The Number and Distribution of Incompatibility Alleles in *Laccaria laccata* var. *moelleri* (Agaricales)

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ABSTRACT

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Sporocarps of the ectomycorrhizal fungus *Laccaria laccata* var. *moelleri* were collected in association with black spruce on peatlands in northern Minnesota and central Canada. Pairings between homokaryotic mating-type reference cultures obtained from the sporocarps indicated an absence of any heterogenic incompatibility barriers. The number of homogenic incompatibility alleles in the species was estimated to be 17 at the A locus and 18 at the B locus. These estimates are much lower than those reported for other basidiomycetes. Outbreeding efficiency was estimated to be 88.6%.

Ectomycorrhizae are beneficial to plant growth and can act as deterrents to feeder root infection by plant pathogens (11). The long-range goal of our research is to obtain basic information on the genetics of ectomycorrhizal fungi so that we might use them more effectively in forest tree nurseries and plantations.

Black spruce (Picea mariana (Mill.) B. S. P.) is naturally abundant on peatlands in Minnesota and much of Canada. A survey of ectomycorrhizal fungi on peatlands in northern Minnesota revealed that *Laccaria laccata* var. *moelleri* Singer commonly was associated with black spruce (Fig. 1., sites 1–8)(5). Our earlier work determined that *L. l. moelleri* possesses a bifactorial (tetrapolar) homogenic incompatibility system that promotes outcrossing. No evidence was found for any heterogenic incompatibility barriers among isolates of *L. l. moelleri* collected at sample locations in eight black spruce stands in northern Minnesota (4). This is in contrast to the related *L. bicolor* (Maire) Orton, an ectomycorrhizal associate of black spruce on mineral soils, in which extensive heterogenic incompatibility barriers divide the species into a number of intersterile incompatibility groups (4). These incompatibility groups may result from host and/or ecological specialization, a situation that could have important implications for those planning to use ectomycorrhizal fungi as part of reforestation efforts.

We have extended our previous work (4) by examining the mating system of *L. l. moelleri* over a larger portion of its natural range. Our first objective was to determine whether the lack of heterogenic incompatibility barriers was restricted to northern Minnesota or whether it extended over a much broader portion of the range of *L. l. moelleri* in North America. Our second objective was to determine the number and distribution of mating-type alleles at the two loci controlling the homogenic incompatibility system.

MATERIALS AND METHODS

Sporocarps of *L. l. moelleri* were collected from 16–25 August 1988 in stands of black spruce on peatlands in central Canada (Fig. 1, sites 11–19). At least one sporocarp from each of three 1-m-diameter sample locations was collected in nine stands. Dried sporocarp voucher specimens and notes on the location, ecology, macro-, and micromorphology for each collection (Minnesota sites 1–8 and Canada sites 11–19) are at the Mycological Herbarium, Department of Plant Biology, University of Minnesota, St. Paul.

Homokaryotic cultures were obtained from fresh sporocarps and stored as described previously (4). One pair of reference cultures with compatible mating-type alleles was identified by sibling pairings for one representative sporocarp for each of the nine sites. Reference cultures with known mating-types had been identified previously for collections of *L. l. moelleri* from eight stands of black spruce on peatlands in Minnesota (4) (Fig. 1).

One pair of compatible mating-type reference cultures for a single sporocarp from each of the eight Minnesota sites was used in this study.

To determine the number and distribution of incompatibility alleles, a total of 34 mating-type reference cultures from Minnesota and Canada were paired in all possible combinations on the migration medium developed for genetic studies of *Laccaria* (4). Pairings between compatible, common A and common B allele cultures were interpreted as described previously (4). The complementary nature of the bifactorial homogenic incompatibility system can be used in studies on allele frequencies to minimize the number of pairings required. In this study, using one pair of compatible mating-type reference cultures instead of both pairs from a sporocarp, we were able to reduce the number of pairings required by approximately 75%.

Dobzhansky and Wright (2) proposed a method of estimating the number of lethal alleles in an infinite population based on sample data from a finite population. Raper et al (14) applied this method to estimate the number of homogenic incompatibility alleles in the basidiomycete *Schizophyllum commune* Fr.:Fr. This estimate is obtained as the inverse of the observed proportion of incompatible pairings. These authors (14) also provided a formula for estimating outbreeding efficiency, a measure of the number of matings expected between compatible mating-types under panmixia. We have employed these methods in this study. The number of incompatible pairings at each locus is distributed as a binomial random variable. We used the normal approximation to the binomial to obtain 95% confidence limits on the proportion of incompatible pairings and inverted these limits to obtain 95% confidence limits on the estimated number of alleles at each incompatibility locus.

RESULTS

No heterogenic incompatibility barriers were detected among any of the collections of *L. l. moelleri* from Canada and Minnesota (Table 1). For the homogenic incompatibility system, if no alleles were repeated among the reference cultures, a theoretical maximum of 34 A and 34 B mating-type alleles could be identified by all possible pairings among mating-type reference cultures from 17 sporocarps. Fourteen A and 14 B alleles were actually identified by the pairings in this study. Three A alleles occurred only once, six occurred twice, two three times, two four times, and one five times. Three B alleles occurred once, five were repeated twice, three three times, and three four times. The mating-type allele combinations A4B7, A5B8, A9B9, and A9B13 each occurred twice among the sites sampled in this survey. These data yielded estimates of 17 A and 18 B alleles in *L. l. moelleri*. The 95% confidence limits for the numbers of alleles are 14 and 20 for the A locus and 15 and 21 for the B locus. Outbreeding efficiency under panmixia is estimated to be 88.6%.

DISCUSSION

The collections of *L. l. moelleri* we sampled represent a large interfertile group with limits yet to be determined. Further collections from the entire species range, including its European portion, will be needed if those limits are to be defined. *L. l. moelleri* is associated with black spruce on peatlands in Minnesota that have relatively homogeneous flora, topography, water movement, and water chemistry (3,7,8,16). This contrasts to the situation in the related *L. bicolor*. *L. bicolor* forms ectomycorrhizal associations with a number of tree species, including black spruce, jack pine (*Pinus banksiana* Lamb.), red pine (*Pinus resinosa* Aiton), and trembling aspen (*Populus tremuloides* Michx.), on a variety of nonpeatland sites in Minnesota (5, *unpublished data*). This diversity of host species and environments is accompanied by a large number of heterogenic incompatibility barriers that act to break the species into a number of intersterile incompatibility groups (4,6,10). Whether physiological specialization based on host or ecological factors occurs in *L. bicolor* and whether this leads to the development of heterogenic incompatibility

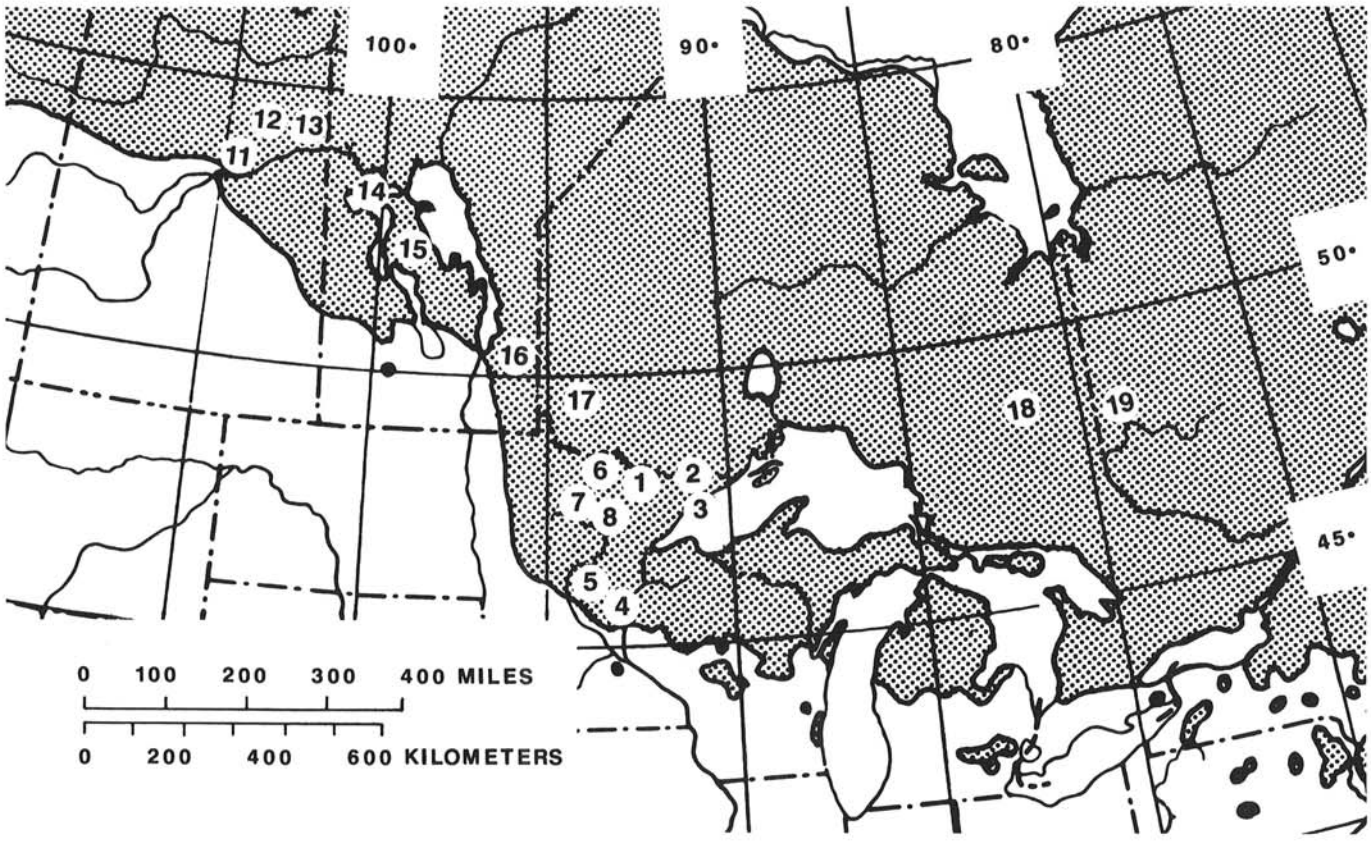

Fig. 1. Collection locations of sporocarps of *Laccaria laccata* var. *moelleri* found in association with black spruce (*Picea mariana*) on peatlands of northern Minnesota in 1986 and of central Canada in 1988. Two compatible mating-type reference cultures were obtained from each sporocarp. Numbers represent sample locations, Minnesota sites 1–8 and Canada sites 11–19. Shaded area is natural range of black spruce.

TABLE 1. Sexual incompatibility reactions of the mating-type reference cultures from seventeen sporocarps of *Laccaria laccata* var. *moelleri* collected in association with black spruce (*Picea mariana*) on peatlands in northern Minnesota and central Canada[a]

	Sporocarp number																	
	11		12		13		14		15		16		17		18		19	
	A11 B9	A4 B11	A4 B7	A12 B11	A13 B8	A7 B4	A1 B12	A14 B9	A9 B13	A11 B1	A2 B13	A14 B14	A9 B9	A4 B7	A9 B13	A1 B11	A5 B8	A13 B6
1[b] A1B1[c]	+[d]	+	+	+	+	+	A	+	+	B	+	+	+	+	+	A	+	+
A2B2	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
2 A1B3	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+
A3B4	+	+	+	+	+	B	+	+	+	+	+	+	+	+	+	+	+	+
3 A4B5	+	A	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+
A5B2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+
4 A6B6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B
A7B7	+	+	B	+	+	A	+	+	+	+	+	+	+	B	+	+	+	+
5 A3B5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
A8B7	+	+	B	+	+	+	+	+	+	+	+	+	+	B	+	+	+	+
6 A5B8	+	+	+	+	B	+	+	+	+	+	+	+	+	+	+	+	AB	+
A9B9	B	+	+	+	+	+	+	B	A	+	+	+	AB	+	A	+	+	+
7 A1B8	+	+	+	+	B	+	A	+	+	+	+	+	+	+	+	A	B	+
A10B5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8 A6B10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
A7B3	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
11 A11B9	AB	+	+	+	+	+	+	B	+	A	+	+	B	+	+	+	+	+
A4B11		AB	A	B	+	+	+	+	+	+	+	+	+	A	+	B	+	+
12 A4B7			AB	+	+	+	+	+	+	+	+	+	+	AB	+	+	+	+
A12B11				AB	+	+	+	+	+	+	+	+	+	+	+	B	+	+
13 A13B8					AB	+	+	+	+	+	+	+	+	+	+	+	B	A
A7B4						AB	+	+	+	+	+	+	+	+	+	+	+	+
14 A1B12							AB	+	+	+	+	+	+	+	+	A	+	+
A14B9								AB	+	+	+	A	B	+	+	+	+	+
15 A9B13									AB	+	B	+	A	+	AB	+	+	+
A11B1										AB	+	+	+	+	+	+	+	+
16 A2B13											AB	+	+	+	B	+	+	+
A14B14												AB	+	+	+	+	+	+
17 A9B9													AB	+	A	+	+	+
A4B7														AB	+	+	+	+
18 A9B13															AB	+	+	+
A1B11																AB	+	+
19 A5B8																	AB	+
A13B6																		AB

[a]The origin of each sporocarp is presented in Fig. 1, Minnesota sites 1–8 and Canada sites 11–19.
[b]The mating-type alleles for reference cultures of sporocarps 1–8 were identified previously (4).
[c]Inferred mating-type genotype of reference cultures.
[d]+ = Dikaryotization of one or both homokaryons; A = common A mating-type alleles; B = common B mating-type alleles; AB = common A and B mating-type alleles.

barriers that reinforce such specialization is not known. If this is the case, we may need to carefully match specific isolates with specific host tree species and sites in reforestation. In contrast, the relatively homogeneous environment encountered by *L. l. moelleri* may eliminate the need for the evolution of heterogenic incompatibility barriers and provide us with greater flexibility in choosing isolates to use in reforestation.

Based on a sample of eight sporocarps collected in Minnesota, we had previously estimated the number of incompatibility alleles in *L. l. moelleri* to be 18 at the A locus and 20 at the B locus (4). The current estimate from a collection of 17 sporocarps, including the eight previously sampled, is 17 at the A locus and 18 at the B locus. This is only a slight change in the estimate despite more than double the sample size and a great increase in the geographic area sampled. Such a small change would be expected for a population with a restricted number of alleles at incompatibility loci.

Estimates of the number of alleles at homogenic incompatibility loci have been reported for a number of basidiomycetes (12). In general, these estimates are higher than the 17 A and 18 B alleles reported in this study. For example, Raper et al (14) reported estimates of 339 A and 64 B alleles in *S. commune*. Few estimates are available for soil fungi, but our estimates are comparable with the 17 H alleles reported for *Thanatephorus cucumeris* (Frank) Donk (1).

With so few alleles present in the species, it is not surprising to find repeated alleles in the population, but it is interesting that several alleles were repeated only at sample locations separated by hundreds of kilometers. For example, allele A13 was detected only at the two most distant sample locations (sites 13 and 19). This could be due to the relatively small sample size covering a relatively large geographic area. More curious, however, is the fact that specific combinations of alleles were duplicated in reference cultures collected from sporocarps separated by hundreds of kilometers. For example, genotype A5B8 was present at sites 6 and 9, and genotype A9B13 was present at sites 15 and 18. There is a great deal of information available on the fine structure of mating-type loci of *S. commune* (12,13,15) and a few other higher fungi (9), and efforts are underway to understand the molecular structure of mating-type loci (17). A comparison of the molecular genetic structure of functionally similar mating-type alleles from sporocarps separated by great distances would be very useful in understanding the mechanisms of action of mating-type loci.

LITERATURE CITED

1. Anderson, N. A., Stretton, H. M., Groth, J. V., and Flentje, N. T. 1972. Genetics of heterokaryosis in *Thanatephorus cucumeris*. Phytopathology 62:1057-1065.
2. Dobzhansky, T., and Wright, S. 1941. Genetics of natural populations. V. Relations between mutation rates and accumulation of lethals in populations of *Drosophila pseudoobscura*. Genetics 26:23-52.
3. Doudrick, R. L. 1988. Ectomycorrhizal associations of black spruce in northern Minnesota. Ph.D. thesis. University of Minnesota, St.

Paul. 77 pp.
4. Doudrick, R. L., and Anderson, N. A. 1989. Incompatibility factors and mating competence of two *Laccaria* spp. (Agaricales) associated with black spruce in northern Minnesota. Phytopathology 79:694-700.
5. Doudrick, R. L., Stewart, E. L., and Alm, A. A. 1990. Survey and ecological aspects of presumed ectomycorrhizal fungi associated with black spruce in northern Minnesota. Can. J. Bot. 68:825-831.
6. Fries, N., and Mueller, G. M. 1984. Incompatibility systems, cultural features and species circumscriptions in the ectomycorrhizal genus *Laccaria* (Agaricales). Mycologia 76:633-642.
7. Heinselman, M. L. 1963. Forest sites, bog processes, and peatland types in the glacial Lake Agassiz region, Minnesota. Ecol. Monogr. 33:327-374.
8. Heinselman, M. L. 1970. Landscape evolution, peatland types, and the environment in the Lake Agassiz Peatlands Natural Area, Minnesota. Ecol. Monogr. 40:235-261.
9. Koltin, Y. 1978. Genetic structure of incompatibility factors–the ABC of sex. Pages 31-54 in: Genetics and Morphogenesis in the Basidiomycetes. M. N. Schwalb and P. G. Miles, eds. Academic Press, New York.
10. Kropp, B. R., and Fortin, J. A. 1988. The incompatibility system and relative ectomycorrhizal performance of monokaryons and reconstituted dikaryons of *Laccaria bicolor*. Can. J. Bot. 66:289-294.
11. Marx, D. H. 1972. Ectomycorrhizae as biological deterrents to pathogenic root infections. Annu. Rev. Phytopathol. 10:429-454.
12. Raper, J. R. 1966. The Genetics of Sexuality of Higher Fungi. Ronald Press Co., New York. 283 pp.
13. Raper, J. R., and Hoffman, R. M. 1974. *Schizophyllum commune*. Pages 597-626 in: Handbook of Genetics. I. R. C. King, ed. Plenum Press, New York.
14. Raper, J. R., Krongelb, G. S., and Baxter, M. G. 1958. The number and distribution of incompatibility factors in *Schizophyllum*. Am. Nat. 92:221-232.
15. Stamberg, J., and Koltin, Y. 1973. Genetic control of recombination in *Schizophyllum commune*: Evidence for a new type of regulatory site. Genet. Res. 22:101-111.
16. Swanson, D. K. 1988. Properties of peatlands in relation to environmental factors in Minnesota. Ph.D. thesis. University of Minnesota, St. Paul. 220 pp.
17. Ullrich, R. C., Novotny, C. P., Specht, C. A., Froeliger, E. H., and Munoz-Rivas, A. M. 1985. Transforming Basidiomycetes. Pages 39-57 in: Molecular Genetics of Filamentous Fungi. W. E. Timberlake, ed. Alan R. Liss, Inc., New York.