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Genetic structure of a population of the ectomycorrhizal fungus *Russula vinosa* in subtropical woodlands in southwest China

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Abstract The genetic structure of a population of the ectomycorrhizal fungus *Russula vinosa* was analyzed using random amplified polymorphic DNA markers. Of 121 bands, 114 (94.2%) were polymorphic and there was a high genetic diversity (H=34.98) in this population. Each sporocarp represented a different genet and the genet size was no larger than 1 m. Pairwise sporocarps closer than 10 m had significantly higher genetic similarity. Second-order analysis indicated clumps with a radius of about 20 m in the whole population as well as in three genetic groups, i.e. simple matching similarity coefficients (S_m) 0.5–0.6, 0.6–0.7, and 0.7–0.8, respectively. The high-genetic-similarity group tended to have small clumps with high density, whereas the low-genetic-similarity group tended to have large clumps with low density. The spatial pattern analysis showed that the population mainly spread by short-distance spore dispersal rather than vegetative growth of dicaryophytic mycelia or long-distance spore dispersal.

Keywords Genet · Genetic similarity · RADP · Second-order analysis · Spatial pattern

Introduction

Ectomycorrhizas (ECM) are mutualistic symbioses between plants and soil fungi (Molina et al. 1992; Redecker et al. 2001). 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 intraspecific interactions and the maintenance of biodiversity in their habitats (Read 1991, 1997; Simard et al. 1997, 2002). Analyses of the genetic structure of ECM fungal populations could, therefore, help to understand the structure and functioning of natural ecosystems.

Because it is not feasible to distinguish "individuals" within EMF populations using morphological methods, somatic incompatibility tests have been introduced and different clones or genets in the field recognized (Fries 1987; Dahlberg and Stenlid 1990, 1994, 1995). Molecular techniques have been used in the identification of closely related individuals of ECM fungi (Smith et al. 1992; Hadrys et al. 1992; Jacobson 1993; Kerrigan et al. 1993; Bastide et al. 1994; Doudrick et al. 1995). Compared with traditional approaches, molecular methods provide more sensitive and effective markers for the individual identification of ECM fungi and recently they have been widely applied in genetic studies of mycorrhizal fungal populations (Timonen et al. 1997; Bonello et al. 1998; Junghans et al. 1998; Sawyer et al. 1999; Bergemann and Miller 2002).

The mycelia of an ECM fungus are generally considered as vegetative clones and sporocarps from the same clone may have an identical genetic background. A group of sporocarps with identical or nearly identical PCR profile patterns is defined as a genet, the basic genetic unit of an ECM fungi population (Zhou et al. 2000). Genet size indicates the vegetative spread capability of the dicaryophytic mycelia and varies among ECM species, ranging from a few meters to 40 m in diameter (Dahlberg 2001; Redecker et al. 2001). Early colonizers of disturbed areas, e.g. *Hebeloma cylindrosporum* and *Laccaria amethystina*, appeared to have small and nonpersistent genets, whereas ECM fungi appearing late in a succession, e.g. *Cortinarius rotundisporus*, are expected to have large and persistent genets (Deacon and Fleming 1992; Dahlberg 2001). The size and spatio-temporal pattern of ECM genets could, therefore, be an

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indicator of succession stage and environmental change in the host forest.

Although considered to be typical colonizers of late succession stages, species of *Russula* and *Lactarius*, e.g. *R. cremoricolor*, *L. xanthogalactus*, were found to have small genets (Redecker et al. 2001). The authors suggested that intrinsic biological traits of the investigated species play an important role in colonization strategies of ECM fungi. Further genetic studies on ECM populations, especially species of *Russula* and *Lactarius*, would help determine the actual factors controlling the structure and dynamics of ECM populations.

Spatial pattern is the arrangement of points, of plants or other organisms, or of patches of organisms in space that shows a certain predictability. The spatial pattern of a plant population could be affected by the growth pattern of plants, biotic or abiotic environmental factors (Dale 1999). In a relatively uniform environment or where environmental factors are randomly arranged, the spatial pattern of a population could indicate biological traits of the species. Second-order analysis, based on the distances between pairs of points, can be considered as an examination of cumulative frequency distribution (Dale 1999). The method is one of the most commonly used for studying the spatial pattern of mapped points (Andersen 1992).

In a survey of ECM in a subtropical broad-leaved forest in Dujiangyan, western China, *Russula vinosa* was found to be one of the most common species. Genetic structure and spatial pattern of the *R. vinosa* population were studied using RAPD markers and second-order analysis, respectively. Our objective here was to determine the relationship between genetic structure and spatial pattern of the fungal population in subtropical woodlands.

Materials and methods

The study site, 6.4 ha of subtropical woodlands, was on a low hill in Dujiangyan at the western edge of the Sichuan Basin in southwestern China (103° 27′ E, 30° 44′ N). The site has a mean annual precipitation of 1,244 mm, a mean annual temperature of 15.2°C, and an altitude of about 780 m above sea level.

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

Sampling procedure

From July to September 2001, sporocarps of *R. vinosa* Lindbl. were collected every other day. The sporocarps were labeled and dried for 24 h at 60°C. Some large trees at the study site were marked and positioned by GPS (EtrexC, Garmin Ltd., USA) and sporocarps were then mapped relative to the marked trees (Fig. 1). One representative was collected from a circle 1 m in diameter, and 32 sporocarps in total were used in this study.

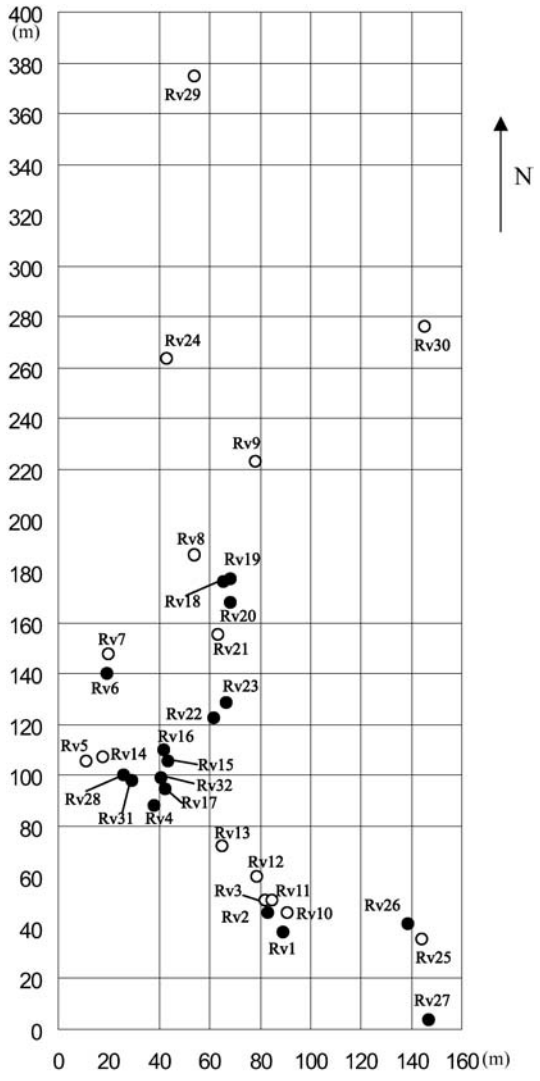

Fig. 1 The relative position of sporocarps in the study site (○ single sporocarps, ● sporocarps collected in aggregations)

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 of 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). After incubation at 65°C for 1 h with occasional gentle swirling, 3 ml of a solution of phenol-chloroform-isoamyl alcohol (25:24:1) was added, well mixed, and the combined solution 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 isopropanol (2/3 volume of the aqueous phase) 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 and 1 mM EDTA pH 8.0).

RAPD assay

Six primers, i.e. S226 (5′-ACGCCCAGGT-3′), S228 (5′-GGACG-GCGTT-3′), S383 (5′-CCAGCAGCTT-3′), S392 (5′-GGGCGG-TACT-3′), S396 (5′-AGGTTGCAGG-3′) and S1216 (5′-CCTT-GCGCCT-3′), were used in this study (Shanghai Sangon Biologic and Engineering Technology and Service Co. Ltd., Shanghai, China). PCR amplification was performed basically following the method of Williams et al. (1990) in an automated thermal cycler (PTC-100, MJ Research, Inc., Watertown, Mass., USA). RAPD-PCRs were 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), 2.5 mM $MgCl_2$, 200 μM of each deoxyribonucleotide triphosphate, 0.4 μM of each primer, ca. 30 ng template DNA, and 1.25 units of *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 45 cycles of 30 s denaturation at 94°C, 1 min primer annealing at 35°C, 2 min extension at 72°C, and a final 10 min extension at 72°C. Five microliters of PCR products from each PCR reaction were 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.

RAPD data analysis

Amplified fragments were scored as 1 for presence and 0 for absence. Statistical analysis of the data was performed using the NTSYS-pc program (version 1.70; Rohlf 1992). The degree of similarity was estimated using simple matching coefficients (Sneath and Sokal 1973): $S_m=(a+d)/(a+b+c+d)$, 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), d=(0,0), with 1 denoting presence and 0 absence of a band. Based on simple matching coefficients, a dendrogram was constructed using SAHN clustering in the UPGMA (the unweighted pair group method with arithmetic means) in NTSYS-pc.

Genetic diversity of this population was estimated using the Shannon-Weaver diversity index (H) calculated by the following formula:

$$\mathrm{H} = -\sum_{i=1}^{k} pi \times \ln pi \quad (1)$$

where k is the total number of kinds of RAPD fragments and pi is the frequency of a given band.

In order to find differences in genetic similarity along the spatial gradient, the pairwise sporocarps were first divided into seven groups according to the spatial distance (less than 10 m, 10–20 m, 20–40 m, 40–80 m, 80–160 m, 160–320 m, and over 320 m), and S_m between groups compared using a t-test.

Spatial pattern analysis

Spatial patterns of the population were determined by second-order analyses based on Ripley's (1976) K-function. In the analyses, a circle of radius t is centered in each sporocarp and the number of neighbors within the circle counted. $K(t)$ is a function of t, with the expected value of πt^2 in randomly arranged populations. Ripley (1976) gave an approximately unbiased estimator for $K(t)$ as

$$\hat{K}(t) = An^{-2}\sum\sum w_{ij}^{-1} I_t(u_{ij}) \quad (2)$$

where n is the number of sporocarps in the analyzed plot, A the area of the plot in m^2, w_{ij} a weighting factor to correct for edge effects, u_{ij} the distance between sporocarp i and j, $I_t(u_{ij})$ is a counter variable, which is set to 1 if $u_{ij} \leq t$ and 0 if $u_{ij} > t$. w_{ij} were obtained according to edge correction methods of Getis and Frankin (1987) and Goreaud and Pélissier (1999). A further parameter, $L(t)$, was also used to determine the spatial pattern of the population (Thioulouse et al. 1997; Dale 1999):

$$\hat{L}(t) = \sqrt{\hat{k}(t)/\pi} - t \quad (3)$$

According to a series of t, from 0 to 80 m in this study, large negative values of $L(t)$ indicate overdispersal and large positive values indicate clumping. In order to find the spatial patterns of sporocarps with different genetic similarities, pairwise sporocarps were divided into three genetic groups according to the simple matching coefficient (0.5–0.6, 0.6–0.7, and 0.7–0.8). The spatial patterns of the three genetic groups were tested using equations (2) and (3); n in equation (2) was adjusted according to the number of pairwise distances:

$$n = 0.5 + \sqrt{2I(u_{ij}) + 0.25} \quad (4)$$

where $I(u_{ij})$ is the number of pairwise distances in the group.

The boundaries of the 99% confidence interval for the null hypothesis of complete spatial randomness (Poisson pattern) were computed using ADS (a free software for spatial data analysis, ver. 2001, available online: http://pbil.univ-lyon1.fr/ADE-4/ADSWebUS.html) in the ADE-4 package (Thioulouse et al. 1997).

Results

Genetic diversity

Genomic DNA of 32 sporocarps was amplified using 6 primers and 121 reproducible RAPD bands obtained, of which 114 were polymorphic. The size of amplified fragments ranged from 220 bp to 2,350 bp.

Genetic similarities between isolates, shown as simple matching coefficients, ranged from 0.487 to 0.885. Each sporocarp represented a different genet and no obvious genetic groups were found in the phenetic tree (Fig. 2). The percentage of single-sporocarp genets, i.e. genets represented by individual sporocarps, was ≥46.9% (15 out of 32 genets or more including aggregations with genets represented by single sporocarps). A high genetic diversity (H=34.98) was also obtained in this population.

Simple matching similarity coefficients of 7 different distance groups are shown in Fig. 3. S_m of group A was significantly higher than that of any other group, whereas there was no significant difference between the six other groups, i.e. B, C, D, E, F, and G.

Spatial pattern

Since 17 out of 32 samples were found in aggregations within a circle 1 m diameter, the population is obviously clumped on a small scale. As small-scale clumping may be due to vegetative growth of the fungus, further analyses were focused on the spatial pattern of genets or clones on a larger scale.

The $L(t)$ values of the population are shown in Fig. 4. The results indicated that the whole population as well as three genetic groups tended to be spatially clumped. $L(t)$ of different groups changed similarly with increase in radius (t): an increase with t from 0 to 20 m, a decrease with t from 20 to 40 m, and shifted where t was higher

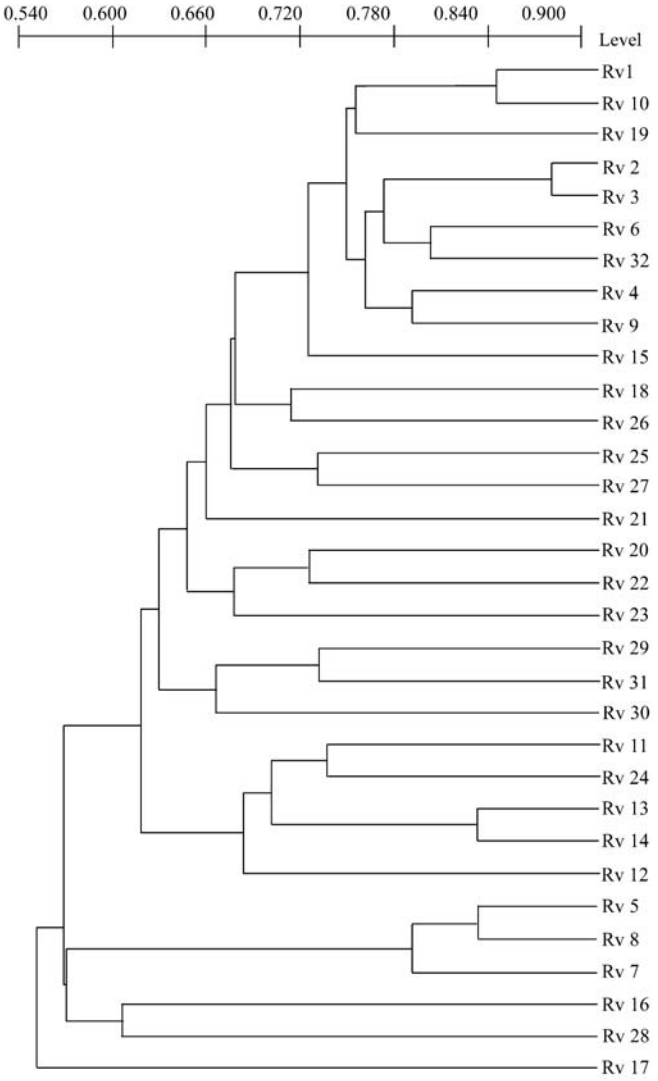

Fig. 2 Phenetic tree obtained from simple matching coefficients of *Russula vinosa* using UPGMA (the unweighted pair group method with arithmetic means) in NTSYS-pc

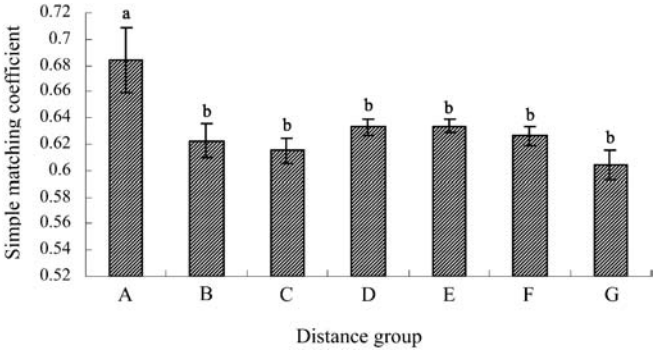

Fig. 3 Genetic similarity (shown as simple matching coefficient) of different distance groups. **A** less than10 m; **B** 10–20 m; **C** 20–40 m; **D** 40–80 m; **E** 80–160 m; **F** 160–320 m; **G** over 320 m. Different letters denote significant differences (α=0.05)

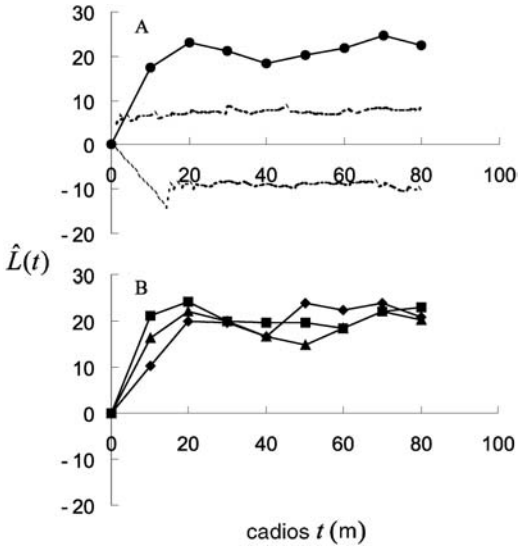

Fig. 4 Second-order analysis of the whole population (**A**) and three genetic groups (**B**) Large negative values of *L(t)* indicate overdispersal and large positive values indicate clumping. The dotted line indicates the boundaries of the 99% confidence interval for the null hypothesis of complete spatial randomness (Poisson pattern) (● the whole population, ◆ genetic group with S_m 0.5–0.6, ▲ genetic group with S_m 0.6–0.7, ■ genetic group with S_m 0.7–0.8)

than 40 m. Since it would increase errors to estimate *L(t)* at a large radius (*t*) due to bias in estimating edge effects, we mainly focused on radii (*t*) of less than 40 m. The largest *L(t)* values of three genetic groups, i.e. simple matching coefficients 0.5–0.6, 0.6–0.7, and 0.7–0.8, were 19.92, 21.98, and 24.19, respectively. If clumps with the same density [same *L(t)*]) were considered, clumps of the high-genetic-similarity group tended to have a smaller radius, e.g. when *L(t)* was 15, the radii of the three genetic groups, from low to high genetic similarity, were 15.22, 9.21 and 7.06 m, respectively. It was interesting that the radius of the clumps increased with decreasing genetic similarity, whereas the density of sporocarps in the clumps decreased.

Discussion

Two processes, growth of dicaryophytic mycelia and spread of basidiospores, are thought to be involved in the construction of ECM populations (Egger 1994; Dahlberg 2001). Short-lived and small genets may suggest colonization from recent sexual spores, whereas persistent and large genets may be from mycelial spread (Dahlberg 2001; Fiore-Donno and Martin 2001).

Our results indicated that the *R. vinosa* population had a high genetic diversity and included many single-sporocarp genets, which agrees with a study of *R. cremoricolor* in northern California (Redecker et al. 2001). The *R. cremoricolor* population was found to be dominated by single-sporocarp genets and had small

genet size (less than 1.5 m). In a recent study, genets represented by single sporocarps were also found in *R. brevipes* populations, three out of nine genets and seven out of 14 genets at two sites (Bergemann and Miller 2002). As no identical RAPD profiles were found, the genet size of *R. vinosa* may be no larger than 1 m in the present study. Single-sporocarp genets or small genet size may suggest low ability of the ECM fungus in mycelial spread and longevity or multiple spore establishment events (Dahlberg 2001; Fiore-Donno and Martin 2001). As to the fungal population, proliferation by sexual spores may play a much more important role than vegetative growth.

Since only one sporocarp was selected from several sporocarps aggregated in a small region (less than 1 m in diameter) in our sampling procedure, determination of the smallest genet size of the *R. vinosa* population at this site will require sampling of more sporocarps, particularly within the circle of 1 m.

The dispersal of sexual spores of ECM fungi is usually by wind and animals (Allen 1991). Because of the relatively unpredictable traits of spore transmission and the heterogeneity of host distribution and abiotic factors in the forest, it is difficult to find a simple correlation between spatial distance and genetic similarity (Fiore-Donno and Martin 2001). The spatial distance between sporocarps, however, should not be arbitrarily considered as marginal factors in the population. The spatial pattern shown as point-to-point distances could also be helpful in understanding the genetic structure of the EMF population.

Considering the low viability of basidiospores, only a small fraction of spores will establish, mate and finally become dicaryophytic mycelia. The density of sexual spores is, therefore, a crucial factor in the establishment of dicaryophytic mycelia and further occurrence of sporocarps. Spore dispersal studies on pathogenic basidiomycetes indicate that the vast majority of spores fall within a few meters of the fruit body (Kallio 1970; Olson and Stenlid 2000). It is likely that the daughter sporocarps only appear in a very limited region around the parent fruit bodies and that the main expansion of a fungal population is by short-distance spore dispersal. Zhou et al. (2001) found that the spread of alleles of *Suillus grevillei* was probably by repeated short-distance rather than long-distance spore dispersal. Compared with long-distance spore dispersal, the genotypes in an ECM Forgal population dominated by short-distance spore dispersal should be spatially more clustered, i.e. the spatial pattern of the population should be more clumped rather than randomly arranged.

Our results indicate that sporocarps within 10 m had significantly higher genetic similarity, and pairwise sporocarps with different genetic similarity had different distribution patterns. The spatial pattern of the whole population as well as of three genetic groups was found to be clumped into clumps of about 20-m radius, which suggested that the population was not established via long-distance spore dispersal. The observation that smaller clumps with higher density are in the high-genetic-similarity group, whereas larger clumps with lower density are in the low-genetic-similarity group, could also be explained by the short-distance spore dispersal model. Clumps of close relatives would be found in a population dominated by short-distance spore dispersal, and these clumps would enlarge with the development of the population. Because of the relatively unpredictable traits of spore transmission, the clumps formed by low-genetic-similarity individuals may greatly overlap and the spatial pattern of such individuals should be more like a random-arranged pattern.

Due to the limited size of the fungal population and the sampling area, only three genetic groups had enough pairwise sporocarps for second-order analysis. More useful information about the correlations between genetic structure and spatial pattern would be obtained in a larger ECM fungal population. Although providing only primary information, such integrative analyses of both spatial pattern and genetic structure of ECM Forgal populations should help to understand gene flow within or between ECM Forgal populations, the functioning of ECM fungal communities and forest succession.

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Reference

Allen MF (1991) The ecology of mycorrhizae. Cambridge University Press. Cambridge, UK, pp 66–68

Andersen M (1992) Spatial analysis of two species interactions. Oecologia 91:134–140

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

Bergemann SE, Miller SL (2002) The size, distribution, and persistence of genets in local populations of the late-stage ectomycorrhizal basidiomycete, *Russula brevipes*. New Phytol 156:313–320

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 bovinus* as indicated by spatial distribution of fungal clones. New Phytol 115:487–493

Dahlberg A, Stenlid J (1994) Size, distribution and biomass of genets in populations of *Suillus bovinus* (L.: Fr.) Roussel revealed by somatic incompatibility. New Phytol 128:225–234

Dahlberg A, Stenlid J (1995) Spatiotemporal patterns in ectomycorrhizal populations. Can J Bot 73:1222–1230

Dale MRT (1999) Spatial pattern analysis in plant ecology. Cambridge University Press. Cambridge, UK, pp 206–241

Deacon JW, Fleming LV (1992) Interactions of ectomycorrhizal fungi. In: Allen MF (ed) Mycorrhizal functioning: an integrative plant-fungal process. Chapman & 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

Egger KN (1994) Molecular analysis of ectomycorrhizal fungal communities. Can J Bot 73: S1415–S1422

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

Fries N (1987) Somatic incompatibility and field distribution of the ectomycorrhizal fungus *Suillus luteus* (Boletaceae). New Phytol 107:735–739

Getis A, Franklin J (1987) Second-order neighborhood analysis of mapped point patterns. Ecology 68:473–477

Goreaud F, Pélissier R (1999) On explicit formulas of edge effect correction for Ripley's *K*-function. J Veg Sci 10:433–438

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

Hadrys H, Balick M, Schierwater B (1992) Application of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol Ecol 1:55–63

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 granulatus*. 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

Kallio T (1970) Aerial distribution of the root-rot fungus *Fomes annosus* (Fr.) Cooke in Finland. Acta For Fen 107:1–55

Kerrigan RW, Royer JC, Baller LM, Kohli Y, Horgen PA, Anderson JB (1993) Meiotic behavior and linkage relationships in the secondarily homothallic fungus *Agaricus bisporus*. Genetics 133:225–236

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 & Hall, New York, pp 357–423

Olson Å, Stenlid J (2000) Functional units in root diseases: lessons from *Heterobasidion annosum*. In: Flood J, Bridge PD, Holderness M (eds) Ganoderma diseases of perennial crops. CAB, Wallingford, UK, pp 139–156

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

Ripley BD (1976) The second-order analysis of stationary processes, J Appl Prob 13:255–266

Rohlf FJ (1992) NTSYS-pc numerical taxonomy and multivariate analysis system, 1.70. Exeter Software, Setauket, New York

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

Smith ML, Bruhn JN, Anderson JB (1992) The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature 356:428–431

Sneath P, Sokal R (1973) Numerical taxonomy. Freeman, San Francisco

Thioulouse J, Chessel D, Dolédec S, Olivier JM (1997) ADE-4: a multivariate analysis and graphical display software. Statistics and Computing 7:75–83

Timonen S, Tammi H, Sen R (1997) Outcome of interactions between genets of two *Suillus* species 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

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl Acid Res 18:6531–6535

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