Incompatibility Factors and Mating Competence of Two *Laccaria* spp. (Agaricales) Associated with Black Spruce in Northern Minnesota

R. L. Doudrick and N. A. Anderson

Department of Forest Resources and Department of Plant Pathology, respectively, University of Minnesota, St. Paul 55108.
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ABSTRACT

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Sporocarps of *Laccaria* were collected in conjunction with a survey of potential ectomycorrhizal fungi associated with black spruce in northern Minnesota. Homokaryons were grown from single spores, and dikaryons were isolated from sporocarp tissue and from surface-disinfested ectomycorrhizae. Pairings of sibling homokaryons indicated a bifactorial (tetrapolar) sexual incompatibility system for all sporocarps. In only six of 37 cases were 15–22 homokaryons insufficient to find the four mating-type factors from a single sporocarp. All pairings between homokaryons obtained from sporocarps collected on peatlands with those from mineral soils were negative. Sporocarps collected in black spruce stands on peatlands were members of a freely interbreeding population, *Laccaria laccata* var. *moelleri*. Sporocarps collected on mineral soils represented several populations of *Laccaria bicolor*, with reduced outbreeding efficiency. The reduced outbreeding efficiency in *L. bicolor* (evident as a decrease in the number of clamp-connections produced in pairings of nonsibling sexually compatible homokaryons) apparently is due to a heterogenic incompatibility system, superimposed on the sexual incompatibility system. Preliminary evidence suggested a possible relationship between site-related factors for collections of *L. bicolor* identified by the survey and their mating competence. Di-mon pairings of *L. l. moelleri* and *L . bicolor* homokaryons with dikaryons isolated from black spruce ectomycorrhizae synthesized in aseptic culture indicated that precise genetic identification of the dikaryotic strains of both species was possible when known A and B mating-type factors were used as markers.

Site preference has been noted for several potential ectomycorrhizal fungi associated with black spruce (*Picea mariana* (Mill.) B.S.P.) on peatlands and mineral soils in northern Minnesota (4). Because a succession of ectomycorrhizal associations occurs during the maturation of a stand (2), those fungi present earliest in stand succession would be expected to be important in seedling establishment. Ectomycorrhizae produced on seedlings by early-stage fungi continue to develop when the seedlings are transplanted into nonsterile soil, whereas those ectomycorrhizae produced by late-stage fungi fail to persist (14). In Minnesota, sporocarps of *Laccaria* spp. are found in black spruce stands of various origins, ages, and qualities. They are commonly the most abundant and frequently the only sporocarps evident in the youngest stands (4). Ectomycorrhizae have been produced in aseptic culture with black spruce seedlings by several isolates of *Laccaria* collected from stands less than 20 yr old, but some collected from older stands with closed canopies failed to produce ectomycorrhizae (4).

A better understanding of the adaptive traits associated with certain sites would aid in identifying strains of ectomycorrhizal fungi that might improve the survival and early growth of seedlings planted in the field. Evaluation of ecological relationships often begins with genetic analyses of population composition and dynamics. The mating system, i.e., the number of alleles and loci involved, and population size and interfertility in the fungus species can be determined by the pairing of homokaryotic mating-type cultures. Until now, however, these techniques have not been applied in the study of ectomycorrhizae, and this neglect consequently has perpetuated problems with seedling survival and growth in planting studies.

The genus *Laccaria* in Minnesota was chosen as a model for a study of the sexual incompatibility systems of ectomycorrhizal fungi because: isolates of *Laccaria* that were believed to represent early-stage fungi that would presumably be candidates for planting studies had been identified; isolates from a wide variety of sites were available; ectomycorrhizae had been produced by several isolates in aseptic culture with black spruce seedlings; and studies elsewhere have shown that strains of *Laccaria* spp. had the potential to be manipulated genetically (6–9). The objective of this study was to determine the species limits and the genetic control of the mating systems in populations of *Laccaria* associated with black spruce in northern Minnesota.

MATERIALS AND METHODS

Sporocarps of *Laccaria* spp. were collected 4 August to 9 September 1986 from a variety of stands in conjunction with a survey of potential ectomycorrhizal fungi associated with black spruce in northern Minnesota (4). A total of 51 sporocarps, three each from 17 1-m-diameter circular sample locations, were collected in 12 stands. Each sporocarp was labeled by date collected, stand, and sample location. Dried sporocarp voucher specimens and notes on the ecology, macro- and micromorphology for each collection were submitted to the Plant Pathology herbarium, University of Minnesota, for preservation and future reference.

Homokaryotic cultures were obtained from single spores germinated on nutrient agar medium. Autoclaved filter paper with a 1-cm-diameter disk removed from the center was moistened and placed in the lid of a petri plate containing modified Melin-Norkrans' agar medium (MMN) (12). Pieces of gill tissue from fresh sporocarps were positioned in petroleum jelly on the petri plate lid and stored in the dark at 20 C. Spores were cast and collected at 24-hr periods for 3 days. Some spores were stored on MMN for up to 4 wk in the dark at 3 C. In preparation for germination, spores were spread across the medium with a sterile glass-rod, and one-half of the agar surface of each plate was covered to a density of approximately 10% with autoclaved, activated charcoal (Sigma Chemical Company, St. Louis, MO). For germination, plates were maintained at 20 C in the dark

and checked every other day for germinating spores for up to 6 wk. Single germinating spores were selected under a binocular dissecting microscope at ×25 and transferred to MMN. Single-spore cultures were grown at 20 C in the dark for at least 3 wk. These cultures were stored at 3 C on MMN slants and also as mycelial pads submerged in sterile water. An attempt was made to obtain at least 16 homokaryotic sibling cultures from each sporocarp.

Dikaryotic strains of *Laccaria* spp. also were cultured. Tissue samples were excised from stipe apices of fresh sporocarps and isolated on malt extract agar medium variously amended or supplemented. The pH of the medium was adjusted to 4.0–4.5 with 1.0 and 0.1 N hydrochloric acid before autoclaving, or with 25% lactic acid after autoclaving. Ten milliliters per liter of 1.0% streptomycin sulfate and/or 10 ml/L of 0.5% penicillin-G were added after autoclaving. Dikaryons were maintained and stored as described for homokaryotic cultures.

Laboratory-synthesized ectomycorrhizae were produced on black spruce seedlings by wild dikaryotic strains of *Laccaria* spp. by methods similar to those of Molina and Palmer (16). Excised ectomycorrhizae were plated on amended malt extract medium, and the fungus symbionts were hyphal-tipped and transferred to MMN.

A medium promoting nuclear migration (5) was adjusted further for the current studies by increasing the potassium phosphate concentration to 1.0 g/L, and reducing sucrose to 2.0 g/L. All other ingredients and concentrations were the same for this medium and MMN. The pH of the migration medium before autoclaving was 6.2, compared with 5.5–5.7 for MMN. Adjustments in concentrations and pH helped to minimize the effects of pH shifts observed in the medium as a result of pairings. The newest medium further intensified macroscopic reactions and facilitated interpretation of pairings. As a precaution, pairings always were evaluated microscopically to confirm macroscopic signs.

To determine the genetic control of mating systems, the following pairings were made. Within a sample location, all possible pairings were made of at least 15 homokaryotic siblings from one sporocarp. Mating-type reference cultures representing each of the four mating-types were selected based on the results of these pairings. To determine the number of mating-type factors, species and population limits, and mating competence, all possible pairings were made among the four mating-type reference cultures from the sample locations. Mating-type factors for other sporocarps collected from a sample location were estimated by pairing at least 15 homokaryotic siblings with the sample location mating-type reference cultures. When the second and/or third sporocarps were found to have arisen from different dikaryons than the reference cultures, then all possible pairings also were made of at least 15 sibling homokaryons from these other sporocarps.

RESULTS

Homokaryotic cultures. Sixteen to 22 single-spore cultures were obtained from each of 37 sporocarps from 14 sample locations (Fig. 1). Sibling pairings indicated that all single-spore cultures were homokaryotic. Adequate numbers of single-spore cultures were not obtained from 14 sporocarps from four sample locations.

Pairings among homokaryotic siblings. Sibling pairings of homokaryons readily indicated a bifactorial (tetrapolar) sexual incompatibility system for each sporocarp. Compatible pairings were identifiable by the presence of clamp-connections and by various macroscopic changes in color, morphology, and growth rate of the dikaryotized hyphae. Common A pairings also produced changes in morphology, evident as localized proliferations of short, distorted hyphae, termed witches'-brooms (Figs. 2 and 3). False clamps were infrequently found in pairings of common B homokaryons, but an interaction zone ridge of tangled hyphae commonly was produced.

Pairings among mating-type reference cultures. Pairings among mating-type reference cultures from eight sporocarps from the peatlands and six sporocarps from mineral soils were all negative,

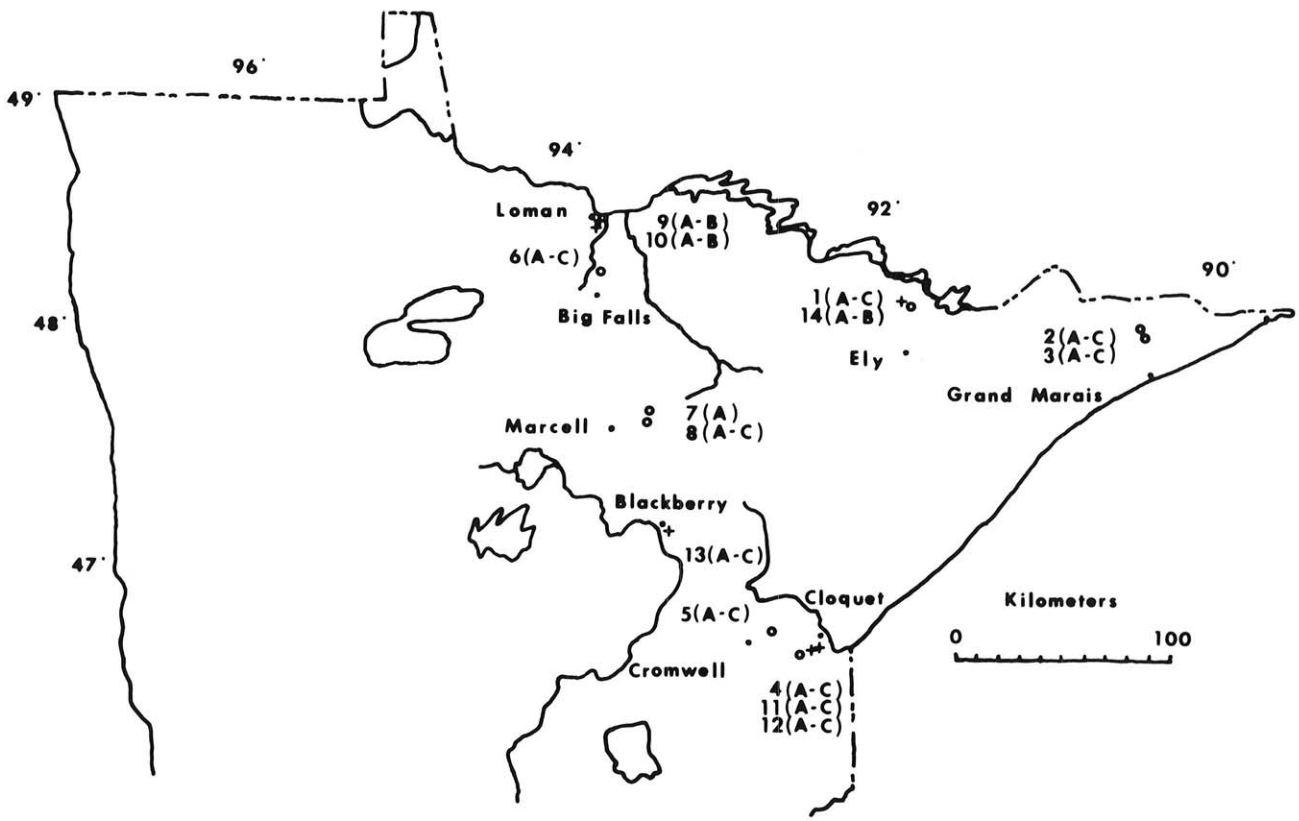
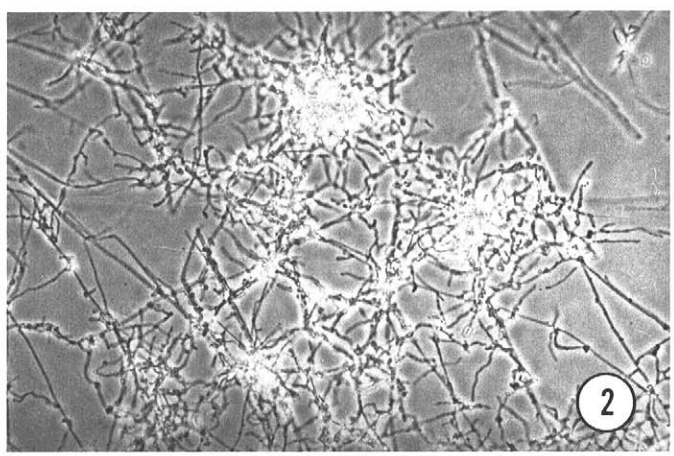


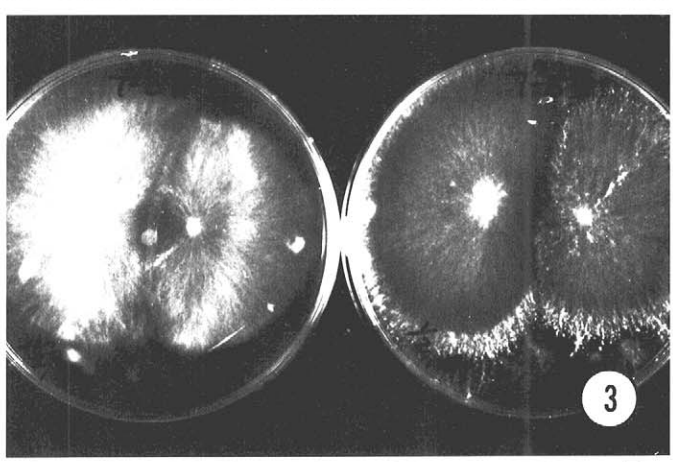
Fig. 1. Location and number of sporocarps of *Laccaria* spp. collected in conjunction with a survey conducted in 1986 of potential ectomycorrhizal fungi associated with black spruce (*Picea mariana*) in northern Minnesota. Nineteen to 22 homokaryons were obtained from each sporocarp. Numbers = different sample locations; A, B, and C = different sporocarps collected at a sample location; o = peatland collection; + = mineral soil collection.

TABLE 1. Results of pairing the four mating-type reference cultures obtained from eight sporocarps of *Laccaria* spp. collected in association with black spruce (*Picea mariana*) on peatlands in northern Minnesota and six sporocarps from mineral soils[a]

Sample location[b]		Reaction												
Peatlands	1A	+												
Peatlands	2B	+	+											
Peatlands	3C	+	+	+										
Peatlands	4B	+	+	+	+									
Peatlands	5C	+	+	+	+	+								
Peatlands	6A	+	+	+	+	+	+							
Peatlands	7A	+	+	+	+	+	+	+						
Peatlands	8A	+	+	+	+	+	+	+	+					
Mineral soils	9A	−	−	−	−	−	−	−	−	+				
Mineral soils	10B	−	−	−	−	−	−	−	−	+	+			
Mineral soils	11A	−	−	−	−	−	−	−	−	+	+	+		
Mineral soils	12C	−	−	−	−	−	−	−	−	+	+	+	+	
Mineral soils	13C	−	−	−	−	−	−	−	−	+	+	+	+	+
Mineral soils	14A[c]	−	−	−	−	−	−	−	−	−	−	−	−	−
		1A	2B	3C	4B	5C	6A	7A	8A	9A	10B	11A	12C	13C	14A

[a]+ = depending on the number of repeated A and B factors, various combinations of the 16 pairings between the mating-type reference cultures of the two sporocarps being tested were compatible; − = none of the 16 pairings between the mating-type reference cultures of the two sporocarps being tested were compatible.
[b]The origin of each sporocarp is presented in Figure 1.
[c]Sample location 14 was in the only mature, natural, boreal conifer stand sampled. All other sample locations on mineral soils were in plantations.





Figs. 2 and 3. 2, Witches' brooming observed in common A factor pairings of *Laccaria laccata* var. *moelleri* (×262). **3,** Comparison of compatible pairing (left) of *Laccaria bicolor* and common A factor pairing (right).

TABLE 2. Mating-type factors identified by pairings of 32 mating-type reference cultures obtained from eight sporocarps of *Laccaria laccata* var. *moelleri* collected in association with black spruce (*Picea mariana*) on peatlands in northern Minnesota and 20 mating-type reference cultures from five sporocarps of *L. bicolor* collected on mineral soils[a]

Sporocarp number[b]	Mating-type factors	Total mating-type factors A	Total mating-type factors B
Laccaria laccata var. *moelleri*			
1A	A(1,2) B(1,2)		
2B	A(1,3) B(3,4)		
3C	A(4,5) B(1,5)		
4B	A(6,7) B(6,7)		
5C	A(3,8) B(5,8)		
6A	A(5,9) B(9,10)		
7A	A(1,10) B(5,9)		
8A	A(6,7) B(3,11)		
		10	11
Laccaria bicolor			
9A	A(1,2) B(1,2)		
10B	A(3,4) B(2,3)		
11A	A(5,6) B(4,5)		
12C	A(7,8) B(6,7)		
13C	A(9,10) B(1,8)		
		10	8

[a]Because it was unclear whether between sample location total incompatibility was an extreme expression of heterogenic compatibility or whether other factors were involved, the mating-type reference cultures for 14A, identified as *L. bicolor*, collected from a mature, natural boreal conifer stand in northeastern Minnesota were excluded from calculations of mating-type factors of *L. bicolor*.
[b]The origin of each sporocarp is presented in Figure 1.

indicating that collections from peatlands and mineral soils were distinct, noninterbreeding species (Table 1). Collections of *Laccaria* from peatlands were identified as *L. laccata* var. *moelleri* Singer and those from mineral soils as *L. bicolor* (Maire) Orton.

The entire collection of *L. l. moelleri* appeared to be a single freely interbreeding population (Table 1). Sixteen A and B mating-type factors could have been identified by all possible pairings among mating-type reference cultures (Table 2). Ten A and 11 B factors were identified by the pairings, four A factors occurred twice and one occurred three times, and three B factors were repeated twice and one B factor three times. Wright's formula (3) can be used to predict the number of mating-type factors from finite samples. By applying Wright's formula, we predict 18 A and 20 B factors. Outbreeding efficiency for collections of *L. l. moelleri* was estimated at 81%.

In contrast, collections of *L. bicolor* did not appear to be freely interbreeding, and results of pairings suggested that a number of subpopulations may exist (Table 1). As seen in Table 1, the mating-type reference cultures for the 14A collection were not dikaryotized by any other collections of *L. bicolor*, although the remainder were interbreeding. The '14' collections were from the only mature natural boreal conifer stand sampled in conjunction

with the survey; all other upland, mineral soil collections were from younger seedling plantation stands.

The dikaryotization efficiency of homokaryotic sibling pairings was greater than nonsibling pairings from different sample locations. Macroscopic signs of dikaryotization seldom were observed in what should have been fully compatible pairings, and, although no quantitative estimate of the reduction in mating competence was possible, clamp-connections were produced less frequently. (Because it was unclear whether between sample location total incompatibility was an extreme expression of heterogenic incompatibility or whether other factors were involved, the mating-type reference cultures for 14A, collected from a mature, natural boreal conifer stand in northeastern Minnesota were excluded from calculations of mating-type factors for collections of *L. bicolor*). Ten A and B mating-type factors could have been identified by all possible pairings of mating-type reference cultures (Table 2). None of the A factors were repeated in the collections, but two B factors were repeated once, so all 10 possible A and eight B factors were identified by these pairings. Wright's (3) formula predicts 23 B factors possible and an infinite number of A factors, because no repeated A factors were detected. The outbreeding efficiency for the collections of *L. bicolor* were estimated at 88% if the sexual incompatibility system alone was considered, but such a figure does not consider that mating competence apparently had been reduced dramatically by heterogenic incompatibility.

Pairings within sample locations. Dikaryotization occurred readily in compatible pairings among mating-type reference cultures and homokaryons obtained from sporocarps collected within the same sample location for both *L. l. moelleri* and *L. bicolor* (Tables 3 and 4).

The four mating-type cultures were obtained from all but two of 22 sporocarps of *L. l. moelleri*. New A and B factors were found at five sample locations, indicating these sporocarps arose from different dikaryons, whereas sporocarps with the same mating-type factors as those of the reference cultures were collected at four sample locations; a sporocarp with a single A and B factor in common with mating-type reference cultures was collected at another sample location. The four mating-type factors were obtained for 11 of 15 sporocarps of *L. bicolor*. From three sporocarps, three mating-type cultures were obtained and, from a fourth, only two mating-type cultures were recovered even after 20 homokaryotic cultures were tested. New A and B factors representing different dikaryons were found in two sample locations, whereas sporocarps with the same mating-type factors as the reference cultures were found at three more, and a sporocarp with a single A and B factor in common with mating-type reference cultures was detected at another sample location.

Di-mon pairings. The mating-type factors of dikaryotic cultures of *L. bicolor* and *L. l. moelleri* were determined by pairings with homokaryons of known mating-type. Patterns of Buller phenomenon dikaryotization were compared for synthesized and wild dikaryotic cultures of both species (Table 5).

DISCUSSION

Percentage and rate at which spore germination occurred was highly variable for both species. The relative rates of spore germination were slower for some collections, and nearly one-fourth of the sporocarps collected were deleted from the study because sufficient numbers of homokaryotic cultures could not be obtained. Conversely, germination began prematurely for six collections of cast spores while they were still in temporary storage. There was no obvious relationship between capacity or rate of spore germination and fungus species or characteristics of the black spruce stand from which a sporocarp was collected. Other studies have suggested that fungal co-cultures are required to induce spore germination in *Laccaria* (6–8). In this study, co-cultures were not beneficial, and, where cultures of *Laccaria* spp. were tested, co-cultures overgrew spores before sufficient germination occurred. Previous studies also indicated that a tree root factor may stimulate spore-germination (15), but this possibility was not explored.

Extensive studies on inbred strains of *Schizophyllum commune* provide direction in determining genetic controls of Hymenomycete mating systems (19,20). Although homokaryon collections were incomplete in previous studies of *Laccaria*, a bifactorial (tetrapolar) sexual incompatibility system was predicted for

TABLE 3. List of A and B mating-type factors identified by pairings among mating-type reference cultures of *Laccaria laccata* var. *moelleri* obtained from eight sporocarps collected in association with black spruce (*Picea mariana*) on peatlands in northern Minnesota and homokaryons of other sporocarps collected at the same sample locations

Sporocarp number[a]		Mating-type factors[b]
1A	Reference cultures	A(1,2) B(1,2)
1B		A(+,+) B(+,+)
1C		A(1,+) B(+,2)
2A		A(+,+) B(+,+)
2B	Reference cultures	A(1,3) B(3,4)
2C		A(1,3) B(3,4)
3A		A(4,5) B(1,5)
3B		A(4,5) B(1,5)
3C	Reference cultures	A(4,5) B(1,5)
4A		A(+,+) B(+,+)
4B	Reference cultures	A(6,7) B(6,7)
4C		A(6,7) B(6,7)
5A		A(3,8) B(5,8)
5B		A(3,8) B(5,8)
5C	Reference cultures	A(3,8) B(5,8)
6A	Reference cultures	A(5,9) B(9,10)
6B		A(+,+) B(+,+)
6C		A(+,+) B(+,+)
7A	Reference cultures	A(1,10) B(5,9)
8A	Reference cultures	A(6,7) B(3,11)
8B		A(+,+) B(+,+)
8C		A(+,+) B(+,+)

[a]The origin of each sporocarp is presented in Figure 1.
[b](+,+) = sporocarp arose from different dikaryon with two different A and two different B factors than the mating-type reference cultures; (#,+) or (+,#) = one factor in common with mating-type reference cultures.

TABLE 4. List of A and B mating-type factors identified by pairings among mating-type reference cultures of *Laccaria bicolor* obtained from five sporocarps collected in association with black spruce (*Picea mariana*) plantations on mineral soils in northern Minnesota and homokaryons of other sporocarps collected at the same sample locations

Sporocarp number[a]		Mating-type factors[b]
9A	Reference cultures	A(1,2) B(1,2)
9B		A(+,0) B(+,+)
10A		A(+,+) B(+,+)
10B	Reference cultures	A(3,4) B(2,3)
11A	Reference cultures	A(5,6) B(4,5)
11B		A(5,6) B(4,5)
11C		A(+,6) B(+,5)
12A		A(7,8) B(6,7)
12B		A(7,8) B(6,7)
12C	Reference cultures	A(7,8) B(6,7)
13A		A(+,+) B(+,+)
13B		A(9,10) B(1,8)
13C	Reference cultures	A(9,10) B(1,8)

[a]The origin of each sporocarp is presented in Figure 1.
[b](+,+) = sporocarp arose from different dikaryon with two different A and two different B factors than the mating-type reference cultures; (+,#) = one factor in common with mating-type reference cultures; (+,0) = one of two factors different than the mating-type reference cultures and the other factor was not obtained.

several species (9). This study showed conclusively the bifactorial nature of the incompatibility systems for *Laccaria* spp. associated with Minnesota black spruce, and confirmed taxonomic work defining species of *Laccaria* (17). *L. l. moelleri* was collected only from peatlands and *L. bicolor* only from mineral soils.

L. l. moelleri was a freely interbreeding population on peatlands, whereas collections on mineral soils appeared to represent at least two distinct subunits of *L. bicolor*. Although pairings of compatible homokaryons of *L. bicolor* within a sample location were dikaryotized readily, formation of the dikaryon resulting from paired homokaryons from different sample locations was greatly reduced. This reduced efficiency of dikaryotization, apparently due to heterogenic incompatibility, was observed not only in pairings among homokaryons from sporocarps collected from different stands, but also in pairings among homokaryons from sporocarps collected from different sample locations within the same stand and from within essentially the same sample location when collections were separated by time (Table 6 and Fig. 4). Dikaryotization of paired, fully compatible homokaryons of *L. l. moelleri* from different sample locations were not affected by heterogenic incompatibility, and clamp connections were formed readily.

A site preference relationship has been observed for several fungus species associated with northern Minnesota black spruce (4). The collections of *L. l. moelleri* apparently represent a sample of a panmictic population adapted to peatlands. These peatlands were relatively homogenous in floristics, topography, water movement, and water chemistry (4,10,11,21). Conversely, there was little similarity among the mineral soil sites from which *L. bicolor* was collected. Site factors would demand that organisms intimately associated with an environment evolve to fill specific and distinctly different niches, thus, creating a number of populations or subpopulations with site specificities within a species. This appears to be the case for *L. bicolor*. Within limits of sample location and time, outbreeding efficiency was still high, but, beyond those limits, mating competence apparently was restricted by heterogenic incompatibility. This seems to reflect the diversity within a stand on a mineral soil site.

Intersterility between several species of *Laccaria* and within *L. laccata* has been described (9). The northeastern Minnesota boreal conifer stand collection of *L. bicolor*, 14A, was incompatible with collections of *L. bicolor* from northcentral areas of the state. This collection may be a population of *L. bicolor* that has become isolated, and, may represent an extreme example of heterogenic incompatibility or an intersterile group as defined by Chase and Ullrich (1). More extensive studies are needed to correctly address all of these population-related issues.

Various nuclear exchange interactions have been observed in compatible di-mon pairings; however, dikaryotization of the homokaryotic mycelium is the predominant pattern (18). In preliminary tests, mating-type factors of dikaryotic strains of *Laccaria* spp. obtained from sporocarps or laboratory-synthesized

TABLE 5. Results of di-mon pairings among homokaryotic mating-type reference cultures and synthesized and wild dikaryons of *Laccaria bicolor* and *L. laccata* var. *moelleri* obtained from sporocarps collected in association with black spruce (*Picea mariana*) in northern Minnesota[a]

	L. bicolor 9A Reference cultures A(1,2) B(1,2) Synthesized dikaryons		
	(A1B1 + A2B2)	(A1B2 + A2B1)	Wild dikaryon
A1B1	+[b]	−	+
A1B2	−	+	−
A2B1	−	+	−
A2B2	+	−	+
			(A1B1 + A2B2)

	L. moelleri 2B Reference cultures A(1,3) B(3,4) Synthesized dikaryons		
	(A1B3 + A3B4)	A1B4 + A3B3)	Wild dikaryon
A1B3	+	−	−
A1B4	−	+	+
A3B3	−	+	+
A3B4	+	−	−
			(A1B4 + A3B3)

[a]The origin of each sporocarp is presented in Figure 1.
[b]+ = dikaryotization of the homokaryon based on presence of clamp-connections; − = homokaryon was not dikaryotized.

TABLE 6. Sexual incompatibility reactions of the four mating-type reference cultures from five sporocarps of *Laccaria bicolor* collected in association with black spruce (*Picea mariana*) plantations on mineral soils in northern Minnesota[a]

		9A[b]				10B				11A				12C				13C			
		A1 B1	A1 B2	A2 B1	A2 B2	A3 B2	A3 B3	A4 B2	A4 B3	A5 B4	A5 B5	A6 B4	A6 B5	A7 B6	A7 B7	A8 B6	A8 B7	A9 B1	A9 B8	A10 B1	A10 B8
9A	A1B1	−	A	B	+	(+)	+	+	+	+	+	+	+	+	+	+	+	B	+	B	+
	A1B2	A	−	+	B	B	(+)	B	+	+	+	+	+	+	+	+	(+)	+	+	+	+
	A2B1	B	+	−	A	+	+	+	+	+	+	+	+	+	+	+	+	B	+	B	+
	A2B2	+	B	A	−	B	(+)	B	+	+	+	+	+	+	+	+	+	+	+	+	+
10B	A3B2					−	A	B	+	+	+	(+)	+	+	+	+	+	+	+	(+)	(+)
	A3B3					A	−	+	B	+	+	(+)	+	+	+	+	+	+	+	+	+
	A4B2					B	+	−	A	+	(+)	+	(+)	+	+	+	+	(+)	+	+	+
	A4B3					+	B	A	−	+	+	+	+	+	+	+	+	+	+	+	+
11A	A5B4									−	A	B	+	+	+	+	+	+	+	+	+
	A5B5									A	−	+	B	+	+	(+)	+	+	+	+	+
	A6B4									B	+	−	A	(+)	+	(+)	+	+	+	+	+
	A6B5									+	B	A	−	+	+	(+)	+	+	+	+	+
12C	A7B6													−	A	B	+	(+)	(+)	+	+
	A7B7													A	−	+	B	(+)	+	+	+
	A8B6													B	+	−	A	+	+	+	(+)
	A8B7													+	B	A	−	+	+	+	(+)
13C	A9B1																	−	A	B	+
	A9B8																	A	−	+	B
	A10B1																	B	+	−	A
	A10B8																	+	B	A	−

[a]The origin of each sporocarp is presented in Figure 1.
[b]+ = dikaryotization of one or both homokaryons; A = common A mating-type factors; B = common B mating-type factors; − = common A and B mating-type factors; (+) = no clamp-connections found in pairing, the reaction was predicted to be compatible based on the results of other pairings.

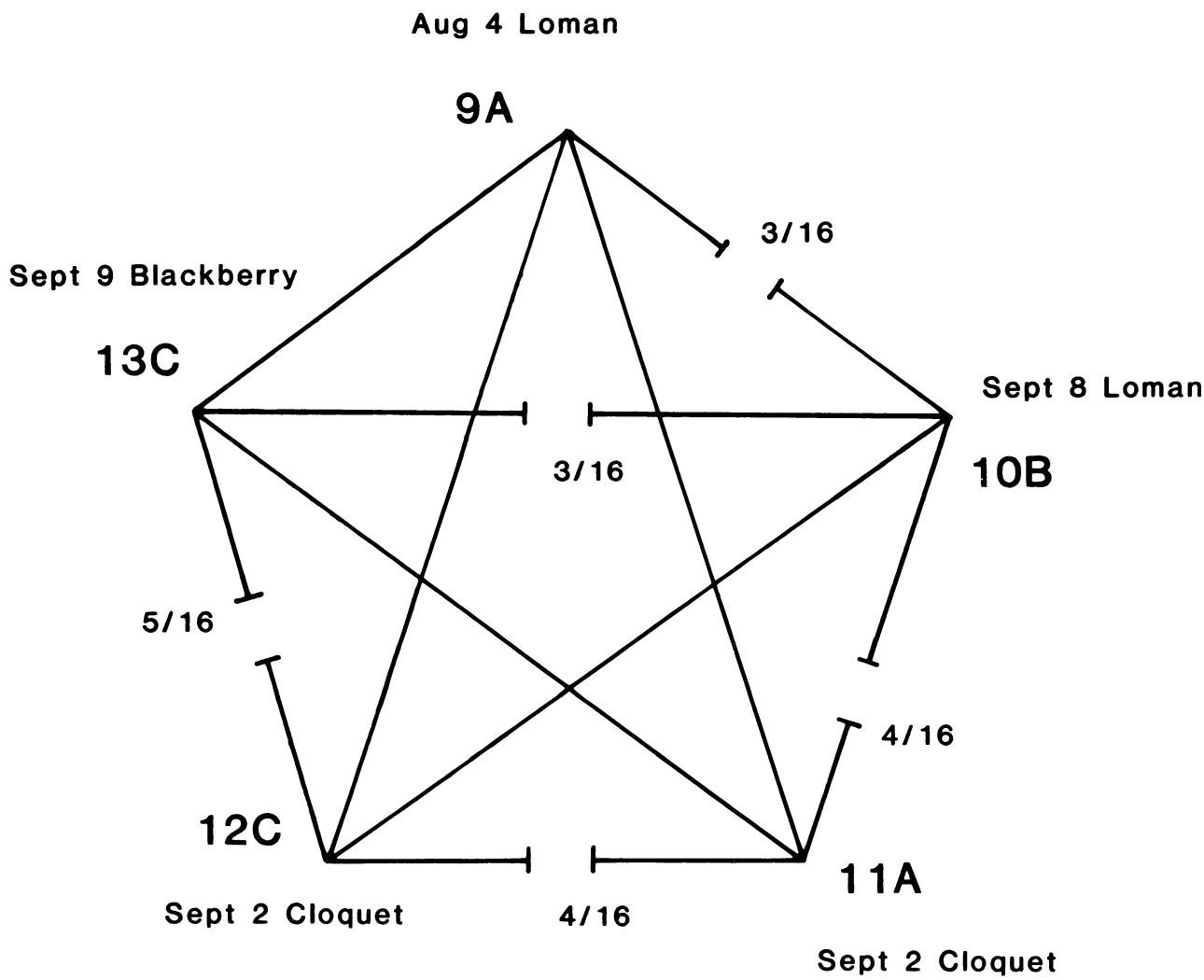


Fig. 4. Sexual and heterogenic incompatibility in pairings of the four mating-type reference cultures obtained from five sporocarps of *Laccaria bicolor* collected in 1986 on the dates indicated in association with black spruce (*Picea mariana*) plantations on mineral soils in northern Minnesota. The number of pairings in which clamp-connections were not found and predicted to be compatible based on sexual incompatibility factors, is expressed as a fraction of the total number of pairings. The origin of each sporocarp is presented in Figure 1.

ectomycorrhizae tentatively were identified by pairings with a library of homokaryons whose A and B mating-type factors were known. Formation of clamp-connections on peripheral hyphae of homokaryotic reference cultures was used to determine the A and B factors of the dikaryon. By using these genetic markers, it is possible to test at any time for the presence or absence of the original strain used to inoculate seedlings planted in the field. The goal of future studies will be to further refine techniques to identify and confirm the identity over time of the mating-type factors of dikaryotic strains of *Laccaria* spp. used in mycorrhizal inoculation studies.

These studies were in conjunction with a survey of potential ectomycorrhizal fungi of black spruce in northern Minnesota (4). The purpose was to identify fungi that might improve survival and early growth of black spruce seedlings planted in northern Minnesota. Survey data and mating-type factor analyses of *Laccaria* spp. have provided a unique opportunity to examine possible relationships of ectomycorrhizal fungi and site-related factors. Results of these studies indicate that the population of *L. bicolor* associated with black spruce on mineral soil sites is composed of several subpopulations that are not freely interbreeding. Studies with *Amanita muscaria* and *Betula pendula* indicated that fungus and host genotypes affect the morphology and physiology of ectomycorrhizae (13). The above suggests that more careful consideration of site-related factors would help insure the success of seedling inoculations and plantings.

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