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Population genetic structure of an ectomycorrhizal fungus *Amanita manginiana* in a subtropical forest over two years

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Abstract The population genetic structure of the late-stage fungus *Amanita manginiana* in a natural forest in Dujiangyan, southwest China was examined over two years using inter-simple sequence repeat (ISSR) markers. Seven ISSR primers were used and 170 bands were obtained in this population: 134/160 and 135/153 bands were polymorphic for sporocarps of 2001 and 2002, respectively. Each sporocarp represented a single genet in 2001 and 2002, and no identical genets were found between the two years. The results of genetic similarity comparison, using unweighted pair group method with arithmetic means, and analysis of molecular variance, indicated that although genetic variances were mainly within individuals of the same year the genetic variance between years was statistically significant ($P<0.001$). Relationships between genetic similarity and spatial distance of pairwise sporocarps were also found to be different in the two years. The differences in genetic structure and genetic similarity between individuals of the two years implied that the sporocarps were not likely to be derived from continuous generations, i.e., the sporocarps collected in 2002 were not developed from sexual spores dispersed by sporocarps of 2001. We suggest that the life-cycle traits of ectomycorrhizal (ECM) fungi should be considered in genetic studies on ECM fungal populations.

Keywords *Amanita manginiana* · Inter-simple sequence repeat · Genetic structure · Life-cycle traits

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Introduction

Ectomycorrhizas (ECM) are mutualistic structures between plants and soil fungi (Molina et al. 1992; Redecker et al. 2001). ECM fungi obtain carbon resources from the host plants and simultaneously provide water and nutrients to the hosts. It is also generally accepted that ECM fungi are beneficial to the growth and abiotic or biotic resistance of the host plants (Harley 1991). Since the majority of host plants in these associations are trees, including many dominant species in forest ecosystems (Molina et al. 1992), ECM fungi play important roles in nutrient transfer, inter- or intra-specific interactions, and maintenance of biodiversity in their habitats (Read 1991, 1997; Simard et al. 1997, 2002).

Molecular techniques have been used to distinguish “individuals” or genets within ECM fungal populations since the early 1990s (Jacobson 1993; Bastide et al. 1994; Doudrick et al. 1995). Compared with traditional methods, such as somatic incompatibility, molecular methods provide more sensitive and effective markers in the individual identification of ECM fungi, and are now being widely applied in genetic studies of mycorrhizal fungal populations (e.g., Timonen et al. 1997; Bonello et al. 1998; Junghans et al. 1998; Sawyer 1999; Wurzburger et al. 2001). Inter-simple sequence repeat (ISSR) markers were introduced to ECM fungi studies in 1997 (Longato and Bonfante 1997; Martin et al. 1997) and have proved a reliable, sensitive and technically simple method of assaying genetic variability (Lanfranco et al. 1998; Zhou et al, 1999, 2000).

The dikaryotic mycelia of an ECM fungus are generally considered as vegetative clones and sporocarps from the same clone may have identical genetic backgrounds. A group of sporocarps that have identical PCR profile patterns is defined as a genet, the basic genetic unit of an ECM fungal population (Zhou et al. 2000). Genet size indicates the ability of vegetative spread of the dikaryotic mycelium, and it shifts between ECM fungal species, ranging from a few meters to 40 m in diameter (Dahlberg 2001; Redecker et al. 2001). Because genet traits of early

and late colonizers of natural forests are generally different (Deacon and Fleming 1992; Dahlberg 2001; Redecker et al. 2001), the size and spatio-temporal pattern of ECM fungal genets may act as an indicator of succession stage and environmental changes of the host forest.

Although considered as typical colonizers of late stages of succession, species of *Amanita* were found to have small genets (Redecker et al. 2001). Redecker et al. (2001) suggested that intrinsic biological traits of the investigated species play an important role in the colonization strategy of the ECM fungus. More genetic studies on ECM fungal populations would therefore be helpful in finding the actual factors controlling the structure and dynamics of ECM fungal populations.

In a survey of ECM fungi in a subtropical broad-leaved forest in Dujiangyan, southwest China, *Amanita manginiana* Pat. et Hariot. was found to be one of the most common species. The genetic structure and spatial pattern of the *A. manginiana* population were studied over two years using ISSR markers. Our objectives were: (1) to compare the genetic structure of the ECM fungal population between the two years, and (2) to find the relationships between genetic structure and spatial pattern within the fungal population.

Materials and methods

Study site

The study site, 10 ha of subtropical woodlands, was on a low hill in Dujiangyan, on the western edge of the Sichuan Basin, southwest China (103°27′E, 30°44′N). Climatic data show a mean annual precipitation of 1,244 mm and a mean annual temperature of 15.2°C. The altitude of the site is about 780 m above sea level.

The study was conducted on a hillside, varying from 0° to 35° in slope (Fig. 1). The woodlands are dominated by evergreen broad-leaved trees, e.g., species of Lauraceae, Fagaceae, and Theaceae. Two subtropical conifers—*Pinus massoniana* Lamb. and *Cunninghamia lanceolata* (Lamb.) Hook.—are also common species there.

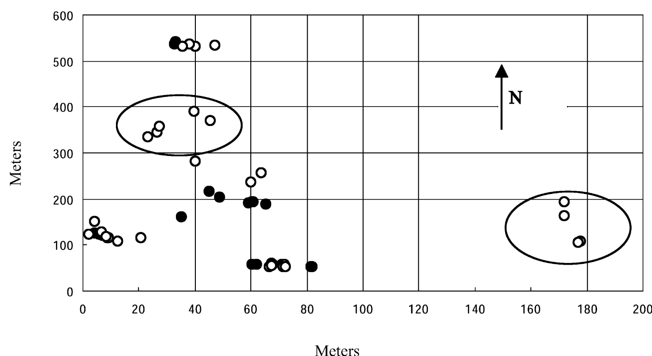

Fig. 1 Relative position of sporocarps in the study site. *Open circles* Sporocarps in 2001, *filled circles* sporocarps in 2002, *large ovals* areas where sporocarps were found only in 2001

Sampling procedure

From July to September of 2001 and 2002, sporocarps of *A. manginiana* appearing in the study site were collected daily. The sporocarps were then labeled, and dried for 24 h at 60°C. Each sporocarp was positioned using a GPS receiver (EtrexC, Garmin, USA) and further adjusted by field measurements (Fig. 1); 29 and 22 sporocarps were collected in 2001 and 2002, respectively.

DNA extraction

Genomic DNA was extracted from dried sporocarps according to the modified CTAB protocol (Guo et al. 2000). Dried pileus tissues (ca. 70 mg) were ground in 2 ml 2× CTAB extraction buffer (2% CTAB, 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl, 1% PVP-40) and ca. 0.2 g sterilized sand (white quartz; Sigma, St. Louis, Mo.). After incubation at 65°C for 1 h with occasional gentle swirling, 3 ml phenol-chloroform-isoamyl alcohol (25:24:1) was added, mixed well, and the solution was centrifuged at 13,000 *g* at room temperature for 15 min. The aqueous layer was extracted with an equal volume of chloroform-isoamyl alcohol (24:1) until no interface was visible. DNA was precipitated with a two-thirds volume of isopropanol at −20°C overnight and then spun at 10,000 *g* for 5 min in a microcentrifuge. DNA was rinsed twice with 70% ethanol, dried at room temperature and dissolved in 70 µl 1× TE buffer(10 mM Tris-HCl; 1 mM EDTA, pH 8.0).

ISSR assay

Seven primers—$(AC)_8YT$, $(CA)_6RY$, $(CA)_6RG$, $(GT)_6RY$, $(CTC)_4RC$, $(GTG)_3GC$, and $(CAC)_4RC$—were used in this study (R=C/G,Y=A/T). PCR amplification was performed in an automated thermal cycler (GeneAmp PCR System 2700; Applied Biosystems, Foster City, Calif.). ISSR-PCR was carried out in a 25 µl reaction volume containing PCR buffer [10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl, pH 8.8, 0.1% TritonX-100], 2.0 mM $MgCl_2$, 200 µM each deoxyribonucleotide triphosphate, 0.4 µM each primer, ca. 30 ng template DNA, and 1.25 U *Taq* DNA polymerase (Sino-American Biotechnology Company, China). A negative control using milliQ water instead of template DNA was included. The thermal cycling program was as follows: 3 min initial denaturation at 94°C, followed by 35 cycles of 30 s denaturation at 94°C, 1 min at the annealing temperature, 2 min extension at 72°C, and a final 10 min extension at 72°C. The annealing temperatures were 60°C for primer $(CAC)_4RC$ and 49°C for all other primers. A 5 µl sample of the products from each PCR reaction was examined by electrophoresis at 70 V for 5 h in a 1.8% (w/v) agarose gel in 0.5× TBE buffer. Gels were stained with ethidium bromide (0.5 µg/ml) and photographed under UV light.

Data and statistical analysis

Statistical analysis was performed using the NTSYS-pc program (version 2.02, Applied Biostatistics; see Rohlf 1997). The degree of similarity was estimated using Jaccard similarity coefficients: S_j=a/(a+b+c), in which the data are defined by a two-way contingency table such that for any pairwise comparison of isolates, a=(1, 1), b=(1, 0), c=(0, 1), with ‘1’ denoting presence and ‘0’ denoting absence of a band. Based on simple matching coefficients, a dendrogram was constructed using SAHN (Sequential Agglomerative Hierarchical Nested) clustering using the UPGMA (the unweighted pair group method with arithmetic means) function of the NTSYS-pc program.

Analysis of molecular variance (AMOVA) was conducted using DCFA1.1 (Zhang and Ge 2002) and WINAMOVA1.55 (Excoffier 1993; Excoffier et al. 1992) to determine the genetic variances within and between the two years. Euclidean squared distances (δ^2_{xy}) instead of Jaccard similarity coefficients were used in

Table 1 Inter-simple sequence repeat (ISSR) bands of the *Amanita manginiana* population over two years

Primer	Totalnumber of bands	Polymorphic/ total bands in 2001	Polymorphic/ total bands in 2002	Common bands of all individuals (bp)	Bands (bp) found only in 2001	Bands (bp) found only in 2002
$(AC)_8$-YT	24	18/22	18/23	–	725	325; 1,300
$(CA)_6$-RY	23	18/23	15/19	535	140; 835; 1,105; 1,630	
$(CA)_6$-RG	23	17/23	16/18	–	120; 170, 745; 1,465; 1,600	
$(GT)_6$-YR	23	14/19	20/23	–		150; 635; 865; 1,485
$(CTC)_4$-RC	23	16/20	21/21	–	300; 650	145; 495; 1,910
$(GTG)_3$-GC	25	22/24	22/24	–	320	180
$(CAC)_4$-RC	29	29/29	23/29	–	120; 500; 625; 1,545	
Total	170	134/160	135/153	–	–	–

AMOVA as suggested by Zhang and Ge (2002) and Excoffier (1993). δ^2_{xy} was obtained by the following equation:

$$\delta^2_{xy} = \sum_{i=1}^{S} (x_i - y_i)^2 \quad (1)$$

where for two individuals x and y, x_i and y_i are presence or absence of a particular molecular marker i, and *S* is the total number of markers in AMOVA.

The average S_J of each year and between the two years were compared using one-way ANOVA (SPSS 10.0 for Windows; SPSS, Cary, N.C.). In order to find the next of kin to each sporocarp, the maximum S_J for each sporocarp in each year as well as the maximum S_J in 2001 for each sporocarp of 2002 were also calculated.

Average genetic diversities in each year and in the whole population were estimated using the Shannon-Weaver diversity index (*H*), calculated as follows: $H = -\frac{1}{k}\sum_{i=1}^{k} p_i \times \ln p_i$, where *k* is the total number of the kinds of ISSR fragments, and p_i is the frequency of a given band.

Spatial distance and genetic similarity

The spatial distance between each two sporocarps was calculated according the relative position of the sporocarps. Regression analyses were conducted to find the relationship between spatial distance and genetic similarity (estimated by S_J) (SPSS 10.0). The spatial distance of the next of kin in each year—possible sisters—were also compared using SPSS 10.0.

Results

ISSR assay

In total, 170 reproducible ISSR markers were obtained during the 2-year study, and 161 of these were polymorphic. No identical profile was found in the population during the two years, which indicated that each sporocarp represented a genet or an individual in the ECM fungal population. For the population of 2001 and 2002, 134/160 and 135/153 were polymorphic, respectively (Table 1); 17 and 10 ISSR markers were found only in individuals of 2001 and 2002, respectively (Table 1). A specific band, $(AC)_8$YT-325, was found in all individuals of 2002 but not found in any individuals of 2001. A universal ISSR band, $(CA)_6$RY-535, was found in all individuals of both years.

The results of SAHN clustering are shown in Fig. 2. Sporocarps designated Am1–Am22 were collected in 2002, and the others were collected in 2001. Two genetic groups were found in 2002. While one genetic group of 2002 had relatively high genetic similarity with a major group (23/29 sporocarps) of 2001, the individuals of the same year tended to cluster together. Jaccard similarity coefficients (mean ± standard error) of population in 2001, 2002 and between the two years were 0.5007±0.0041, 0.4717±0.0083, and 0.4188±0.0029, respectively. All the differences were statistically significant (F=154.42, P<0.001), which indicated that genetic similarities between individuals of 2001 were higher than those of 2002 and genetic similarities within years were much higher than those between years. Genetic similarities of the next of kin (i.e., the individual with the highest S_J to each sporocarp), however, showed contrasting trends, i.e., average genetic similarity of the next of kin in 2001 was significantly lower (P=0.012) than that in 2002 (0.6531 vs 0.7202). Regarding individuals in 2002, average genetic similarity of their next of kin in 2001 was 0.5021, which was significantly lower than those of either 2001 or 2002 (P<0.001).

The average genetic diversities for 2001, 2002 and both years, shown as mean ± standard error, were 0.4240 ±0.0186, 0.4425±0.0185, and 0.4768±0.0151, respectively. Genetic diversity for 2002 was slightly higher than that for 2001, but the difference was not significant (P=0.480). Since the genetic diversities for 2001 and 2002 were a high proportion of the whole population (88.9%

Table 2 Analysis of molecular variance (AMOVA) for 51 individuals of *A. manginiana* in two years

Source of variation	*df*	SSD	MSD	Variance component	Total (%)	*P*-value
Between years	1	142.8	142.8	4.647	14.9	<0.001
Within years	49	1,301.7	26.6	26.566	85.1	<0.001

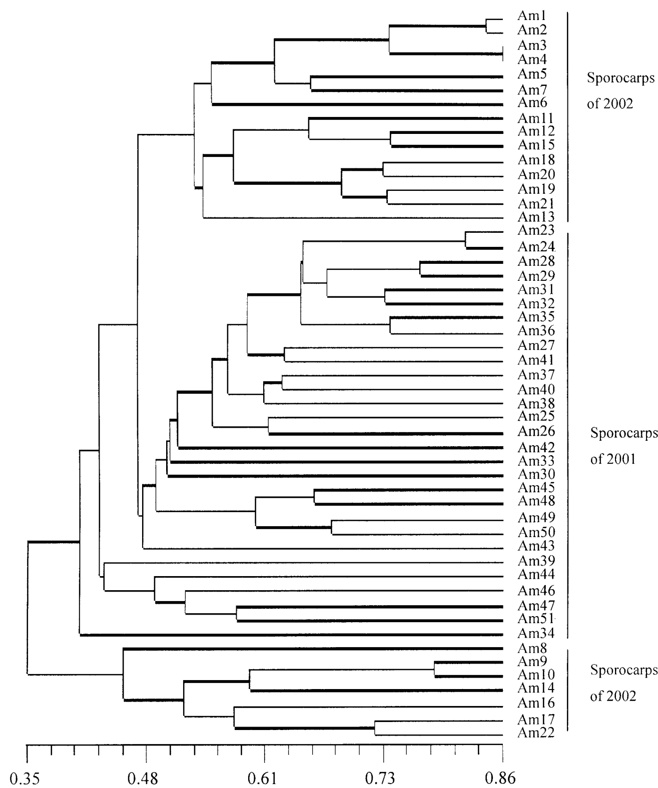

Fig. 2 Phylogenetic tree obtained from Jaccard similarity coefficients of *Amanita manginiana* using unweighted pair group method with arithmetic means (UPGMA) in the program NTSYS-pc

and 92.8% respectively), genetic variances primarily occurred within years.

The results of AMOVA are listed in Table 2. Genetic variances, which were similar to genetic diversity analysis using the Shannon-Weaver index, were found mainly within years (85.1%). Similar to the genetic similarity assay above, genetic variances between years, accounting for 14.9% of the total genetic variances, were also significantly different (P<0.001).

Genetic similarity and spatial distance

Relations between genetic similarity (S_J) and spatial distance are shown in Fig. 3. Genetic similarity between pairwise individuals of 2001 was found to be negatively correlated with spatial distance (r=0.20, P<0.001). A quadratic rather than linear regression was found between S_J and spatial distance in 2002 individuals (r=0.37, P<0.001). The lack of data between 170 and 300 m is due to the absence of sporocarps in the two areas in the year 2002 (see Fig. 1).

The average distance of the next of kin to each individual in 2001 was significantly higher than that in 2002 (137.5 vs 48.3, P=0.016). Most of the distances (55%) of 2001 were in the range of medium distance (from 20 to 200 m), whereas most distances (77%) of 2002 were very limited (less than 20 m).

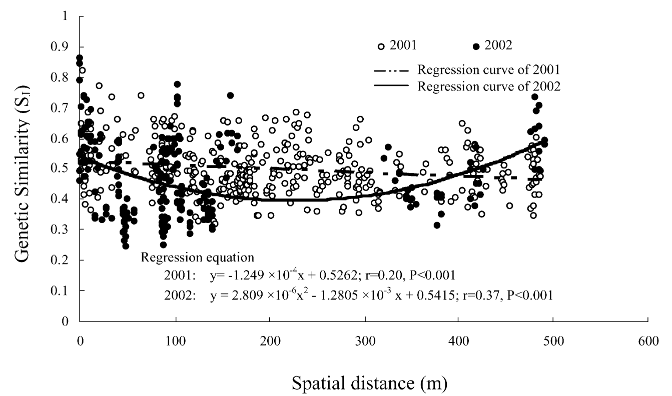

Fig. 3 Regression analysis between spatial distance and genetic similarity (S_J) of pairwise sporocarps collected in 2001 and 2002

Discussion

While sporocarps of some ECM fungal species, e.g. *Cantharellus cibarius* (Danell and Camacho 1997), *Pisolithus tinctorius* (e.g., Gong et al. 1994), *Hebeloma westraliense* (Malajczuk et al. 1994), and *Laccaria laccata* (Malajczuk et al. 1994), have been obtained in some inoculation experiments, and the moisture, temperature and nutrients requirements of some species have been well documented, the biological and lifecycle traits of ECM fungi in natural forest are still poorly understood (Watling 1997).

Molecular techniques have made it feasible to study the genetic structure of an ECM fungal population by providing sensitive markers for individual identification and assay of genetic variation within populations. Recent studies have focused mainly on genet assays and have made progress in understanding the size and distribution of genets of some ECM fungi (e.g., Zhou et al. 1999, 2000, 2001; Dahlberg 2001; Fiore-Donno and Martin 2001; Redecker et al. 2001).

Genetic studies on ECM fungal populations have shown that early colonizers, e.g., *Laccaria* and *Hebeloma* species, tend to have small and non-persistent genets, whereas late-stage fungi, e.g. *Suillus* and *Cortinarius* species, tend to have large and persistent genets (Dahlberg 2001). Some late-stage species, e.g., *Lactarius xanthogalactus*, *Russula cremoricolor* and *Amanita francheti*, however, were also found to have small genets in recent studies (Redecker et al. 2001). Our results indicate that each genet of *A. manginiana* in the study site was represented by a single sporocarp, which agreed with Redecker et al. (2001). The genet traits of *Amanita*, *Russula* and *Lactarius* fungi imply that besides early or late-stage classification, more biological traits should also be considered in genetic studies on ECM fungal populations.

The dynamic characteristics of ECM fungal populations, indicating shifts and trends of fungal succession, are an important aspect of genetic studies on these ECM fungi. It has been found that genet size tends to become larger as forests mature (Dahlberg and Stenlid 1990; Frankland 1998). Short-term variation in ECM fungal

populations, e.g., the number of sporocarps, the number of genets, and the spatial distribution and size of genets, have also been the focus of some studies (e.g., Zhou et al. 2000, 2001; Redecker et al. 2001).

Some biological traits of the fungi might also be involved in the dynamics of ECM fungal populations. The first trait is persistence of genets or clones. ECM fungi with persistent genets would tend to have relatively stable populations whereas rapid turnover would result in large shifts between years. The second trait is the mode of expansion. An ECM fungal population established by vegetative growth of mycelia would be relatively stable, whereas an ECM fungal population formed by spore dispersal would depend more on changes in environmental factors. The third trait is the lifecycle of the fungi. The time-span from sexual spores to sporocarps, and the sporocarp-forming frequency of each genet, would also influence the dynamics of an ECM fungal population. The first two traits of ECM fungi are related to genet size and are generally considered integratively. ECM fungal populations with large genets are generally expected to have persistent genets and to be established mainly by vegetative growth, whereas those with small genets are expected to have non-persistent genets and to be established mainly by sexual spores (e.g., Frankland 1998; Redecker et al. 2001). The lifecycle traits of ECM fungi, however, are not mentioned in most studies.

In our study, the *A. manginiana* population showed different genetic structures in the two years: (1) no identical genets were found in 2001 and 2002; (2) genetic similarities between individuals of 2002 were much lower than those of 2001; (3) the genetic variance between years was statistically significant; (4) a linear regression was found between S_J and spatial distance in 2001, whereas a quadratic regression was found in 2002.

It is also interesting that the average genetic similarity as well as the maximum S_J (genetic similarity of parent and child of continuous generations or of siblings in one generation) for each individual between years was significantly lower than those within a year, since parent and child as well as siblings share 50% of their genes. A probable reason was that the individuals of 2001 were not the parents of most individuals of 2002, i.e., the majority of sporocarps in 2002 were not developed from the sexual spores dispersed by individuals of 2001. This is reasonable, since it generally takes more than 1 year to obtain a sporocarp around the inoculated seedlings in controlled conditions (Gong et al. 1994; Malajczuk et al. 1994; Danell and Camacho 1997), and it might take an even longer period in natural forests.

The lifecycle of an ECM fungus of basidiomycotina generally consists of four stages, i.e., sporocarps, sexual spores, primary mycelia (N) and secondary mycelia (2N). Because it is not feasible to study primary and secondary mycelia in soil, most studies on the genetic structure of ECM fungal populations are based on data for sporocarps. The span between sporocarps of continuous generations depends greatly on the underground development of the mycelia. If sexual spores can develop into sporocarps

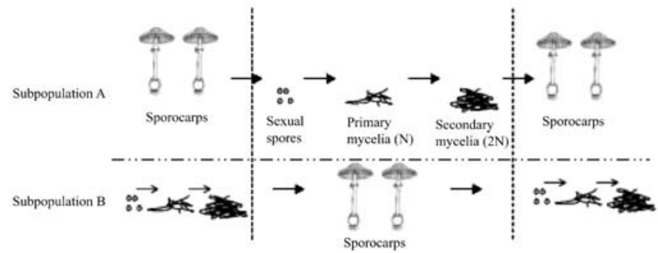

Fig. 4 Structure of an ectomycorrhizal (ECM) fungal population showing the effects of lifecycle traits. The population consists of non-persistent genets and the span of the underground development of the fungus is 2 years. *Vertical dotted lines* Borderline between different years, *horizontal dashed line* temporal isolation between subpopulations

within a year, the sporocarps of different years could form continuous generations. If the development from basidiospores to sporocarps needs more than 1 year, however, offspring (sporocarps) of individuals of 1 year will not be found in the next year. If the span of development is constant and over 1 year (e.g., 2 years) for all individuals of an ECM fungal population, the local population will be composed of two subpopulations appearing on the surface in different years (Fig. 4). Because of the poor viability of basidiospores and primary mycelia of late-successional ECM fungi, individuals in different subpopulations will have no chance to mate and a kind of "temporal isolation" could be found in the population of a non-persistent ECM fungal species. Such temporal isolation differs from geological isolation, and depends greatly on the lifecycle traits of the fungal species. For some fungal species with variable lifespan, the temporal isolation will be broken by gene flow between subpopulations. In a local ECM fungal population, with relatively homogeneous plant composition and environmental factors, most individuals should have a relatively constant span of development, and gene flow between subpopulations would be carried by some individuals with faster or slower development.

Other biological traits of ECM fungal species should also be considered in the study of an ECM fungal population. Temporal isolation between subpopulations could be found in ECM fungal populations with non-persistent genets, and could be affected by persistence of genets and fruiting ability of ECM fungal species. If the ECM fungus has persistent genets and fruits in continuous years, the genetic variance between years would be negligible and there would be only a continuous population with overlapping generations in the forest. If the fungal species has persistent genets and fruits irregularly in different years, the population structure will be much more complex and relatively unpredictable.

Our results also imply that there were two genetic groups of sporocarps in 2002. The relationship between these two groups and the individuals in 2001 should be determined from long-term studies on this ECM fungal population. Since only the above-ground period of the ECM fungal lifecycle was the subject of the present study, our further studies on root-tips and mycelia underground using more specific markers (e.g., SCAR, SSR) will help

describe the whole lifecycle of the fungus and to understand the spatial-temporal structure of this ECM fungal population.

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References

Bastide PD, Kropp BR, Piché Y (1994) Spatial distribution and temporal persistence of discrete genotypes of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) Orton. New Phytol 127:547–556

Bonello P, Bruns T, Gardes M (1998) Genetic structure of a natural population of the ectomycorrhizal fungus *Suillus pungens*. New Phytol 138:533–542

Dahlberg A (2001) Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. New Phytol 150:555–562

Dahlberg A, Stenlid J (1990) Population structure and dynamics in *Suillus bovines* as indicated by spatial distribution of fungal clones. New Phytol 115:487–493

Danell E, Camacho FJ (1997) Successful cultivation of the golden chanterelle. Nature 385:303

Deacon JW, Fleming LV (1992) Interactions of ectomycorrhizal fungi. In: Allen MF (ed) Mycorrhizal functioning: an integrative plant-fungal process. Chapman and Hall, New York, pp 249–300

Doudrick RL, Raffle VL, Nelson CD, Furnier GR (1995) Genetic analysis of homokaryons from a basidiome of *Laccaria bicolor* using random amplified polymorphic DNA (RAPD) markers. Mycol Res 99:1361–1366

Excoffier L (1993) Analysis of molecular variance (AMOVA) ver 1.55. Genetics and Biometry Laboratory, University of Geneva, Switzerland

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: applications to human mitochondrial DNA restriction data. Genetics 131:479–491

Fiore-Donno AM, Martin F (2001) Populations of ectomycorrhizal *Laccaria amethystine* and *Xerocomus* spp. show contrasting colonization patterns in a mixed forest. New Phytol 152:533–542

Frankland JC (1998) Fungal succession—unraveling the unpredictable. Mycol Res 102:1–15

Gong M-Q, Wang F-Z, Chen Y (1994) Advances of ectomycorrhizal research for Eucalyptus. Acta Pedol Sin 31[Suppl]:127–133

Guo LD, Hyde KD, Liew ECY (2000) Identification of endophytic fungi from *Livistona chinensis* based on morphology and rDNA sequences. New Phytol 147:617–630

Harley JL (1991) Introduction: the state of the art. In: Norris JR, Read DJ, Varma AK (eds) Methods in microbiology, vol 23. Techniques for the study of mycorrhiza. Academic press, London, pp 1–23

Jacobson KM, Miller OK, Turner BJ (1993) Randomly amplified polymorphic DNA markers are superior to somatic incompatibility test for discrimination genotypes in natural populations of ectomycorrhizal fungus *Suillus granulats*. Proc Natl Acad Sci USA 90:9159–9163

Junghans DT, Gomes EA, Guimarães WV, Barros EG, Araújo EF (1998) Genetic diversity of the ectomycorrhizal fungus *Pisolithus tinctorius* based on RAPD-PCR analysis. Mycorrhiza 7:243–248

Lanfranco L, Perotto S, Bonfante P (1998) Applications of PCR for studying biodiversity of mycorrhizal fungi. In: Bridge PD, Arora DK, Reddy CA, Elander RP (eds) Applications of PCR in Mycology. CAB International, Wallingford, pp 107–124

Longato S, Bonfante P (1997) Molecular identification of mycorrhizal fungi by direct amplification of microsatellite regions. Mycol Res 101:425–432

Malajczuk N, Grove TS, Bougher NL, Dell B, Gong M-Q (1994) Ectomycorrhizas and nutrients: their importance to Eucalyptus in China. In: Brown AG (ed) Australian tree species research in China. ACIAR proceedings, no. 48. Canberra, pp 132–139

Martin F, Costa G, Delaruelle C, Diez J (1997) Genomic fingerprinting of ectomycorrhizal fungi by microsatellite-primed PCR. In: Varma A (ed) Mycorrhizal manual. Springer, Berlin Heidelberg New York, pp 463–474

Molina R, Massicotte H, Trappe JM (1992) Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen MF (ed), Mycorrhizal functioning: an integrative plant-fungal process. Chapman and Hall, New York, pp 357–423

Read DJ (1991) Mycorrhizas in ecosystems. Experientia 47:376–391

Read DJ (1997) Mycorrhizal fungi—the ties that bind. Nature 388:517–518

Redecker D, Szaro TM, Bowman RJ, Bruns TD (2001) Small genets of *Lactarius xanthogalactus*, *Russula cremoricolor* and *Amanita francheti* in late-stage ectomycorrhizal successions. Mol Ecol 10:1025–1034

Rohlf FJ (1997) NTSYS-pc, version 2.02. Exeter Software, Applied Biostatistics Inc.

Sawyer NA, Chambers SM, Cairney JWG (1999) Molecular investigation of genet distribution and genetic variation of *Cortinarius rotundisporus* in eastern Australian sclerophyll forests. New Phytol 142:561–568

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R (1997) Net transfer of carbon between ectomycorrhizal tree species in the field. Nature 388:579–582

Simard SW, Jones MD, Durall DM (2002) Carbon and nutrient fluxes within and between mycorrhizal plants. In: van der Heijden MGA, Sanders IR (eds) Mycorrhizal ecology. Springer, Berlin Heidelberg New York, pp 34–74

Timonen S, Tammi H, Sen R (1997) Outcome of interactions between genets of two *Suillus* spp. and different *Pinus sylvestris* genotype combinations: identity and distribution of ectomycorrhizas and effects on early seedling growth in N-limited nursery soil. New Phytol 137:691–702

Watling R (1997) The business of fructification. Nature 385:299–300

Wurzburger N, Bidartondo MI, Bledsoe CS (2001) Characterization of *Pinus* ecotmycorrhizas from mixed conifer and pygmy forests using morphotyping and molecular methods. Can J Bot 79:1211–1216

Zhang F-M, Ge S (2002) Data analysis in population genetics. I. Analysis of RAPD data with AMOVA. Biodiv Sci 10:438–444

Zhou ZH, Miwa M, Hogetsu T (1999) Analysis of genetic structures of a *Suillus grevillei* population in a *Larix kaempferi* stand by polymorphism of inter-simple sequence of repeat (ISSR). New Phytol 144:55–63

Zhou ZH, Miwa M, Hogetsu T (2000) Genet distribution of ectomycorrhizal fungus *Suillus grevillei* populations in two *Larix kaempferi* stands over two years. J Plant Res 113:365–374

Zhou ZH, Miwa M, Hogetsu T (2001) Polymorphism of simple sequence repeats reveals gene flow within and between ectomycorrhizal *Suillus grevillei* populations. New Phytol 149:339–348