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Population structure and mycelial phenotypic variability of the ectomycorrhizal basidiomycete *Laccaria bicolor* (Maire) Orton

Abstract Mating type allele distribution and phenotypic variability were investigated in field populations of *Laccaria bicolor*. Sporophores associated with Norway spruce (*Picea abies*), colonized by natural sources of inoculum and growing in a seed orchard, were sampled to obtain dikaryotic strains and assay their phenotypic variability for traits important to the symbiosis. Basidiospores were also collected for mating type analysis of different mycelia. Four sporophore mating types were identified containing seven A and five B factors. Outbreeding efficiency was estimated at 73.8% and the population was slightly inbred. Crosses with previously characterized *L. bicolor* strains from two nearby populations identified in total six sporophore mating types and ten A and nine B factors, for an estimated outbreeding efficiency (85.7%) similar to previous studies of more spatially disparate *Laccaria* spp. populations. Dikaryotic strains were tested for mycelial growth rate, as a measure of their competitive ability, on agar media containing a soluble (NaH_2PO_4), or an insoluble ($CaHPO_4$) phosphate source. Their ability to solubilize the latter was also tested to assess their relative capacity to access insoluble, inorganic phosphate. In most cases, significant variation was detected among strains from the same site for all variables. On three sites (VC4, VC5 and VC7), each determined previously to possess a uniform mycelial genotype, phenotypic variability was likely due to epigenetic variation among different strains of the same genotype. Possible evidence for dikaryon-monokaryon crosses was observed in vivo on one sample site (VC2) where adjacent mycelia shared two mating factors. The phenotypic variability of different mycelial genotypes reflected their genetic variability observed as mating type allele diversity and underlined the importance of basidiospore dispersal in introducing new genotypes into the population. The reproductive strategies of *L. bicolor* are discussed and compared to those of other basidiomycete species.

Key words *Laccaria bicolor* · Clone development · Population ecology · Genetic variability · Reproductive strategies

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Introduction

Basidiomycete fungi include a diverse array of species which exploit different ecological niches as saprophytes, pathogens or root symbionts. Basidiomycete populations are initially established by basidiospore dispersal or vegetative inoculum and can develop over time with further contributions from these sources. Mycelial growth, resource availability, and interactions between individuals will also shape these populations (Carlile 1987; Rayner 1991).

The majority of Holobasidiomycete species are heterothallic and possess multiallelic bipolar or tetrapolar mating systems (Burnett 1975; Rayner and Todd 1982a; Carlile 1987). These mating systems favor crosses between nonsibling, monosporous mycelia. Heteroplasmon formation and possibly recombination among elements of the cytoplasmic genome may also occur (Caten 1987). Primary or secondary homothallism and other mechanisms favoring self fertility (Nasmyth 1983; Ullrich et al. 1985) will reduce but not prevent outcrossing among strains of some species (Burnett 1975; Carlile 1987). Somatic incompatibility interactions prevent the

transfer of genetic material between sexually incompatible individuals of a species by the induction of antagonistic responses, thus influencing their spatial and genetic limits (Rayner and Todd 1982a,b; Rayner 1991). For more distantly related strains from different intersterility groups, intersterility genes likely determine compatibility (Chase and Ullrich 1990a,b). They are distinct from the mating type genes and may contribute to speciation by restricting gene flow (Rayner and Todd 1982a; Fries 1983; Carlile 1987; Mueller and Gardes 1991). The population biology of basidiomycete species will thus be shaped by these different mechanisms, which influence outbreeding and maintain the genetic integrity of a mycelium.

Ectomycorrhizal basidiomycetes constitute an important element of forest fungal communities (Fogel 1981; Villeneuve et al. 1989; Vogt et al. 1991) and their unique characteristics as root symbionts make them an interesting subject for studies of population ecology and genetics. Their mycelia maintain a close physiological link to host-tree feeder roots (Harley and Smith 1983; Read 1992). Conidia are absent among most species (Hutchison 1989), with only a few forming chlamydospores (Agerer 1991). These fungi therefore rely upon basidiospores, mycelial fragmentation and occasionally sclerotia (Molina et al. 1992) as their principal means of dispersal and reproduction. Only preliminary information has been gathered assessing the population structure, genetic variability and reproductive strategies of their natural populations. Most studies have examined the genera *Laccaria* (Fries 1983; Kropp and Fortin 1988; Doudrick and Anderson 1989; Doudrick et al. 1990; Mueller and Gardes 1991; Baar et al. 1994) and *Suillus* (Fries 1987; Zhu et al. 1988; Dahlberg and Stenlid 1990; Sen 1990; Keller 1992; Jacobson et al. 1993; Jacobson and Miller 1994).

Ectomycorrhizal fungi play an important role in the phosphorus nutrition of their host plants (Harley and Smith 1983; Kropp and Langlois 1990). In forest soils, phosphorus occurs primarily as insoluble compounds unavailable to plants (Brady 1974; Stevenson 1986). Some ectomycorrhizal fungi may improve the host's ability to utilize insoluble phosphate by producing acid phosphatase enzymes (Bartlett and Lewis 1973; Ho 1988; Kroehler et al. 1988; Kropp 1990a) or organic acids (Cromack et al. 1979; Lapeyrie et al. 1987), which will solubilize organic and inorganic mineral phosphate, respectively. Insoluble salts of phosphate are formed principally with calcium, iron, aluminum or manganese in forest soils and the relative abundance of each varies with soil type and pH. These inorganic compounds can constitute an important pool of phosphate for ectomycorrhizal trees (Brady 1974; Binkley 1986).

Mycelial growth rate is a polygenic trait that can be useful in assessing the competitive ability of different genotypes. Competitive interactions among ectomycorrhizal fungi during root colonization are thought to occur primarily by competitive exclusion, with mycelial growth rate, inoculum potential and other biotic/abiotic factors influencing the success of suitable mycobionts (Deacon and Fleming 1992). In addition to its role in root colonization, mycelial growth rate may also affect the ability of the extramatrical mycelium to exploit available soil resources and transport them to the host plant (Read 1992).

Laccaria bicolor (Maire) Orton is an early-stage species of temperate and boreal forests with a broad host range (Last et al. 1987; Villeneuve et al. 1989) whose variability has been described in vitro (Kropp et al. 1987; Kropp and Fortin 1988; Wong et al. 1989; Gardes et al. 1990b; Kropp 1990a,b; Gardes et al. 1991a,b). Its' mating system is bifactorial heterothallic with multiallelic mating type factors and includes several intersterility groups (Fries and Mueller 1984; Kropp and Fortin 1988; Mueller and Gardes 1991). The objectives of the present study were to describe the variability of a small *L. bicolor* field population for sporophore mating type and the variability of their dikaryotic tissue isolates for phenotypic traits of importance to the symbiosis.

Materials and methods

Site description and sampling

Sporophores of *L. bicolor* were sampled from four different sites around grafted Norway spruce [*Picea abies* (L.) Karst.] trees in a seed orchard at the Valcartier forest station of Forestry Canada in Valcartier, Québec, Canada. Features of the sample location, all cultural treatments applied and the origins of the tree stock were described by de la Bastide et al. (1994), with the exception of site VC2. For this site, stem stock was taken from 4-year-old seedlings obtained from the Bas Saint-Maurice nursery (Ministère de l'Énergie et des Ressources du Québec, MERQ) at Saint-Maurice, Québec and grafted to the common root stock of 4-year-old Norway spruce, obtained as nonmycorrhizal, bare-root seedlings from the Duchesnay nursery (MERQ) at Sainte-Catherine de la Jacques Cartier, Québec. Site VC2 trees were kept in pots at the Valcartier forest station for 5 years before outplanting, with all other trees, at their current locations in 1986.

Intersite distances among sample sites were 19.38 m (VC2-VC4), 17.50 m (VC2-VC5), 20.94 m (VC2-VC7), 13.44 m (VC4-VC5), 3.13 m (VC4-VC7) and 11.56 m (VC5-VC7). All trees had been colonized by natural inoculum and *Thelephora terrestris* Fr. and *Hebeloma* spp. sporophores were also observed in the orchard. Sporophores of *L. bicolor* were collected from August until the first hard frost and their coordinates noted on a sampling grid to prepare distribution maps. Sampling grid dimensions were 2×2 m (VC2), 2×2 m (VC4), 1.6×1.6 m (VC5) and 0.8×0.8 m (VC7) and delimited the maximal distribution of sporophores detected during the sample period for each site. Site VC2 was sampled one season (1990), site VC7 over two (1990–1991), site VC5 over three (1990–1992) and site VC4 over four (1990–1993) consecutive seasons. All mature sporophores were collected, and those connected at the base of the stipe were counted separately. Basidiospores were collected from sporophores by fastening caps to the lid of sterile Petri plates with lanolin and stored in the dark at 4° C until use. Sporophore distribution and genotypic persistence around host trees were reported by de la Bastide et al. (1994).

Culture isolation, maintenance and mating studies

Dikaryotic strains were obtained by plating clean fragments of sporophore tissue onto Petri plates containing modified Melin-

Norkran (MMN) agar medium (Marx 1969), augmented with 10–20 mg of an undiluted streptomycin/tetracycline/benomyl (10:5:3 by weight) mixture. For each sample site, 36 of 91 (VC2), 6 of 21 (VC4), 18 of 29 (VC5) and 7 of 16 (VC7) dikaryotic strains collected from sporophores in 1990 were successfully isolated and later used for phenotypic analyses. Dried voucher specimens of these sporophores, including those used for mating type analysis, were kept at the Département des Sciences du Bois et de la Forêt, Université Laval.

For mating type analysis, adequate spore samples collected from selected sporophores were used to obtain the four mating type monokaryons (tester strains) for sporophores from each site. Tester strains were identified for 7 (VC2), 7 (VC4), 3 (VC5) and 1 (VC7) sporophores from the different sites. Monokaryotic strains were isolated from germinated basidiospores with the method of Fries (1983) and were verified for their lack of clamp connections before use. All strains were maintained on MMN medium. Monokaryon mating types for each sporophore were determined by pairing selected strains in all possible combinations on MMN agar. After 3 weeks growth at 22° C, the presence of clamp connections at the interface indicated a compatible mating (Kropp and Fortin 1988; Mueller and Gardes 1991). Mating types were subsequently assigned according to their compatibility patterns and the tester strains from each sporophore were then paired in all possible combinations among sporophores to determine the allelic distribution.

The correct sporophore mating types were assigned by pairing their testers with those of previously characterized *L. bicolor* strains. These included the testers of a sporophore collected in 1982 in Sweden by G. Mueller (Fries and Mueller 1984) and the testers of sporophores identified as QBF19861 (collected at Sainte-Étienne-de-Lauzon, Québec), QBF19877 (collected on the campus of Laval University, Sainte-Foy, Québec) and QBF19862 [collected 13 March 1985 in a growth chamber at Laval University associated with a host seedling (*Pinus banksiana* Lamb.) which was previously inoculated with a dikaryotic strain from a sporophore collected in 1981 by C. Godbout at Burt Lake, Ontario] (Kropp and Fortin 1988). All tester crosses were performed twice to verify initial results.

The number of mating type factors at each incompatibility locus was estimated for the population studied and compared to the number detected for Valcartier sites alone and together with other Québec strains (Kropp and Fortin 1988). Crosses with the other biological species (QBF19862) and Swedish strains of *L. bicolor* were not used for these calculations. This method was developed by Raper et al. (1958) for *Schizophyllum commune* Fr:Fr and used the formula $N=n(n-1)/2/r$, where N is the number of A or B factors estimated, n is the number of individuals (strains) in the sample and r is the number of pairs of identical factors occurring in the sample. Estimated outbreeding efficiency (Raper et al. 1958) is a measure of the number of successful matings expected between compatible strains under panmixia. This was calculated with the formula $E_T=1-(1/N_A+1/N_B)$, where E_T is the outbreeding efficiency and N_A and N_B are the estimated numbers of A and B factors, respectively. The 95% confidence limits for N_A and N_B were determined using the 95% confidence limits on the proportion of incompatible pairings, as calculated by a normal approximation to the binomial distribution, which was then converted to a $\pm N$ value ($N=n(n-1)/2/\pm r$) (Doudrick et al. 1990).

Phenotypic variability of dikaryotic isolates

Selected dikaryotic isolates of sporophores collected from each site in 1990 were tested in vitro for phenotypic variability. Their relative ability to solubilize an insoluble mineral phosphate (P) was determined in assays on agar medium containing calcium phosphate ($CaHPO_4 \cdot 2H_2O$) as the P source (adapted from Goldstein and Liu 1987). The medium contained $(NH_4)_2SO_4$ (1.25 g), $MgSO_4 \cdot 7H_2O$ (0.15 g), KCl (0.275 g), $CaCl_2$ (0.05 g), thiamine (40 μg), biotin (0.4 μg), D-glucose (5.0 g), bacto agar (10.0 g) and $CaHPO_4 \cdot 2H_2O$ (0.860 g) in 1 l of distilled water and was adjusted to pH 5.5 before autoclaving. Calcium phosphate was autoclaved separately in 500 ml of distilled water, filtered when cool and washed three times with sterile, distilled water on a 20-μm filter paper to separate the insoluble P from any soluble P in solution. It was then resuspended in 10 ml of sterile, distilled water and added to the autoclaved medium at 55° C. The ratio of the solubilization zone diameter to colony diameter ($CaHPO_4$ ratio) was determined 14 days after inoculation. The solubilization zone was defined as the annular region of the medium adjacent to the colony free of undissolved calcium phosphate granules and was easily observed. Those strains with higher values were considered to have a comparatively greater ability to solubilize mineral P.

Mycelial growth rate (mm diameter $week^{-1}$) was determined on this medium over the 14-day period and on a similar medium containing instead sodium phosphate ($NaH_2PO_4 \cdot 2H_2O$) as a soluble P source (0.780 g l^{-1}). For each assay, 10 ml of medium was added to each Petri plate, followed by inoculation with a 3-mm-diameter plug obtained from the margin of a 2-week-old culture, for three replicates of each strain tested. Plates were completely randomized for each assay and cultured at 22° C under low light (2.5 μE m^{-2} s^{-1}). Data were analyzed using an ANOVA and the multiple range Scott-Knott method for mean separation (Scott and Knott 1974; Gates and Bilbro 1978) to test for both within- and among-site variability; a pooled mean for each site for each variable measured was used in the latter method.

Results

Four sporophore mating types were identified in this population; two were associated with the site VC2 and one each with sites VC4, VC5 and VC7, the latter two possessing the same mating type (Table 1). Site VC4 sporophores VC4-6 and -20 (1990), VC4-4 and -21 (1991), and VC4-17, -28 and -42 (1992) shared no mating type alleles with those of other sites. Sites VC5-19 and -21 (1990), -8 (1991) and VC7-7 (1990) sporophores shared one allele (B_7) with the VC2-56 and -60 sporophores and two alleles (B_7 and B_8) with the VC2-64, -72, -73, -75 and -87 sporophores (Fig. 1). The two VC2 sporophore mating types shared the alleles A_2 and B_7. Dikaryotic cultures of sporophores VC2-56, -60 and -73, VC4-6 and -20, and VC5-21 were not successfully isolated, but their mating types were determined. The estimated number of mating factors for the Valcartier population was 16 A and five B factors, as compared with seven A and five B factors identified. Four A factors occurred once and three occurred twice, while three B factors occurred once, one occurred three times and one occurred four times among sporophores. The 95% confidence limits of estimates were 8 and 32 for the A and 3 and 7 for the B locus, with an E_T of 73.8%.

When testers of the Valcartier population were crossed with those of previously characterized *L. bicolor* cultures, they shared very few alleles (Table 2). QBF19861 testers shared the allele A_2 with the VC2 groups, but did not share alleles with VC4 or VC5 sporophores. QBF19877 testers shared no alleles with the Valcartier population. QBF19862 testers were completely incompatible with the Valcartier testers, indicating that they belong to a different intersterility group. Testers of the Swedish sporophore shared the alleles B_4

Table 1 Mating interactions among tester strains of Valcartier sporophore mating type groups for sites VC2, VC4, VC5 and VC7. Negative (−) crosses indicate shared mating type alleles. Sporophores included in each group are indicated

		VC2-56, -60				VC2-64, -72, -73, -75, -87				VC4-6, -20, -4, -21, -17, -28, -42				VC5-19, -21, -8, VC7-7			
		A_2B_7	A_7B_7	A_2B_4	A_7B_4	A_2B_7	A_8B_7	A_2B_8	A_8B_8	$A_{10}B_9$	$A_{10}B_{10}$	$A_{11}B_9$	$A_{11}B_{10}$	A_9B_7	A_9B_8	A_3B_7	A_3B_8
VC2-56, -60	A_2B_7	−	−	−	+	−	−	−	+	+	+	+	+	−	+	−	+
	A_7B_7	−	−	+	−	−	−	+	+	+	+	+	+	−	+	−	+
	A_2B_4	−	+	−	−	−	+	−	+	+	+	+	+	+	+	+	+
	A_7B_4	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
VC2-64, -72, -73, -75, -87	A_2B_7					−	−	−	+	+	+	+	+	−	+	−	+
	A_8B_7					−	−	+	−	+	+	+	+	−	+	−	+
	A_2B_8					−	+	−	−	+	+	+	+	+	−	+	−
	A_8B_8					+	−	−	−	+	+	+	+	+	−	+	−
VC4-6, -20, -4, -21, -17, -28, -42	$A_{10}B_9$									−	−	−	+	+	+	+	+
	$A_{10}B_{10}$									−	−	+	−	+	+	+	+
	$A_{11}B_9$									−	+	−	−	+	+	+	+
	$A_{11}B_{10}$									+	−	−	−	+	+	+	+
VC5-19, -21, -8, VC7-7	A_9B_7													−	−	−	+
	A_9B_8													−	−	+	−
	A_3B_7													−	+	−	−
	A_3B_8													+	−	−	−

with the VC2-60 group and A_3 with VC5 sporophores; one cross between VC5 and apparently compatible Swedish tester monokaryons ($A_9B_8 \times A_3B_4$) was consistently negative, despite several recrosses using different VC5 tester strains. Self (control) crosses among previously characterized strains confirmed their monokaryon mating types. For the Valcartier and the compatible Québec strains together, the estimated number of mating factors was 19 A and 11 B, as compared with 10 A and nine B factors identified. Seven A factors occurred once, two occurred twice and one occurred three times, while seven B factors occurred once, one occurred three times and one occurred four times among sporophores. The 95% confidence limits of estimates were 12 and 29 for the A and 7 and 14 for the B locus, with an E_T of 85.7%.

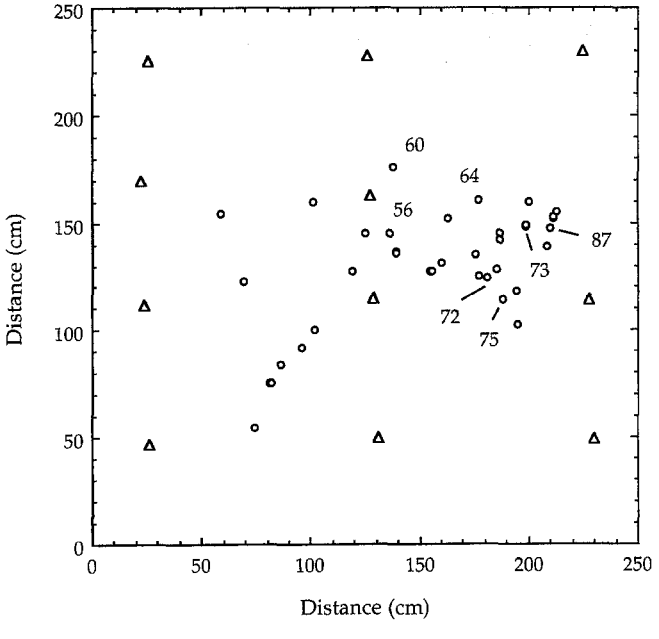

Fig. 1 The distribution of sporophores (○) on site VC2. *Numbers* indicate the position of mating typed sporophores; the locations of Norway spruce trees (Δ) are shown

The phenotypic variability of all dikaryotic sporophore isolates tested is summarized in Table 3. Significant variation was detected among strains from the same site for all variables, except for the growth rate of VC7 strains on soluble P. Significant variation was detected for all variables among the strains isolated from the sporophores VC2-64, -72, -75 and -87. Mating type analysis determined that these strains all possessed the same mating type alleles (Table 1). In addition, the corresponding sporophores occurred in close proximity on site VC2 and were separated by less than 50 cm (Fig. 1). Table 4 summarizes the results for among-site variability, based on a pooled site mean for each variable. Significant variation was observed for mycelial growth rate on both media, but not for the $CaHPO_4$ ratio. Strains collected on sites VC5 and VC7, where sporophores possessed the same mating type alleles (Table 1), were not significantly different for the phenotypes tested. Strains with different mating type alleles, collected on sites VC2 and VC4, had a significantly slower growth rate on insoluble P than the former strains, while only site VC2 strains grew more slowly on soluble P (Table 4).

Discussion

The number of factors identified among four sporophores (seven A and five B) in the Valcartier population were fewer than and the number of factors identified among seven sporophores (10 A and nine B) in the combined Valcartier and Québec populations were similar to those described by Doudrick and Anderson

Table 2 Crosses among tester strains of previously characterized *Laccaria bicolor* isolates and Valcartier mating type groups. Negative (−) crosses indicate shared mating type alleles. Control crosses among testers of the former group confirmed their mating types

		Québec 19861				Sweden				Québec 19877				Québec 19862			
		A_1B_1	A_1B_2	A_2B_1	A_2B_2	A_3B_3	A_3B_4	A_4B_3	A_4B_4	A_5B_5	A_5B_6	A_6B_5	A_6B_6	$A_1'B_1'$	$A_1'B_2'$	$A_2'B_1'$	$A_2'B_2'$
VC2-60	A_2B_7	+	+	−	−	+	+	+	+	+	+	+	+	−	−	−	−
	A_7B_7	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	A_2B_4	+	+	−	−	+	−	+	−	+	+	+	+	−	−	−	−
	A_7B_4	+	+	+	+	+	−	+	−	+	+	+	+	−	−	−	−
VC2-72	A_2B_7	+	+	−	−	+	+	+	+	+	+	+	+	−	−	−	−
	A_8B_7	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	A_2B_8	+	+	−	−	+	+	+	+	+	+	+	+	−	−	−	−
	A_8B_8	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
VC4-20	$A_{10}B_9$	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	$A_{10}B_{10}$	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	$A_{11}B_9$	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	$A_{11}B_{10}$	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
VC5-19	A_3B_7	+	+	+	+	−	−	+	+	+	+	+	+	−	−	−	−
	A_3B_8	+	+	+	+	−	−	+	+	+	+	+	+	−	−	−	−
	A_9B_7	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	A_9B_8	+	+	+	+	+	−	+	+	+	+	+	+	−	−	−	−
Québec 19861	A_1B_1	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−
	A_1B_2	−	−	+	−	+	+	+	+	+	+	+	+	−	−	−	−
	A_2B_1	−	+	−	−	+	+	+	+	+	+	+	+	−	−	−	−
	A_2B_2	+	−	−	−	+	+	+	+	+	+	+	+	−	−	−	−
Sweden	A_3B_3					−	−	−	+	+	+	+	+	−	−	−	−
	A_3B_4					−	−	+	−	+	+	+	+	−	−	−	−
	A_4B_3					−	+	−	−	+	+	+	+	−	−	−	−
	A_4B_4					+	−	−	−	+	+	+	+	−	−	−	−
Québec 19877	A_5B_5									−	−	−	+	−	−	−	−
	A_5B_6									−	−	+	−	−	−	−	−
	A_6B_5									−	+	−	−	−	−	−	−
	A_6B_6									+	−	−	−	−	−	−	−
Québec 19862	$A_1'B_1'$													−	−	−	+
	$A_1'B_2'$													−	−	+	−
	$A_2'B_1'$													−	+	−	−
	$A_2'B_2'$													+	−	−	−

(1989) in a population study of *L. bicolor* conducted in Minnesota. They identified 10 A and eight B factors and estimated 23 B and an infinite number of A factors, for five sporophores collected at disparate locations in Minnesota black spruce [*Picea mariana* (Mill.) BSP] forests. In our study, the E_T was moderate in the Valcartier population (73.8%), indicating that it was slightly inbred, while it was relatively high for the combined Valcartier and Québec populations (85.7%) and similar to that of the Minnesota study (88%). However, the authors considered this value probably to be an underestimate, due to reduced mating competence caused by intersterility barriers to strain compatibility within and among sample sites, which they suggested was linked to site heterogeneity. Such interactions were only observed with our QBF19862 testers, strains of a different intersterility group (Kropp and Fortin 1988).

The sample sites of Doudrick and Anderson (1989) were much more spatially disparate than our own, separated by as much as 210 km. Valcartier sites were less than 25 m apart, while the QBF19877 and QBF19861 sites were 26.5 and 36.5 km distant, respectively, from Valcartier and 13.5 km apart. Considering the small sample size and the homogeneity of the Valcartier sites, the number of alleles detected is surprisingly large compared with the Minnesota study, where sporophores from the same sample location often shared alleles. Valcartier and Québec sites combined possessed a comparable E_T and allelic diversity on a smaller scale than the Minnesota study. The detection of a large number of mating type factors in our study site confirms the role of basidiospore dispersal in establishing new *L. bicolor* genotypes, a preferred reproductive strategy of early-stage species on disturbed sites (Last et al. 1987; Dahlberg and Stenlid 1990). Basidiospore dispersal will maintain genetic exchange among allopatric populations, providing that intersterility barriers to mating do not restrict gene flow (Raper et al. 1958).

A similar study (Doudrick et al. 1990) examining *Laccaria laccata* var. *muelleri* Singer populations, sampled at 19 locations across Minnesota and central Canada, identified 14 A and 14 B and estimated 17 A and 18 B factors. Many alleles were shared among sites and the E_T of 88.6% was similar to our combined popula-

Table 3 Mean values for mycelial growth rate (mm diameter week^{-1}) on a soluble P medium, an insoluble P medium and the $CaHPO_4$ ratio for dikaryotic sporophore isolates of sites VC2, VC4, VC5 and VC7. Significant differences among means within each site are indicated by differing letters within a column for each variable (Scott-Knott, $P<0.05$)

Site and isolate[a]	Mean growth rate (mm week^{-1})		$CaHPO_4$ ratio
	Soluble P	Insoluble P	
VC2-15	3.92 c	12.00 b	1.74 d
VC2-19	3.67 c	9.17 d	2.04 a
VC2-20	4.25 b	13.17 b	1.78 d
VC2-21	3.42 c	12.33 b	1.86 c
VC2-22	3.67 c	11.17 c	1.76 d
VC2-24	3.42 c	13.17 b	1.70 d
VC2-25	4.25 b	12.33 b	1.73 d
VC2-26	3.92 c	13.67 b	1.69 d
VC2-27	4.42 b	15.50 a	1.56 e
VC2-28	3.75 c	13.83 b	1.75 d
VC2-29	3.92 c	12.50 b	1.86 c
VC2-31	4.33 b	11.33 c	1.74 d
VC2-32	3.75 c	11.83 b	1.84 c
VC2-33	3.92 c	11.67 c	1.78 d
VC2-34	4.25 b	9.83 d	1.80 c
VC2-35	3.75 c	11.33 c	1.72 d
VC2-36	5.67 a	12.67 b	1.70 d
VC2-37	3.92 c	12.67 b	1.80 c
VC2-38	3.75 c	16.33 a	1.59 e
VC2-39	4.17 b	11.50 c	1.64 e
VC2-40	4.38 b	12.67 b	1.69 d
VC2-41	4.42 b	14.67 a	1.38 f
VC2-42	4.08 b	10.50 c	1.84 c
VC2-43	4.33 b	10.83 c	1.87 c
VC2-45	3.25 c	12.33 b	1.77 d
VC2-64*	3.92 c	9.67 d	2.13 a
VC2-69	3.25 c	11.83 b	1.81 c
VC2-70	3.42 c	10.83 c	1.86 c
VC2-71	3.75 c	10.83 c	1.96 b
VC2-72*	4.33 b	9.50 d	1.98 b
VC2-75*	3.83 c	9.50 d	2.02 a
VC2-78	3.83 c	9.17 d	2.04 a
VC2-82	3.83 c	9.33 d	2.08 a
VC2-87*	3.83 c	10.83 c	1.90 b
VC2-89	3.33 c	9.33 d	2.09 a
VC2-91	3.75 c	8.83 d	1.97 b
VC4-4	5.83 b	9.33 b	1.59 b
VC4-5	5.08 b	10.00 b	1.95 a
VC4-8	8.17 a	9.67 b	2.08 a
VC4-14	5.75 b	12.17 a	1.87 a
VC4-15	7.17 a	10.50 b	1.50 b
VC4-17	5.25 b	10.17 b	2.08 a
VC5-2	5.17 b	18.33 a	1.64 b
VC5-3	4.33 c	17.17 a	1.73 a
VC5-4	4.42 c	17.33 a	1.63 b
VC5-5	5.50 b	17.33 a	1.71 a
VC5-6	4.17 c	18.50 a	1.64 b
VC5-9	4.42 c	17.83 a	1.62 b
VC5-10	5.75 b	19.33 a	1.65 b
VC5-11	5.42 b	11.67 b	1.82 a
VC5-13	5.75 b	18.83 a	1.62 b
VC5-14	5.33 b	14.83 b	1.75 a
VC5-15	5.58 b	18.33 a	1.60 b
VC5-17	5.42 b	14.67 b	1.67 b
VC5-18	6.25 a	18.50 a	1.63 b
VC5-19†	6.67 a	14.83 b	1.69 b
VC5-23	6.25 a	14.17 b	1.76 a
VC5-24	6.33 a	15.50 b	1.76 a
VC5-27	4.42 c	17.00 a	1.62 b
VC5-29	6.17 a	13.67 b	1.69 b
VC7-1	6.33 a	17.67 a	1.65 b
VC7-3	5.42 a	15.83 b	1.70 b
VC7-5	5.08 a	15.33 b	1.77 a
VC7-6	4.92 a	16.00 b	1.66 b
VC7-7†	5.42 a	14.50 c	1.76 a
VC7-14	6.00 a	13.67 c	1.77 a
VC7-15	5.42 a	17.50 a	1.74 a

[a] Isolates for which mating type was also determined: $A_2B_7A_8B_8$ (*); $A_3B_7A_9B_8$ (†)

Table 4 Comparisons among sites for the variables measured, using pooled means of all strains from a given site. Significant differences among sites for each variable are indicated by differing letters

Site	No. of strains	Mean growth rate (mm week^{-1})		$CaHPO_4$ ratio
		Soluble P	Insoluble P	
VC2	36	3.93 b	11.63 b	1.82 a
VC4	6	6.21 a	10.31 b	1.85 a
VC5	18	5.41 a	16.55 a	1.68 a
VC7	7	5.51 a	15.79 a	1.72 a

tions. However, the number of alleles detected and the maximum distance between sites (1800 km) were greater. Sporophores from the same location often had the same alleles, as detected by Doudrick and Anderson (1989) for *L. bicolor*. Unlike the *L. bicolor* populations, all *L. laccata* collections were interfertile (Doudrick et al. 1990).

Mueller and Gardes (1991) identified three intersterility groups within the *L. bicolor sensu lato* complex, which correspond to three distinct North American species, including *L. bicolor sensu stricto*. This species showed a limited compatibility with the other intersterile groups and a relatively high incidence of negative pairings within the species. The large number of mating factors seen in our restricted populations and on a larger scale by Doudrick and Anderson (1989) underline the importance of sexual reproduction in *L. bicolor*. However, a parallel intersterility compatibility system appears to be active both intra- and interspecifically, possibly reflecting recent speciation events or the establishment of new sterility barriers as a precursor to speciation (Doudrick and Anderson 1989; Mueller and Gardes 1991). Such barriers were not evident between our Québec isolates and testers from Sweden, which supported the conclusions of Mueller and Gardes (1991) that the latter are contaxic with North American representatives of *L. bicolor sensu stricto*. Intercompatibility has been observed between European and North American populations of saprobic basidiomycete

species (Boidin 1986; Brasier 1987; Hallenberg 1991). Allopatric population compatibility could be due to incomplete geographic isolation, retarding the development of intersterility barriers.

Within-site phenotypic variability was observed among dikaryotic strains on all sites. The analysis of many of the same strains from sites VC4, VC5 and VC7 with RAPD genetic markers identified a single, unique fungal genotype on each site (de la Bastide et al. 1994). Intrasite phenotypic variability was most likely due to epigenetic variation, or differences in gene expression that produced a variable phenotype for strains of the same genotype. In addition, growth rate is a polygenic character and mineral phosphate solubilization does not involve the production of specific enzymes (Cromack et al. 1979; Lapeyrie et al. 1987). The genetic basis of these traits could have thus contributed to their phenotypic variability, for dikaryotic strains isolated from the same individual. In a study of mineral phosphate solubilization, Cromack et al. (1979) found evidence of substantial variation in oxalic acid production within individual mycelial mats of *Hysterangium crassum* (Tul. and Tul.) Fischer. Intraspecific phenotypic variability of *L. bicolor* has been observed previously among strains of the same genotype for the morphology of ectomycorrhizal structures (Wong et al. 1989).

Mycelial growth rate on both agar media varied among sites for their pooled means. This may reflect the differing capacity of genotypes on different sites to colonize host roots and exploit soil resources. These data agree with the results of mating type analysis; sites VC5 and VC7 were similar for mating type, phenotype and almost identical for RAPD markers (de la Bastide et al. 1994), suggesting that these genotypes may have originated from the sporophore progeny of the same mycelium. Compared with VC5 and VC7, sites VC2 and VC4 were variable for both mating type and growth rate. Among-site variability for phenotypic traits in the Valcartier population reflected the genetic variability of mycelia observed as mating type allele diversity and underlined the importance of basidiospore dispersal in introducing new genotypes into the population.

Modes of propagation for *L. bicolor* include sexual basidiospores and a vegetative mycelium. The former permits the generation of genetic variability, dispersal, colonization of new hosts and genetic exchange among compatible populations. The latter facilitates the expansion of successful genotypes in suitable habitats. The reproductive strategy of *L. bicolor* will likely vary under different environmental conditions. In a study of another ectomycorrhizal species, *Suillus bovinus* (L. ex Fr.) O. Kuntze, Dahlberg and Stenlid (1990) determined that clone size increased and fruiting declined with increasing forest age and reduced disturbance. The relative importance of colonization by mycelial expansion versus basidiospores apparently changed with stand development. A young, regenerating forest contained many small clones, probably established by spore inoculum, while older stands contained fewer clones with much larger dimensions, likely resulting from mycelial spread and interclone competition. The young forest resembles our Valcartier site, where several genetically distinct *L. bicolor* mycelia were identified. As the orchard ages, *L. bicolor* mycelia may follow the same trend as those of *S. bovinus*. Clone size has increased over time and remained genetically stable for up to 3 years (de la Bastide et al. 1994). It is likely that the best-adapted genotypes will eventually predominate. A mycorrhizal *L. bicolor* mycelium is the sole source of inoculum available for much of the growing season, with a greater inoculum potential than basidiospores, whose viability is limited (Fries 1983). Colonization by basidiospore mycelia will be difficult, as mycorrhizal mycelia will rapidly colonize available roots. There is, however, the possibility of dikaryon-monokaryon (di-mon) crosses (the Buller phenomenon) with established mycelia (Buller 1958).

Common mating type strains from site VC2 varied in mycelial growth rate and insoluble P use, suggesting the presence of a single phenotypically variable genotype, produced by epigenetic variation, or several mycelial genotypes of the same mating type. The latter could be the product of sibling sporophore progeny crosses or potentially parent-progeny, di-mon crosses that formed new nuclear combinations. These genotypes could thus vary in their competitive ability (Deacon and Fleming 1992), due to their variable growth rate, and their capacity to use insoluble P (Cromack et al. 1979; Lapeyrie et al. 1987). The adjacent site VC2 mycelia shared the alleles A_2 and B_7 and could have arisen from two independent monokaryon-monokaryon (mon-mon) crosses, each involving a monokaryon A_2B_7, or from a di-mon cross between an ancestral dikaryon and a monokaryon of mating type A_7B_4 or A_8B_8.

In vitro evidence supports the existence of di-mon crosses for *L. bicolor*. *Laccaria laccata* (Scop. ex Fr.) Cke. basidiospore germination in pure culture is stimulated by the presence of a dikaryotic mycelium (Fries 1983). For mycorrhizal strains of *L. bicolor* cultured axenically, a monokaryotic strain was dikaryotized by a coinoculated dikaryon and maintained its original mitochondrial genome when reisolated from ectomycorrhizae (Gardes et al. 1990a). The occurrence of a di-mon cross is thus possible for natural *L. bicolor* populations and will yield new nuclear/cytoplasmic genome combinations. Mycelial individualism (Rayner 1991) may perhaps not exclude di-mon crosses for *L. bicolor*. However, it is not certain whether the dikaryotic mycelia will maintain physiological continuity or prevent cytoplasmic exchange through somatic incompatibility interactions. These are often used to identify the limits of individuals (Rayner 1991), but provide inconsistent results in vitro for *L. bicolor* and were not used for our study.

Di-mon crosses may be relevant to the symbiotic function of ectomycorrhizal fungi, whose ability to uti-

lize recalcitrant nutrient sources, especially P, is one of the primary benefits of the symbiosis to the host (Kropp and Langlois 1990). A new genotype of mycorrhizal mycelium formed by a di-mon cross may have an improved ability to access P in the soil, due to its superior P solubilizing ability and mycelial growth rate. It may be of greater benefit to the host than the original dikaryotic genotype and consequently enhance its own development over time.

The reproductive strategies of other Basidiomycete species can vary greatly compared with *L. bicolor*. The relative importance of spores versus mycelial expansion for each is often reflected in their population structure. Fairy ring fungi (Mallett and Harrison 1988; Dowson et al. 1989) display annular mycelial growth and isolates from the same ring likely possess a uniform genotype. A similar situation exists for the tree-root pathogen *Armillaria bulbosa* (Barla) Romagn. A single clone was genetically uniform over a 15-ha area, despite abundant basidiospore production (Smith et al. 1992). For both examples, clones were most likely established by dispersed basidiospores. Subsequent development occurred entirely by mycelial expansion and the incorporation of new genetic material seems to have been restricted. Vegetative growth becomes more important for *L. bicolor* in undisturbed soils, though it utilizes a more discrete nutritional substrate than these species.

In contrast, both spore colonization and vegetative growth are important in the population development of the parasite *Heterobasidium annosum* (Fr.) Bref. (Stenlid 1985). Clone size varies greatly for this species and host infection can occur by basidiospore and conidia colonization of open wounds or stump surfaces, as well as by mycelial invasion of root tissues. For the wood decomposer *Pleurotus ostreatus* (Jacq. ex Fr.) Krummer, basidiospore dispersal is the principal mechanism of new dikaryon establishment (Kay and Vilgalys 1992). Up to 15 individuals may be present on a single log, demonstrating the importance of dispersal in colonizing these discrete substrates, also a requirement of *L. bicolor* in disturbed environments.

Based on this small sample, it appears that basidiospore colonization plays a principal role in the initial establishment of *L. bicolor* populations. The different dikaryotic genotypes produced may vary for phenotypic traits that will influence their success as a mycobiont and the nutrition of the host plant. The incorporation of new genetic material into established mycelia may also potentially occur with di-mon crosses. The relative importance of mycelial expansion will likely increase with time in the absence of disturbance. A long-term study of *L. bicolor* populations is needed to provide a clearer understanding of their reproductive strategies and genetic development at different stages of forest succession.

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