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ORIGINAL PAPER

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Founder effect in a young *Leccinum duriusculum* (Schultzer) Singer population

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Abstract The genetic diversity of a *Leccinum duriusculum* population growing under <20-year-old *Populus alba* on former farming soil was analysed from 1998 to 2001 and compared in 2000 to the two nearest populations found under >70-year-old *P. alba*. Genets were recognized using RAPD amplifications with three different primers, while their conspecificity was assessed by sequencing the nuclear ITS and mitochondrial large ribosomal subunit. The young population was colonized by a large genet that persisted from 1998 to 2001 (most distal sporophores were 10.4 m apart in 2001) and a second genetically related genet appeared in 2001. Five and six genets, respectively, of smaller size were found in the two other populations, while the investigated area was slightly smaller (72.25 m^2) and the three populations were strongly divergent genetically (>33%). The genetic uniformity, as well as the high speed of radial growth of the lasting genet under <20-year-old *P. alba* (radial growth: 1 m/year), are interpreted in the framework of a founder effect. The slow recruitment of genets is proposed to lower the intraspecific competition and to entail large, fast-growing genets. The differences from ectomycorrhizal populations due to secondary colonization, which have been investigated often, are also emphasized.

Keywords Founder effect · *Leccinum duriusculum* (Schultzer) Singer

Introduction

The dynamics of plant communities are tightly linked to the diversity of the soil microflora (Bever et al. 1997). In the successional process occurring during colonization of new areas in terrestrial ecosystems, changes in soil microbial communities induce modifications in the plant community. Here, soil pathogens and symbionts are mainly responsible (Watkinson 1998), by interfering with plant/plant competition and plant/soil interface. Reciprocally, successional appearances and extinctions in the plant community drive evolution of the soil microbial communities. The settlement of ectomycorrhizal fungi, which symbiotically colonize the roots of trees and shrubs (Smith and Read 1997), requires the presence of the host species. Ectomycorrhizal fungi are lacking in former farmed soils and their arrival occurs with the planting of trees (Last et al. 1987; Selosse 2001), with a progressive recruitment of the ectomycorrhizal community. This has been well documented thanks to sporophore surveys, e.g. under *Betula* (Ford et al. 1980; Mason et al. 1982, 1983; Last et al. 1983), *Eucalyptus* (Lu et al. 1999) and conifers (Dighton et al. 1986; Richter and Bruhn 1993). Sporophore patches were reported to enlarge and move outwards from the tree from one year to the next (Ford et al. 1980; Mason et al. 1982; Last et al. 1987; de la Bastide et al. 1994). This progressive colonization was also documented underground by analysing ectomycorrhizal diversity directly on roots (e.g. Deacon et al. 1983; Natarajan et al. 1992).

Studies on ectomycorrhizal populations have focused up to now on populations from forest stands. Populations were analysed at old forest sites with long-established fungal populations (Selosse 2001), for example for species of *Amanita* (Redecker et al. 2001; Sawyer et al. 2001), *Laccaria* (Gherbi et al. 1999; Fiore-Donno and Martin 2001), and *Suillus* (Dahlberg and Stenlid 1994; Zhou et al. 2000). In some cases, populations were investigated on younger tree stands (de la Bastide et al. 1994; Selosse et al. 1998a, b, 1999), sometimes allowing a comparison with older stands (Dahlberg and Stenlid 1994; Dahlberg 1997; Gryta et al. 1997). In every case, these ectomycorrhizal populations were growing in or close to an already established forest, after a disturbance (e.g. fire or clearcut) that gave rise to a new tree generation. These data thus reflect populations from secondary successions (the so-called "second generation" in Last et al. 1987). Little is known of the genetic structure and diversity of the pioneer ectomycorrhizal

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populations observed during primary succession. In several cases, early-stage species still occur under older trees (Newton 1992; de la Bastide et al. 1994; Kranabetter and Wylie 1997), allowing for comparisons. *Hebeloma cylindrosporum* in French coastal dunes provide populations in colonization zones, at the forest border, as well as in mature forests (Gryta et al. 1997, Guidot et al. 2002). As *H. cylindrosporum* recolonizes sandy patches resulting from anthropic perturbations in mature forests, it is unsure whether this provides ectomycorrhizal populations of typical mature forests.

Here I considered a specific ectomycorrhizal species, *Leccinum duriusculum* under *Populus alba*, which is unlikely to exist before planting of its host (Last et al. 1987) because of its specificity (Lannoy and Estadès 1995). I followed its appearance in a small garden plantation of *P. alba* growing on former farmed soil. I propose it here as a model for studying an ectomycorrhizal population at the time of its establishment. The structure of the population was assessed by RAPD, a method providing polymorphic, dominant markers, which have been used already in studies of ectomycorrhizal populations (e.g. de la Bastide et al. 1994; Selosse et al. 1998a, b, 1999). The genetic structure was followed over 4 years and revealed an unexpectedly lower genetic diversity than in populations on the nearest old *P. alba* stands.

Materials and methods

Sampling sites

Three *L. duriusculum* populations under *P. alba* were surveyed in Belle-Ile-en-Mer, an island off Brittany in France (47°20’N and 5°30’W, 50 m above sea level). The average annual precipitation is 1,100 mm, evenly distributed over the year, with a mean annual temperature of 12.3°C (less than 9 days of freezing per year; data since 1950). The forest cover of the island was totally clearcut for farming purposes and there have been no forest stands at least since the 19th century (Chasle de la Touche 1852). However, *Salix* and spp. and *Populus* spp. hedgerows still grow at the borders of wet fields. Population A was found in my family garden in the town of Borgrouaguer, on a soil that was previously farmed, where six 5-year-old poplar trees were outplanted in 1983 near two perpendicular hedgerows of non-ectomycorrhizal shrubs, one planted with *Eleagnus* sp. (hedgerow 1) and the second with *Spartium junceum* (hedgerow 2) (Fig. 1). The outplanted poplar trees were grown in a nursery situated 3 km away. Due to the plateau nature of the site, this stand is unlikely to have harboured any spontaneous poplar trees in the past. *Leccinum duriusculum* sporophores were found here for the first time in 1997 (M.-A. Selosse, unpublished data). This population was surveyed daily during the fruiting period (May to November) in 1998, 2000 and 2001, allowing an extensive sporophore survey during those 3 years (9, 34, and 14 sporophores, respectively, were collected; no sampling was performed in 1997 and 1999). Populations B (Douët de Borgrouaguer) and C (Kerland) were found under *P. alba* trees at least 70 years old and where *L. duriusculum* has been fruiting yearly at least since 1983 (M.-A. Selosse, unpublished data). Population B grows under the poplar stand nearest to population A (200 m away). Populations A and B are situated 1.35 km away from population C. In populations B and C, a 8.5 m × 8.5 m area (i.e. an area slightly smaller than that of population A in 2001) was arbitrarily chosen and surveyed daily during the fruiting period in 2000. This allowed the sampling of 24 sporophores in population B and 31 sporophores in population C. The ground location of the sporophores was mapped in every case with a precision of 5 cm. Sporophores were dried for transport and frozen at –80°C before DNA extraction.

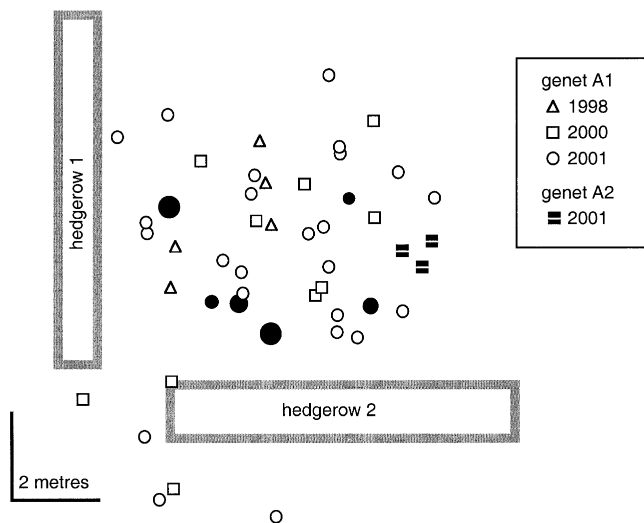

Fig. 1 Map of population A, with positions of the *Leccinum duriusculum* sporophores found in 1998, 2000 and 2001 (symbols indicating the year of collection and genet of origin, B1 or B2, are explained in the box at the right). Trees are located by *black dots* with sizes reflecting tree diameter; several closely growing sporophores are represented by a single symbol

DNA extraction

Total DNA from frozen sporophores was extracted following the protocol of Henrion et al. (1994) for the 1998 and 2000 sampling and using the DNeasy Plant Mini Kits (Qiagen S.A., Courtaboeuf, France), according to the manufacturer’s instructions, for the 2001 sampling. In both cases, the DNA was resuspended in distilled water and kept at –80°C.

RAPD analysis

RAPD reactions were carried out twice using primers 152C (5’-CGCACCGCAC-3’), 155 (5’-CGTGCGGCTG-3’) and 156 (5’-GCCTGGTTGC-3’) that were previously selected for their ability to generate reproducible and polymorphic patterns on this sample set (data not shown). RAPD amplification was performed using the enhanced protocol of Selosse et al. (1998a), followed by electrophoretic separation on 8% acrylamide gels in 1× Tris-borate-EDTA buffer, as described in Selosse et al. (1998a). To compare the various RAPD patterns found, comigrations and side-by-side migrations were performed in every permutation for all genets. The presence/absence of each RAPD fragment was recorded, excluding the fragments of low intensity and of a size greater than 1,400 bp that are less reproducible. A matrix of the number of differences between genets was established using the Mega programme (Kumar et al. 2000). This was then used to build a phenetic tree using the Neighbour-Joining method, with the same software. The percentage differences within or among populations were calculated as the mean percent of fragments by which genets differed from each other, considering every permutation.

Ribosomal DNA amplification and sequencing

For all sporophores, two loci were amplified. A fragment of the mitochondrial large ribosomal subunit DNA (LrDNA) was amplified with primers ML5 and ML6 (Bruns et al. 1998) under the PCR conditions described by Selosse et al. (1998b), and the ITS sequence (encompassing the ITS1, 5.8S and ITS2 sequences) was amplified with primers ITS1F and ITS4 (Gardes and Bruns 1993) under the same conditions as in Selosse et al. (2002). The sequencing was carried out for one randomly chosen sporophore of each genet on an automated sequencing system, as in Selosse et al. (2002), using the amplification primers as well as primers ITS2 and ITS3 internal to the ITS. Sequences were assembled using Sequencher 3.11 for MacOS from Genes Codes (Ann Arbor, USA) and deposited in the GenBank of the National Center for Biotechnology Information (NCBI). Searches for sequence identities were conducted using the Blast algorithm at the http://www.ncbi.nlm.nih.gov/blast/Blast.cgi page of the NCBI, using default settings (Altschul et al. 1997).

Results

Lack of genetic diversity of population A over 4 years

Population A growing under young *P. alba* produced a total of 57 sporophores in 1998, 2000 and 2001 (see positions on Fig. 1). Most sporophores showed the same RAPD pattern (Fig. 2A) and putatively belonged to the same genet, called A1. Only four sporophores collected in 2001 shared another pattern and belonged to a second genet (A2). RAPD patterns of genets A1 and A2 had 19 and 13 fragments, respectively, of which eleven were common to both genets (not shown). Genet A1 was thus characterized by eight fragments, while genet A2 was characterized by two fragments. A map of the sporophores (Fig. 1) showed that the four sporophores of genet A2 clustered on a small area in 2001 (< 1m^2), while A1 was present over the whole area, with a continuous increase in size between 1998 and 2000.

Genetic diversity of populations B and C in 1998

In order to compare population A with populations growing on older *P. alba* stands, sporophores were collected in a 8.5 m × 8.5 m area from populations B and C during 2000. This produced a number of sporophores (24 and 31, respectively) roughly similar to the sporophore number of population A in the same year. RAPD analysis produced a total of 55 fragments and showed that several genets were present on those areas (Fig. 2, Fig. 3), namely five (B1–B5) in population B and six (C1–C6) in population C. A phenetic comparison between the RAPD patterns showed that the genets of each population strongly clustered together (Fig. 2B). The inter-population difference in RAPD pattern between genets was always more than 30% (Table 1), while intra-population differences were less numerous (less than 25%, Table 1), emphasizing a high proportion of RAPD

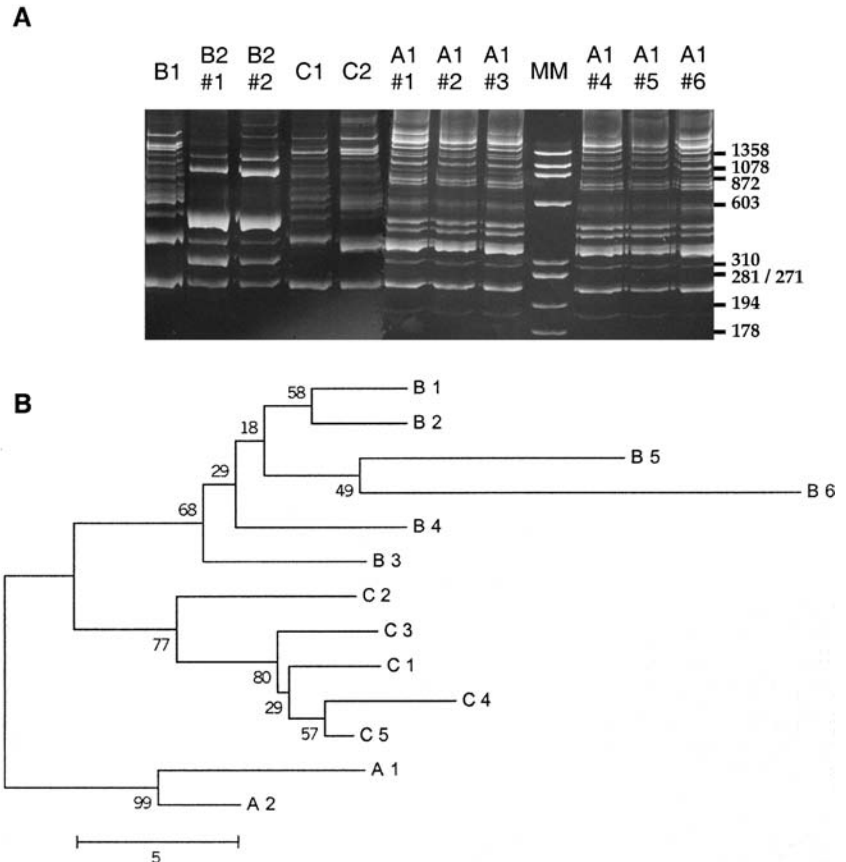

Fig. 2A, B RAPD polymorphism in the investigated *L. duriusculum* populations. **A** An example of RAPD patterns obtained with primer 152C, separated on 8% acrylamide gel. Patterns of several sporophores from genets B1, B2 (*1, 2*), C1, C2 and A1 (*1–6*) are given to show the reproducibility of the typing. *MM* is a molecular size marker (Phi-X-174 digested by *Hae*III). Fragment sizes (bp) are given at the right of the gel. **B** Unrooted neighbour-joining tree comparing the genets from populations A, B and C with bootstrap values on the branches; *scale*: number of differences

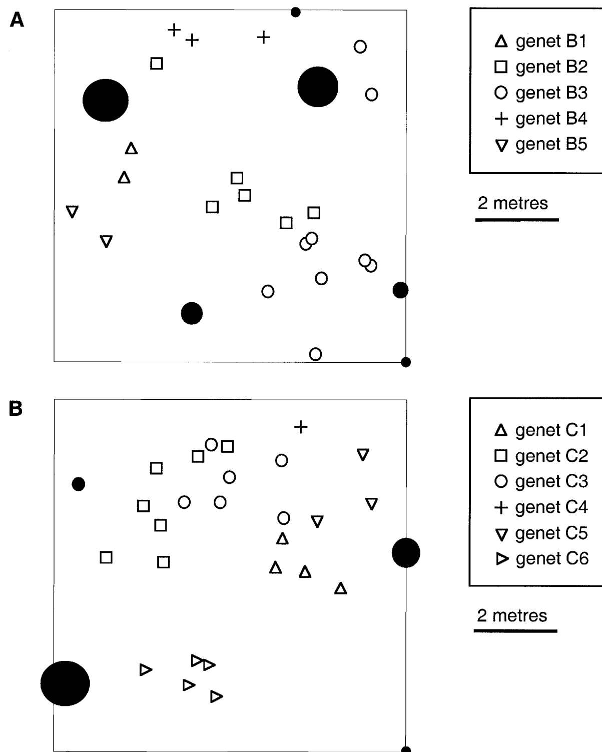

Fig. 3A, B Map of *L. duriusculum* populations occurring under <70-year-old populations in 2000. **A** Population B. **B** Population C. Symbols used for sporophore location indicate the genet of origin, B1 to B5 or C1 to C6, as explained in the box at the right. Trees are located by *black dots* with sizes reflecting tree diameter. Several closely growing sporophores are represented by a single symbol

fragments characteristic of each population and a spatially structured metapopulation. The map (Fig. 3) shows that sporophores of a given genet tended to cluster together (except perhaps for genet B3, Fig. 3).

Table 1 Polymorphism of the RAPD patterns, expressed as the mean percent of fragments by which each genet differs from the others. Values were calculated within populations and between pairs of populations

	A	B	C
A	13.6 ± 4.3	37.1 ± 4.1	33.5 ± 4.3
B		24.2 ± 2.8	35.8 ± 4.0
C			13.0 ± 2.7

All sampled populations belong to the same species

Five out of the 66 RAPD fragments generated in this study were common to all investigated sporophores (not shown). Since no fragments of similar size were found in previous studies using the same RAPD primers on other species (Selosse et al. 1998b, 1999, and unpublished data), this suggests the relatedness of all genets. In order to determine whether the strongly divergent RAPD pattern reflects interspecific polymorphism among populations, the mitochondrial LrDNA and nuclear ITS were amplified from all sporophores and sequenced for one sporophore of each genet. All LrDNA sequences were identical in size and sequence, showing similarities to *Leccinum* spp. sequences deposited in GenBank (Table 2). The ITS did not amplify from several sporophores. It was amplified from at least one sporophore of each genet, except B2, C3 and C4. Whenever it was amplified, the ITS fragment was 1,250 bp long. This is larger than for many basidiomycetous ITS and may explain the amplification problems experienced with some sporophores. Sequences were identical among genets and related to other *Leccinum* spp. sequences (Table 2). Numerous repeated sequences, including a GA_3 motif, were responsible for this unusual ITS length (data not shown), as in *Leccinum* sequences mentioned in Table 2 and other *Leccinum* ITS sequences (H.C. den Baker, personal communication).

Discussion

RAPD typing of *L. duriusculum* sporophores allowed recognition of 13 genets among the three investigated populations growing under *P. alba*. While populations C and B harboured various genets in 2000, population A harboured a single genet in 1998 to 2000, with an additional small genet in 2001. The strongly divergent RAPD phenotypes among populations raised the possibility that they were related to different *Leccinum* cryptic species, since reproductive isolation can occur within morphologically homogeneous *Leccinum* species (Mezhzherin and Mezhzherina 2000). However, identical LrDNA and ITS sequences were found in the three populations, suggesting that all sporophores are conspecific (note that an identity due to retention of ancestral haplotypes in divergent species cannot be ruled out). Comparison

Table 2 Closest GenBank relatives of the mitochondrial LrDNA and nuclear ITS sequences from the sampled *Leccinum duriusculum* populations. The closest two GenBank sequences were found by BLAST analysis. Percent identity over the part of the sequences shared by the closest two GenBank accessions is shown with the length of this alignment given in parentheses. The ITS sequence has less than 2% differences to several other unpublished *L. duriusculum* sequences (H.C. den Baker, personal communication)

Locus	GenBank accession no.	GenBank sequences	Identity	
LrDNA	AF484444	AD001605	*Leccinum holopus*	99.5 (397 bp)
		AD001606	*Leccinum manzanitae*	99.7 (372 bp)
ITS	AF484445	AF438580	*Leccinum* sp. Bremen 26	82.9 (596 bp)
		AF438581	*Leccinum* sp. Bremen 27	81.5 (634 bp)

between *L. duriusculum* populations associated with poplar trees of different ages is, therefore, allowed. Populations C and B, sampled under >70-year-old *P. alba*, have been established since 1983 at least, while population A, sampled under trees outplanted less than 20 years ago, is probably 4–5 years old (as suggested by the fact that no sporophore was found before 1997). The latter population may result from the outplanting and survival of genets from the nursery where *P. alba* were first germinated, but this seems unlikely since (1) sporophores were lacking between 1983 and 1998 in population A and (2) *Leccinum* spp. sporophores were never observed in nurseries at Belle-Ile-en-Mer (personal observations). Thus, this young population likely results from recent colonization by *Leccinum* basidiospores from neighbouring *Populus* spp. stands. The similarity to the nearest population (population B, 200 m away) is not greater than that to the distant population C, suggesting that at least one founder spore of population A came from a fourth population. Alternatively, but less likely as mentioned above, the differences between populations B and A may suggest that the latter arose from the poplar nursery.

Populations B and C harboured 5–6 genets on an area similar to population A. The comparatively low genetic diversity of population A (1 to 2 genets) over 4 years can be explained by a founder effect. A single genet, namely A1, either originating from the *P. alba* nursery or from a single colonization event, implying the germination of two basidiospores followed by mating, could explain this low diversity by a bottleneck effect. Alternatively, one could imagine that the population is made of several very closely genetically related genets, due to inbreeding. Inbreeding has been shown to occur in ectomycorrhizal populations (Gryta et al. 2000) and is likely to imply monomorphism of the genetic markers, associated with homozygosity at many loci. In this scenario, identical RAPD fragments would not distinguish between individuals. To rule out such a situation, at least some RAPD markers should be heterozygous. Since heterozygous markers are likely to be lost during inbreeding, their retention would constitute stronger evidence of genetic identity. Unfortunately, attempts to germinate spores (following the protocol described in Selosse et al. 1998a, b) were unsuccessful (data not shown), precluding any segregation analysis. However, such a monomorphic population can also be explained by a bottleneck effect: colonization by two genetically related basidiospores, followed by inbreeding, could lead to the observed reduced RAPD diversity. Since the RAPD markers were polymorphic in populations B and C and since entirely homozygous strains are unlikely in an organism that is suspected to be panmictic (Mezhzherin and Mezhzherina 2000), the latter interpretation is unlikely. Lastly, it may be that the use of sporophores underestimates the true number of genets, since several genets may survive without fruiting (as demonstrated in Selosse et al. 1998a, 1999). However, sporophores have been shown to reflect the underground ectomycorrhizal population in some abundantly fruiting species such as *Suillus grevillei* (Zhou et al. 2001) and *Hebeloma cylindrosporum* (Guidot et al. 2001). In addition, the same underestimation could apply to populations B and C, so the relatively reduced genetic diversity in population A could still be significant.

Genet A1 lasted at least 3 years, a survival term that has been reported for genets of various ectomycorrhizal fungi, either pioneer species such as *Laccaria bicolor* (Selosse et al. 1998a, b, 1999) and *Hebeloma cylindrosporum* (Gryta et al. 2000) or late-stage species such as *Amanita francheti* (Redecker et al. 2001), *Pisolithus* sp. (Anderson et al. 2001), *Suillus* spp. (Dahlberg and Stenlid 1994; Dahlberg 1997; Bonello et al. 1998; Zhou et al. 2000) and *Xerocomus* spp. (Fiore-Donno and Martin 2001). Jahn and Jahn (1986) observed albino sporophores in a *L. aurantiacum* population over 23 years, suggesting a long-living genet; however, this could result from several short-living genets in a population where an albino allele was maintained at a high frequency. The data presented here constitute the first rigorous demonstration of longevity for a *Leccinum* genet.

Three years after the first fruiting, population A included a new genet. This indicates that recruitment is not finished and that genetic diversity can increase in this founder population. Genet A2 was genetically related to genet A1 (11 out of its 13 RAPD fragments were also found in genet A1) but was not a product of selfing, since it showed two RAPD fragments absent from genet A1. More likely, genet A2 arose by mating of an A1 basidiospore with a migrant basidiospore from another population. The slow recruitment in this *Leccinum* spp. population is compensated by a vigorous vegetative growth that ensured the development of genet A1. The fruiting area was extended from 1998 to 2001, moving outwards from the host trees, as often described under newly established trees (Ford et al. 1980; Mason et al. 1982). This is congruent with experiments showing that *Leccinum* spp. propagate through mycelial strands, using host-derived photosynthates in the colonization of new roots, since severing established roots reduces mycorrhizal colonization by *Leccinum* (Deacon et al. 1983; Fleming 1984; Newton 1992). Results of the latter severing experiments also imply that establishment from spores is a rare event, as observed in population A, explaining why *Leccinum* spp. are late fruiters of new tree stands, not fruiting before 5 years under *Betula* spp. (Mason et al. 1982, 1983; Last et al. 1987).

This is, to my knowledge, the first report on the genetic structure of early ectomycorrhizal populations of newly established tree stands. From the study of forest sites harbouring trees of different ages, mainly with the *Suillus* model (Dahlberg and Stenlid 1994; Dahlberg 1997), it was inferred that long-standing populations of ectomycorrhizal fungi were first made of several small genets whose size increased with time. Competitive elimination of genets was supposed to lead to populations made of less numerous but larger genets. A first challenge to this view came from the reports of several small, and thus possibly recently established, genets in old popula-

tions, either alone (Gherbi et al. 1999; Redecker et al. 2001) or coexisting with larger and older genets (Anderson et al. 2001; Sawyer et al. 2001). The present data now challenge the idea that young populations are made of smaller genets. In this study, the most distant sporophores of genet A1 were 10.4 m away in 2001. This is the same order of magnitude as for genets in old populations (e.g. Dahlberg 1997; Bonello et al. 1998; Sawyer et al. 2001), but strikingly exceeds that of the genet from older *L. duriusculum* populations reported here. The largest genet was B3 with most distant sporophores being 6.5 m away. However, the sizes may be underestimated because (1) the sampling zone may not reveal the whole fruiting area and (2) the fruiting area may be more reduced than the vegetative area. The present data are strikingly congruent with those of Gryta et al. (1997, 2000) on *Hebeloma cylindrosporum* from French coastal dunes, which forms large and lasting genets in the colonization zone, while genets are small and annual in older forest zones (Guidot et al. 2002). However, *H. cylindrosporum* genets may be limited by other factors, such as the size of the secondarily disturbed sandy zones where they grow. Although not in a primary succession context, a similar trend arose in *Suillus grevilleii* populations under 35-year-old *Larix kaempferi*, as compared to a population under >85-year-old trees (Zhou et al. 2000). The former harboured larger genets, a condition that was explained by (1) greater animal disturbance, contributing to mycelial dispersal, (2) presence of other tree species (that are clearly not involved here) and (3) by the fact that fewer genets were present, thus reducing intraspecific competition. In the present study, I favour the latter explanation.

A slow recruitment during the initial stand colonization can in fact allow a fast vegetative growth of a few genets that rapidly reach a large size. Since the most distant A1 sporophores were 10.4 m away in 2001 and since the genet probably established in 1996, this means a diameter growth of 2.08 m/year, i.e. a radial growth from the centre of the patch at about 1 m/year. This may be overestimated since genet A1 was perhaps present without fruiting before 1996, but the distance between outermost sporophores in 2000 and 2001 is of the same magnitude. Similar fast growth has been reported for Basidiomycetes (Bonello et al. 1998; Selosse et al. 1999; Selosse 2001 and references therein). This rapid growth may also be enhanced by poplar root growth. Poplars are fast-growing organisms, especially in such low-density populations. In primary colonizations, the low inoculum pressure may allow sizes that would not be reached in competition with other genets. The observed growth of genet A1 may, therefore, be another outcome of the founder effect. In earlier reports on populations found under relatively young trees (de la Bastide et al. 1994; Dahlberg and Stenlid 1994; Dahlberg 1997; Zhou et al. 2000), concerning secondary colonizations, such large and fast-growing genets were not detected. A higher inoculum level (mycelium and spores from neighbouring stands, or surviving in soil) likely allows the settlement of numerous genets that are reciprocally slow and limit their growth by competition. Such a situation probably occurs in populations B and C, where the growth rate is unlikely to exceed that of genet A1, because of the limited size reached by these genets.

More studies on primary successions are needed to investigate the colonization and growth strategies of ectomycorrhizal fungi on newly established tree stands. Similarly, the diversity of these strategies among ectomycorrhizal species awaits further work. As is well known at the community level (Last et al. 1987), population structure in primary and secondary successions can be expected to differ significantly.

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