement compatibles avec des spécimens d'Europe, de Turquie et du Canada. Les auteurs ont mis en évidence des différences nettes entre les sous-espèces dans les patrons des bandes obtenues par positionnement isoélectrique des protéines mycéliennes en solution tamponnée. Le auteurs proposent que le type d'interstérilité rencontré est intraspécifique et devrait être considéré comme un élément d'une stratégie de propagation.

Mots clés : spéciation, évolution, basidiomycètes, positionnement isoélectrique, gale d'insecte, essai de croisement.

[Traduit par la rédaction]

Introduction

Peniophora cinerea (Fr.) Cooke is a corticioid basidiomycete, usually growing on dead branches of deciduous trees where it belongs to the primary decaying flora. The species is widely distributed in boreal and temperate areas over the northern hemisphere. Excellent description of fruit body morphology is given in Eriksson et al. (1978).

Like other *Peniophora* species, *P. cinerea* grows readily in culture, and delimitation studies have been done by the use of crossing tests (Eriksson 1950; Hallenberg 1984, 1986; Hallenberg and Larsson 1991). As a result of this experimental approach, a sibling species complex was detected (Hallenberg 1986). Specimens growing on decorticated branches of *Fagus silvatica* L. in Central Europe were incompatible to a high degree with specimens from Europe growing on other kinds of substrata. Intercompatibility was found within each group. Furthermore, a high degree of compatibility was observed between both these groups and specimens from Canada. This indicated that a relatively recent speciation event had taken place in Europe and that the speciation was of more or less sympatric nature. Chamuris (1992) continued to investigate mating behaviour in this species using isolates from the northeastern United States. He confirmed earlier results but did not find evidence of a similar speciation among United States isolates.

In the present study, additional cultures of *P. cinerea* have been used in crossing tests and electrophoretic studies. New light has been shed upon speciation processes and the application of a biological species concept. Protein banding patterns from electrophoresis showed variation between the sibling species and isolated populations.

Materials and methods

Mycelia arising from single spores (SS) and polyspores were isolated after spore dispersal on common malt agar (1.25% malt extract). Matings between single spore isolates were made for each specimen to find compatible testers to be used in intercompatibility tests. For details in procedure see Hallenberg (1984).

For electrophoresis, buffer-soluble mycelial proteins were extracted according to a procedure described in Hallenberg and Larsson (1991). Briefly, protein extraction involved incubation of polyspore mycelia in liquid medium (1.5% malt extract in distilled water). Drained and rinsed mycelium was homogenized in triethanolaminehydrochloride buffer (TEA) in a mortar standing on ice. The suspension was centrifuged and the supernatant recovered for electrophoretic runs.

For isoelectric focusing (IEF) we used an LKB 2117 Multiphor II Electrophoresis System connected to a Pharmacia Constant Power Supply (ECPS 3000/150). Samples were run on ultrathin polyacrylamide gels (0.5 mm) with ampholytes added (pH range 3.5–9.5) according to instructions in the laboratory manual of LKB 2117. The electrophoresis cell was kept at 6°C. Gels were prefocused for 1 h at 8 W, and maximum voltage of 2000 V was set throughout the run. Samples of 15 μL were applied on paper wicks and focused for 80 min at 16 W, then increased to 20 W for the last 20 min. Wicks were removed after 30 min. The gels were stained with Coomassie

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Brilliant Blue G 250 (LKB). Standard proteins (pI markers, LKB, range 3–10) were used to determine pH gradient in the gels.

Cultures used in the study of Peniophora cinerea

The sibling species found in Europe are designated Main European type and European *Fagus* type in accordance with Hallenberg and Larsson (1991). Specimens from Canada and Taiwan are referred to as Canadian type and Taiwanese type, respectively. The name of the collector of specimens is mentioned if other than Hallenberg. The cultures are stored in the culture collection at the Department of Systematic Botany, University of Göteborg, and the original specimens, together with spore prints, are kept in the herbarium (GB).

Main European type

GB 0004/ *Salix*/ Sweden, Göteborg Botanical Garden/ Coll. Hallingbäck. GB 0213/ *Alnus glutinosa*/ Sweden, Göteborg Botanical Garden. GB 0312/ *Ulmus glabra*/ Sweden, Närke. GB 0895/ *Syringa*/ Sweden, Göteborg Botanical Garden/ Coll. Nordin. GB 1177/ Deciduous tree/ Norway, Sogn & Fjordane. GB 1179/ *Fagus* (cortex)/ Norway, Rogaland. GB 1263/ *Crataegus*/ Sweden, Skåne. GB 1403 (=LY 4584)/ *Betula*/ France, Ain/ Coll. Boidin. GB 1442/ *Rhamnus*/ Sweden, Göteborg Botanical Garden. GB 1469/ Deciduous tree/ Romania, Iasi. GB 1479/ Deciduous tree/ Romania, Iasi. GB 1483/ *Tilia*/ Romania, Iasi. GB 1485/ *Alnus*/ Romania, Covasna. GB 1486/ Deciduous tree/ Romania, Iasi. GB 1487/ Deciduous tree/ Austria/ Burgenland. GB 1501/ Deciduous tree/ Romania, Iasi. GB 1761/ *Fraxinus*/ Sweden, Västergötland. GB 1810/ *Betula*/ France, Pyrenees Orientale. GB 2026/ *Quercus*/ Denmark, Jylland. GB 2054/ *Fagus* (cortex)/ Sweden, Västergötland/ Coll. Lindqvist. GB 2205/ *Rhododendron*/ Turkey, Trabzon. GB 2207/ *Fagus* (cortex)/ Turkey, Trabzon. GB 2211/ *Alnus*/ Turkey, Trabzon. GB 2225/ Deciduous wood/ Turkey, Trabzon. GB 2296/ *Fagus* (cortex)/ Sweden, Göteborg Botanical Garden.

European Fagus type

GB 1007/ *Fagus* (decorticated)/ Romania, Bistrita-Nasaud. GB 1474/ *Fagus* (decorticated)/ Romania, Brasov. GB 1477/ *Fagus* (decorticated)/ Romania, Iasi. GB 1484/ *Fagus* (decorticated)/ Romania, Neamt. GB 1488/ *Fagus* (decorticated)/ Romania, Brasov. GB 1788/ *Fagus* (decorticated)/ Spain, Huesca. GB 1910/ *Fagus* (decorticated)/ Denmark, Jylland.

Canadian type

GB 0532/ Deciduous wood/ Quebec, Cantley. GB 0594/ *Ostrya*/ Quebec, Cantley. GB 0596/ *Ostrya*/ Quebec, Cantley. GB 0772/ *Ostrya*/ Ontario, S. of Ottawa. GB 1991/ *Alnus*/ British Columbia, Vancouver Island. GB 2330/ *Tilia americana*/ Ontario, Simcoe Co./ Coll. Thorn. GB 2331/ *Prunus virginiana*/ Ontario, Simcoe Co./ Coll. Thorn.

Taiwan type

GB 2176/ *Ficus virgata*/ Nantou Shiahn/ Coll. Wu. GB 2181/ Deciduous wood/ Nantou Shiahn/ Coll. Wu. GB 2182/ Deciduous wood/ Miaoli Shiahn/ Coll. Wu.

Results

The results are divided into four parts: (*i*) intercompatibility tests between newly obtained cultures and tester strains from earlier investigations; (*ii*) detection of insect galls associated with fruit bodies of *Peniophora cinerea*, European *Fagus* type; (*iii*) electrophoresis of mycelial proteins from the new accessions; and (*iv*) study of the variation in barrier formation found in both ordinary mating tests between single spore mycelia and between different secondary mycelia. Intergroup compatibility for specimens of the Main European type and the European *Fagus* type, listed under Materials and methods, was reported earlier (Hallenberg 1986; Hallenberg and Larsson 1991).

Compatibility between GB 2026, GB 2296, and both sibling species in Europe

The Main European type differs from the *Fagus* type in substrate selectivity. The *Fagus* type seems to be restricted to decorticated branches of *Fagus*. Specimens of the Main type have been collected on corticated *Fagus* and other hardwoods while few specimens have also been found on decorticated wood of hardwoods but not *Fagus*. The two cultures GB 2026, on *Quercus*, and GB 2296, on *Fagus* (cortex), are examples of the Main European type but display some unusual mating behaviours. In earlier studies (Hallenberg 1986), the sterility barrier between the two sibling species in Europe is quite distinct, since only in a few cases were clamped mycelia formed upon intergroup mating (ca. 10% of all intergroup matings). In these few positive matings, a distinct barrier was formed on the junction line between the two single spore mycelia, and clamped hyphae were found to be restricted to this barrier. GB 2026 and GB 2296 clearly belong to the Main European type, and in all mating tests performed with this group, a vigorous secondary mycelium is formed, with constant clamp connections on the hyphae. Surprisingly, in matings between these two cultures and representatives of European *Fagus* type, the same kind of vigorous secondary mycelium was formed in most cases; however, a few instances of partial compatibility were also found: (*i*) GB-2296-SS-1 × GB-1910-SS-1,4,5,7 produced a distinct reddish barrier on the junction line, but vigorously growing, aerial mycelium with clamps on hyphae was spreading all over the plate. (*ii*) GB-2296-SS-2 × GB-1910-SS-1,4,5,7 lacked both distinct barrier and clamped hyphae, and no intermingling occurred between the two mycelia. Aerial mycelium was totally absent, the agar was stained brown, and extensive hyphal lysis had taken place at the junction line.

These mating tests clearly indicate that the previously reported (Hallenberg 1986) intersterility between the two sibling species in Europe is not shared by all European specimens. Thus, the traditional definition of a biological species is not applicable to the two sibling species in *P. cinerea*. Moreover, these mating results indicate a close genetic relationship between the two sibling species, which is reinforced by the capacity of both to mate with Canadian (Table 1; Hallenberg 1986) and United States (Chamuris 1992) specimens. The results are quite in accordance with the theory that a simple genetic system may be operative in intersterility reactions as shown in *Heterobasidion annosum* (Fr.) Bref. (Chase and Ullrich 1990*a*, 1990*b*) and *Bulbillomyces farinosus* (Bres.) Jül. (Hallenberg 1988).

Connections between the European Fagus *type and insect galls*

In the fruit bodies of three of the seven specimens brought into culture, prominent brownish pustules were present (GB 1007, GB 1474, GB 1910; Fig. 4). The characteristics of these pustules were overlooked earlier (Hallenberg 1986) but are highly interesting. The brown, fibrous, rounded pustules range from a few millimetres to 15 mm in diameter when coalesced. They are built with fiber hyphae (skeletals), and in the interior there are small galleries containing insects in the pupal stage.

The existence of insect galls on the fruit bodies of *P. cinerea* has not been mentioned earlier in the literature, but on the label of a herbarium specimen from Bavaria, Germany, Herman Hahn made this observation (H. & M. Jahn, 1977-11-10, in GB). In our herbarium (GB) there are a few specimens with such insects galls from Central Europe,

TABLE 1. Intraspecific and intergroup matings of single-spore cultures in *Peniophora cinerea*

	Turkey				Europe		Canada		Taiwan		
	2205	2207	2211	2225	2026	2296	2330	2331	2176	2181	2182
Turkey											
2205		+	+	+	+	+					
2207	+		+	+	+	+			+	1/2	
2211	+	+		4/10	+	+	−	+			
2225	+	+	4/10		+	+	−	5/8			
Europe											
Main type											
1810					+	+			+	+	+
2026	+	+	+	+		+	1/30	+	+	+	+
2296	+	+	+	+	+		2/4	+	1/2	+	
Fagus type											
1488	2/4	−	1/4	−	2/4	+	−	+			
1788	2/8	1/8	3/8	−	+	+	−	+			
1910					2/4	4/8			+	+	+
Canada											
0594					+	5/8	+	+			
0772							+	+	+	+	
1991	+	+	+	+	+	+	−	+	−	+	+
2331			+	5/8	+	+	+				

NOTE: Numbers refer to cultures (without GB prefix) as described in Materials and methods. +, compatible matings that produced clamps; −, incompatible matings that did not produce clamps. Partial compatible matings are indicated by a fraction in which the denominator is the total number of matings and the numerator is the number of compatible matings. Each symbol represents the results of mating two to four single-spore cultures from each partner, i.e., 4−16 matings. Taiwanese cultures are polysporous and matings with them were performed with di−mon tests.

Denmark, and northeastern Turkey, but there are no examples from the voluminous Scandinavian collections. In all cases of insect galls in association with fruit bodies of *P. cinerea*, the substrate has been decorticated *Fagus* branches.

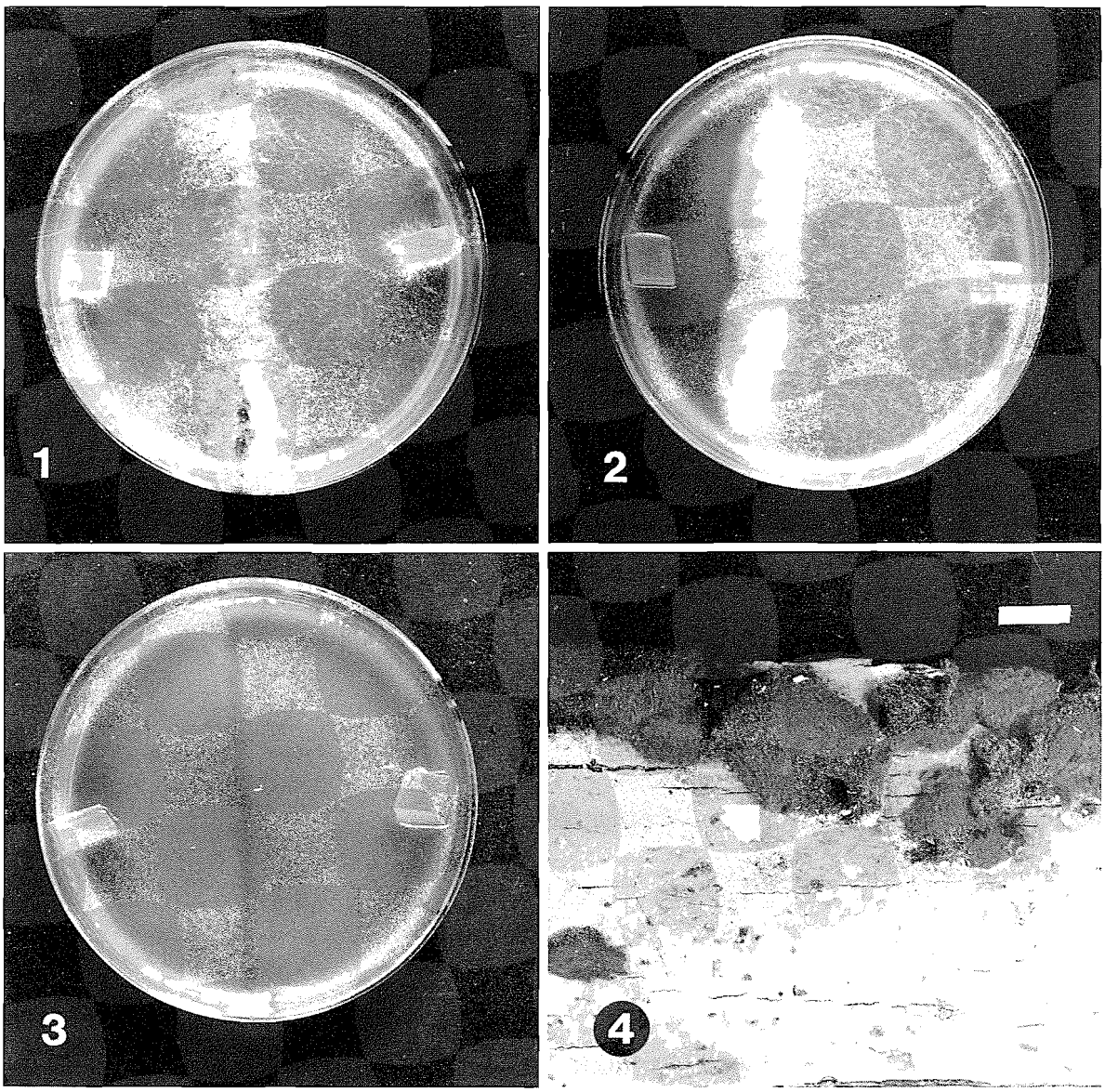
It is interesting to note that the galls are made of skeletal hyphae that clearly belong to *P. cinerea*. Skeletal hyphae are not known from any *Peniophora* species, but they are obviously the result of insect activity. The skeletals originate from thin-walled generative hyphae. They are aseptate, unbranched, 2−4 μm wide, several hundred micrometers long, and thick-walled except for the apical part. The thick-walled parts are yellowish brown in phase contrast. Thick-walled, yellowish brown hyphae are found in basal parts of the fruit bodies, but here they are always regularly septate with clamps. This intimate relationship suggests that insects can modify a fungal organism. If this indicated coadaptation has any relationship to the speciation process in *P. cinerea* is, however, still unknown.

Mating tests with additional material from Canada

Three new cultures from Canada (GB 1991, GB 2330, GB 2331) exhibited unexpected properties in their mating behaviour (Table 1). Earlier results, based on material from Quebec and Ontario, indicated that the Canadian material was intercompatible to a high degree with both sibling species in Europe (Hallenberg 1986). In relation to the European cultures, the new Canadian accessions behaved like earlier ones with one exception. GB 2330 was perfectly compatible with all three specimens from Ontario and Quebec but almost completely incompatible with seven other specimens, including GB 1991 from Vancouver Island. A distinct barrier was formed on the junction line in all incompatible crossings. The exceptions were, not unexpectedly, GB 2296, which was partially compatible with GB 2330, and GB 2026, but here only 1 successful mating out of 30 was recorded. The secondary mycelium formed in this mating was differentiated from the single spore mycelia into a distinct sector. Hyphae formed after mating were constantly clamped but a great part of the clamps was not complete (false clamps). Once again, this is an indication that a simple genetic factor is responsible for the intersterility recorded. Fruit bodies of these new accessions from Canada had gloeocystidia that became blackish when treated with sulfovanilline (GB 2330 and GB 2331), as did the specimens from Quebec and Ontario that had been investigated earlier (Hallenberg 1986). In GB 1991 from British Columbia, however, no such staining reaction was observed.

Mating tests with specimens from Turkey

A number of cultures from Aphyllophoraceous fungi were obtained after collecting in northeastern Turkey in 1989. Results from intercompatibility tests with these cultures showed that the Corticiaceae flora found in Turkey is closely linked genetically to the European flora, despite the phytogeographical isolation of northeastern Turkey (Hallenberg 1991*b*). Four cultures of *P. cinerea* were obtained, and all belong to the Main European type, being intersterile or only partially compatible with the European *Fagus* type (Table 1). One combination, however, was fatal. In 6 of 10 matings between GB 2211 and GB 2225 no secondary mycelium was formed, and existence of homogenic incompatibility cannot explain this incompatibility. No intermingling between the two haploid mycelia occurred and no barrier was formed. Aerial mycelium, which is typical for successful matings, was absent; the agar became brownish, and extensive lysis of hyphae was observed along the junction line. A similar reaction was also found in three of eight matings between GB 2225 and GB 2331 (Canada). Here, a kind of intersterility factor has been active that is obviously different from incompatible matings with GB 2330, but the behaviour is similar to that observed in matings of GB-2296-SS-2 and GB-1910-SS-1,4,5,7 (see above).



FIGS. 1–3. *Peniophora cinerea* ssp. *cinerea*. Variation in barrier formation in somatic incompatibility tests between synthetized secondary mycelia. Petri dishes are 55 mm in diameter. Fig. 1. Barrier with extensive, white aerial mycelium formed on the junction line. The secondary mycelia originates from matings between GB 2207-SS-5 × GB 2296-SS-1 (left) and GB 2205-SS-1 × GB 2296-SAS-1 (right). Fig. 2. Barrier with extensive, white mycelium on the junction line but left culture lacks aerial mycelium. The synthetized mycelia are GB 2207-SS-4 × GB 2225-SS-2 (left) and GB 2205-SS-1 × GB 2296-SS-1 (right). Fig. 3. A narrow lytic zone developed between the two mycelia, aerial mycelium absent, agar stained brown. The synthetized mycelia are GB 2207-SS-4 × GB 2205-SS-2 (left) and GB 2205-SS-2 × GB 2296-SS-2 (right). FIG. 4. *Peniophora cinerea* ssp. *fagicola* (GB 1474). Fruit body with insect galls (arrow). Scale bar = 2.5 mm.

Mating tests with specimens from Taiwan

Three polyspore cultures of *P. cinerea* were obtained from Dr. Sheng Hua Wu, Taipei. Di–mon matings were performed between these cultures and tester strains from Europe and Canada. A high degree of intercompatibility was found in these matings, but some combinations were negative (Table 1). Mating relationships between Taiwanese and European cultures were similar to the relationship between Canadian and European ones (Hallenberg 1986). Gloeocystidia in Taiwanese specimens of *P. cinerea* did not darken in sulfovanilline.

Barrier formation

In intercompatibility tests between single spore mycelia, various fungal responses were observed as a result of confrontations. In compatible matings, a secondary mycelium developed that soon covered the Petri dish. This mycelium was characterized by vigorous growth and an abundance of whitish, aerial mycelium, or the agar became brownish stained and only sparse aerial mycelium developed. Pairings between some cultures resulted in partial compatibility, i.e., some combinations of single spore mycelia were incompatible while others produced secondary mycelia. In the latter case, a distinct barrier was frequently formed with much aerial mycelium restricted to the junction line. Clamps were restricted to this barrier mycelium. In incompatible matings, a distinct barrier with aerial mycelium was mostly formed on the junction line, or a narrow lytic zone developed between the two mycelia, aerial mycelium disappeared, and the agar became stained

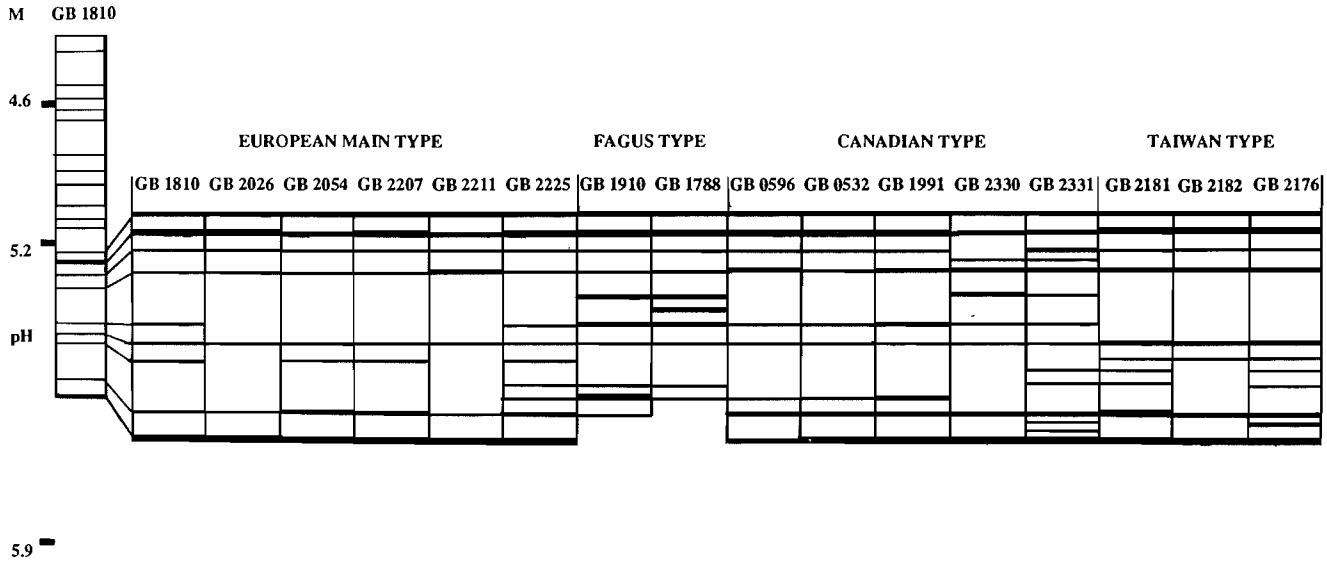


FIG. 5. Isoelectric protein banding patterns of pI markers (M) and mycelial protein solutions, transformed and partly magnified from a gel. Culture numbers and population designations given are assigned to cultures described in Materials and methods. Sample application not shown but located at pH 7.

TABLE 2. Percent staining in secondary mycelia from intraspecific matings in *Peniophora cinerea* ssp. *cinerea*

Single spore cultures*	W	B	Sectored
W × W	73	7	20
W × B	53	39	8
B × B	36	64	0

*W, white mycelium; B, brown-staining mycelium.

brownish. A similar pattern of barrier formation was observed by Boddy and Rayner (1982) in their study of *Stereum gausapatum* Fr.

To gain insight into the genetic background of the variation in barrier formation observed in *P. cinerea*, a special test was set up. Among the single spore cultures isolated in *P. cinerea*, some stained the agar brown while others remained unchanged. It was supposed that this ability to stain agar brown could be associated with subsequent variation in barrier formation. Single spore cultures from six specimens (GB 1991, GB 2205, GB 2207, GB 2211, GB 2225, GB 2296) were selected. These specimens had been found to be completely intercompatible (with the exception of GB 2211 × GB 2225; see above). Altogether, seven single spore cultures stained agar brown and seven did not. In various combinations, 70 matings were performed and resulting secondary mycelium became (*i*) whitish with an abundance of aerial mycelium and without staining the agar; (*ii*) sparse aerial mycelium with brown stained agar; or (*iii*) sectored mycelium in white and brown areas. Depending on different combinations of white and brown staining haploid mycelia, the proportions of resultant secondary mycelia varied (Table 2).

From this comparison it was determined that multiallelism is involved in the regulation of this character in secondary mycelia. Polyspore mycelia, originally isolated from specimens tested here, were all white and among all available polyspore mycelia of *P. cinerea* about four out of five were white and one out of five were brown, which suggest that the brown-staining character is recessive.

Some of the synthesized secondary mycelia were also paired against each other in somatic compatibility tests. After 2 months, patterns of barrier formation were studied and found to be similar to the variation observed in incompatible or partially compatible matings (Figs. 1–3). In all combinations between two brown mycelia a lytic reaction took place at the junction line (Fig. 3).

Electrophoresis

Isoelectric focusing of buffer-soluble mycelial proteins was performed for polyspore cultures of 16 specimens. The purpose was to evaluate the variation in protein banding patterns that should be independent of morphology or mating behaviour. From visual comparison of the different banding patterns, it follows that representatives of the European *Fagus* type are distinguished from all other specimens investigated. No such clear segregation, however, can be seen in any of the other populations (Fig. 5).

Results from compatibility tests, electrophoresis, as well as the observed ecological specialization support the segregation of the European *Fagus* type as a new subspecies that is proposed:

Peniophora cinerea (Fr.) Cooke ssp. ***fagicola*** n.ssp.

Peniophorae cinereae ssp. *cinereae* simillima, sed differt hoc, quod ligno Fagi decorticato habitat.

HOLOTYPUS: Denmark, Jylland/ on decorticated branch of *Fagus*/ 1987-08-21/ N. Hallenberg 10343 (GB 1910).

Discussion

There has been much discussion concerning evolution among fungi, whether allopatric or sympatric speciation is the general mode (Brasier 1987; Burnett 1983; Hallenberg 1991*a*; Vilgalys 1992). Several examples exist where genetic differentiation due to allopatric isolation has been proposed (Boidin and Lanquetin 1983, 1984, 1987; Hallenberg 1986, 1991*a*, 1991*b*; Vilgalys 1992; Vilgalys and Johnson 1987). Morpho-

logical differences and reduced genic identity have been observed between populations from America and Europe.

On the other hand, there are several indications of sympatric speciation in some species complexes. For example, sibling species occurring in the same or adjacent areas may differ in their substrate selectivity (Hallenberg 1986, 1991*a*; Nakasone and Micales 1988). In some cases both interincompatible sibling species may be compatible with representatives from another continent (Boidin and Lanquetin 1984; Hallenberg 1991*a*). In *Heterobasidion annosum* (Fr.) Bref. and *Bulbillomyces farinosus* (Bres.) Jül. it has been shown that intraspecific intersterility may occur, and this intersterility seems to be regulated by simple genetic determinants (Chase and Ullrich 1990*a*, 1990*b*; Hallenberg 1988). Among European populations of *H. annosum*, intersterility has been linked with ecological adaptations. These results support the hypothesis that sympatric speciation may occur.

In this study, we present evidence that intersterility between two sibling species of *P. cinerea* may be regulated by simple genetic determinants. *Peniophora cinerea* ssp. *fagicola* is ecologically segregated from other populations over the northern hemisphere, but intersterility reactions with ssp. *cinerea* are geographically restricted to Europe and the observed intersterility is not complete. An explanation could be connected with a possible intimate relationship with an insect in dispersal of the fungus. It is also shown that intersterility may occur between specimens not having any connection to decorticated *Fagus* or any insect relationship. At present, there is no evidence that this intersterility is the result of speciation events.

A certain differentiation between allopatric populations has been observed in this study. Specimens from Ontario and Quebec all have sulfocystidia, they are completely intercompatible, and one collection, GB 2230, is intersterile with almost all other specimens tested here. The specimen from Vancouver Island differs from the other Canadian specimens by the above-mentioned intersterility and by the lack of sulfocystidia. With exception of GB 2330, all five Canadian specimens had a high degree of intercompatibility with both sibling species in Europe (see also Hallenberg 1986). Such a high degree of intercontinental compatibility was also shown by Chamuris (1992). The isolated position of the Taiwanese specimens is indicated by a slightly reduced compatibility with Canadian and European specimens. From the banding patterns in electrophoresis, however, it follows that the main evolutionary event observed hitherto in *P. cinerea* is the segregation of *P. cinerea* ssp. *fagicola*.

Returning to the question on allopatric versus sympatric speciation, it must be concluded that in principle both modes are possible. A common interpretation of allopatry in fungi, however, has been that populations are occurring on different continents, while sympatry means occurrence on the same continent. This interpretation assumes that basidiomycetes in general are organisms with the capacity for effective long-distance dispersal. Adaptations for long-distance dispersal, however, are not the same as possibilities for establishment of long-distance dispersed spores under normal circumstances. Hallenberg (1991) strongly questioned that long-distance dispersal of a basidiomycete between similar habitats in real life could result in establishment of mycelia. From this it also follows that speciation that takes place on one continent is not necessarily sympatric but may be allopatric or parapatric. In fact, the assumption made here, i.e., that the supposed speciation event in Europe for *P. cinerea* appears to be sympatric, is based mainly on observations that intersterility between the two subspecies is most obvious in intergroup pairing among European isolates. It is possible that the establishment of a new divergent lineage adapted for growth on decorticated *Fagus* branches took place in a local area where *P. cinerea* was absent earlier. The difference between allopatry and sympatry will then be strongly reduced compared with the interpretation above. It is supposed, however, that intersterility was developed to obstruct genetic exchange between ecologically different lineages that were located close enough to make this isolation advantageous.

Very few examples exist where geographical isolation is the causal agent for intersterility and morphological differentiation among species in Corticiaceae, at least over the northern hemisphere. Saprophytic fungi living on dead plant tissues have remained unchanged for a long time, probably depending on a constant selective pressure. The actual wide distribution found among basidiomycetes could depend on a contemporary distribution with forests to which specific fungal communities are closely connected, at least in boreal and temperate areas over the northern hemisphere. The existence of a sibling species complex such as *P. cinerea* could be looked upon as an effect of a propagation strategy for a species. Simple intersterility barriers that are linked to ecological adaptations with occupation of new niches may enhance the possibility for survival of the species. A similar propagation strategy is found in several species complexes in Corticiaceae, where homothallic forms exist in parallel with heterothallic ones. In some species complexes found in Corticiaceae, such as *Phlebia subochracea* (Bres.) Erikss. & Ryv., the two sibling species are both widely distributed in temperate areas but consistently intersterile. The great similarity between the two sibling species exemplifies the conservative character of fruit body morphology, while there are slight differences in culture characters (Hallenberg and Larsson 1991). Obviously, these sibling species are less genetically related than those in *P. cinerea*. The practical delimitation of species in general makes it necessary to use structural and ecological criteria. Pairing tests are excellent instruments for locating the limits between closely related taxa in heterothallic organisms, but the splitting of a complex into two species cannot be based exclusively on pairing tests.

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