J. Plant Res. **114**: 179-185, 2001

Journal of Plant Research

Spatial Distribution of the Subterranean Mycelia and Ectomycorrhizae of *Suillus grevillei* Genets

Zhihua Zhou, Makoto Miwa[1], Yosuke Matsuda[2] and Taizo Hogetsu*

Symbiotic Function Research Unit, Asian Natural Environmental Science Center, The University of Tokyo, Midori-cho 1-1-8, Nishitokyo-shi, Tokyo, 188-0002 Japan

To analyze the distribution of subterranean parts of ectomycorrhizal genets, species-specific polymorphic markers are necessary. In this study, we examined the species-specificity of five polymorphic simple sequence repeat (SSR) markers of *Suillus grevillei*, and found that one, SG-5, is species-specific to *S. grevillei* and polymorphic. Using the SG-5 marker, we analyzed for the first time the horizontal and vertical distribution of the subterranean parts of several *S. grevillei* genets. The spatial distribution of *S. grevillei* genets in the soil demonstrated that the development of *S. grevillei* sporocarps is correlated with that of extra-radical mycelia and ectomycorrhizae of the same genet, which are distributed in a narrow area. However, sporocarps are not always centered over the subterranean parts and the amount of subterranean mycelia and mycorrhizae is not always correlated to the number of sporocarps formed on them. No *S. grevillei* mycelia and mycorrhizae were detected beneath the positions where *S. grevillei* sporocarps emerged in the previous year. The observation indicates the rapid alteration of the subterranean parts of *S. grevillei* genets, and suggests that *S. grevillei* genets change location rather than merely extend their habitat year after year.

Key words: Ectomycorrhizae — Genet — *Larix* — Microsatellite (SSR) — Species-specific — *Suillus grevillei*

Some Basidiomycetes and Ascomycetes fungi form symbiotic ectomycorrhizae on tree roots and contribute to the stability of terrestrial ecosystems through their role in the overall circulation of nutrients (Harley and Smith 1983, Smith and Read 1997). Therefore, there is much interest in these fungal symbionts, especially how they extend their habitat. Ectomycorrhizal (ECM) fungi exist as a complex of ectomycorrhizae, extraradical mycelia and sporocarps that develop from the mycelia. An ECM genet is defined as a genetically identical complex of ectomycorrhizae, extraradical mycelia and sporocarps. Due to difficulty in identifying genets in subterranean ectomycorrhizae and extraradical mycelia, the distribution and size of an ECM genet are inferred from the location and area occupied by genetically identical sporocarps, on the assumption that the genet determined by clonal sporocarps represents the subterranean parts (De la Bastide *et al.* 1994, Dahlberg and Stenlid 1994, Dahlberg 1997, Anderson *et al.* 1998, Bonello *et al.* 1998, Zhou *et al.* 1999, Gherbi 1999). However, there is no direct evidence that the genet inferred from the area occupied by clonal sporocarps is consistent with the true genet, including the subterranean parts. On the contrary, given that sporocarp production varies with environmental conditions and can be sporadic, the distribution of clonal sporocarps is believed to be an inaccurate indicator of genets. Moreover, many studies have indicated that the production and location of sporocarps within a genet vary greatly annually (De la Bastide *et al.* 1994, Dahlberg 1999, Zhou *et al.* 2000). Without information about the subterranean parts of a genet, it is difficult to ascertain whether the annual variation in sporocarp production and development location reflects similar variation in the subterranean parts or only the variation of environmental conditions. To elucidate the characteristics of ECM fungal genets and their dynamics, it is indispensable to analyze the spatial distribution of the subterranean parts of ECM genets and their variation.

Identifying the subterranean parts of an ECM genet is a problem that has been tackled by many investigators. In the soil layer, the microorganism community around a root system is complex and many species of ECM fungi may coexist in the root system of one host plant. DNA directly isolated from ectomycorrhizae and ECM fungal mycelia may be contaminated with DNA from other microorganisms and host plant tissue in ectomycorrhizae. PCR fragments amplified from the contaminants may hinder analysis of DNA polymorphism in the target ECM fungal symbiont. Therefore, highly polymorphic DNA markers that amplify only template from the target ECM fungus are needed to study the identity of the subterranean parts of genets. Sequences flanking the simple sequence repeat (SSR) loci of two Ascomycetes species, *Mycosphaerella fijiensis* and *Candida albicans*, are polymorphic and species-specific (Bretagne *et al.* 1997, Neu *et al.* 1999). SSR markers may be ideal DNA markers for analyzing polymorphisms of subterranean

* Corresponding author: tel/fax, 0424-65-5601; e-mail, ho@uf.a.u-tokyo.ac.jp

[1] Present address: Center for Environmental Science in Saitama, Kumitamadare 914, Kisai Town, Saitama, 347-0115 Japan

[2] Present address: Faculty of Bioresources, Mie University, Kamihama-cho, 1515 Tsu-shi, Mie, 514-8507 Japan

ectomycorrhizae and mycelia.

In a previous study, we developed five polymorphic SSR markers (SG1 to SG5) that distinguished genets of *Suillus grevillei* to a considerable extent (Zhou *et al.* 2001). In this study, we examine whether the five SSR markers are specific to *S. grevillei* and identify subterranean ectomycorrhizae and extraradical mycelia of *S. grevillei* genets using a species-specific DNA marker, then map their horizontal and vertical distributions.

Materials and Methods

Description of test site

The investigation was carried out in a *Larix kaempferi* (Lamb.) Carriere stand located by Lake Yamanaka at the foot of Mt. Fuji (35°24′N, 135°52′E) as in our previous studies (Zhou *et al.* 1999, 2000 and 2001). The genets of *S. grevillei* (Klotz.) Sing were analyzed by examining ISSR polymorphisms of sporocarps sampled in 1997 and 1998.

Sampling soil blocks

One horizontal plot and four longitudinal soil sections were made surrounding or under *Suillus grevillei* sporocarps in the autumn of 1999 during the fruiting season of *S. grevillei*. The horizontal plot and three longitudinal sections were located in the *L. kaempferi* stand, while the other section was located outside the stand (Fig. 1). Soil blocks were then sampled from the horizontal area and the longitudinal sections. The area surrounding the sporocarps of two genets which were distinguished by polymorphisms of both ISSR and SG-5 markers was divided horizontally into 20×20 cm squares, as shown in Fig. 2. From the center of each square, soil blocks approximately 10 cm in diameter were removed at depths from 0 to 15 cm and 15 to 30 cm. The longitudinal soil sections were made with a scoop and a shovel through positions of sporocarp emergence. The surface of each section was divided into 10×10 cm squares (Fig. 3), then 5 cm thick soil blocks were sampled. Samples were stored at 4 C until use.

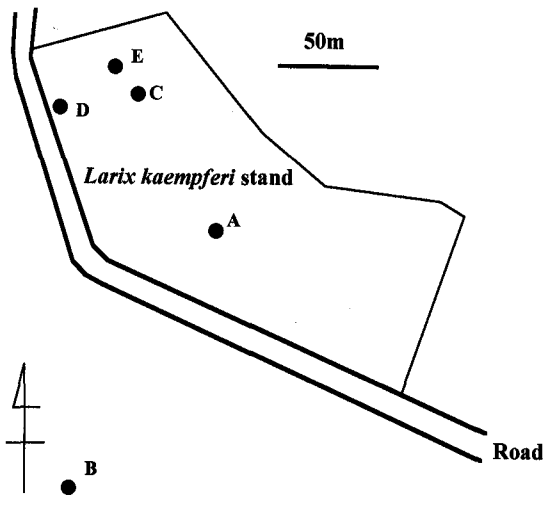


Fig. 1. The spatial relationship between the horizontal plot (A) and longitudinal soil sections (B-E).

Sampling mycelia and ectomycorrhizae

All the visible mycelial strands were removed from each soil block using tweezers under a dissecting microscope and put into a petri plate with water. From this pool of mycelia, about four strands were chosen at random and transferred into a 1.5-ml micro-tube. Several tubes of mycelia were prepared from each soil block, depending on the total number of mycelia in the block. The ectomycorrhizae in the remainder of each soil sample were collected by flushing the soil through a set of sequential sieves, of which the finest had a 0.5 mm mesh, with running water. The ectomycorrhizae collected were classified by morphology according to Durall's glossary (Durall *et al.* 1996), under a dissecting microscope. All ECM root tips from one soil block were pooled, and about one-tenth of the pooled mycorrhizal root tips were chosen randomly and allotted into 1.5 ml micro-tubes at 3-4 tips per tube. All sampled mycelia and ECM root tips were frozen quickly in liquid nitrogen and then stored at −80 C until use.

DNA isolation and polymorphism analysis

DNA from the sampled sporocarps, mycelia, and ectomycorrhizae was extracted in micro-tubes with a micro-pestle set using the modified CTAB method (Gardes and Bruns 1993). The quality of fungal DNA extracted from mycelia and ectomycorrhizae was confirmed by positive PCR amplification from the extracted DNA using a fungi-specific ITS primer pair (ITS1 and ITS4) (White *et al.* 1990).

Amplifications of SSR loci of the samples with positive ITS PCR amplification were performed in a reaction solution containing 5 ng of template DNA, 0.4 mM of each dNTP, 1× GC buffer I (Takara Shuzo Co.) including 2.5 mM Mg^{2+}, 0.5 U LA Taq DNA polymerase (Takara Shuzo Co.) and 0.2 μM of each designed primer, of which one was labeled with Texas Red. PCR was carried out in a PCR Thermal cycler (TP3000, Takara Shuzo Co.) under a schedule of 29 cycles of 1 min at 94 C, 1 min at annealing temperature (Ta) and 1 min at 72 C, then followed by one cycle of 1 min at 94 C, 1 min at Tm and 5 min at 72 C.

The PCR product (5 μl) was mixed with 2 μl of loading buffer (50% glycerine, 1 mM EDTA, 0.25% xylene cyanol FF; Wako Co. Osaka, Japan) and subjected to electrophoresis on 1.5% argarose gels with ethidium bromide. The band patterns were visualized on a UV transilluminator. The samples with bands of expected size were regarded as containing of *S. grevillei* mycorrhizae and mycelia.

Another portion of the PCR product of *Suillus grevillei* samples was denatured at 94 C for 4 min and separated on 6% sequencing gels made of 6% Longer Ranger acrylamide (FMC Bioproducts Co., ME, USA), 6.1 M urea and 1.2×TBE (0.1 M tris (hydroxymethyl) aminomethane, 3.0 mM EDTA and 0.1 M boric acid), by a sequencer (SQ-5500, Hitachi Co., Tokyo, Japan). The resulting banding pattern analyzed using software "FRAGLYS Version 2.0" (Hitachi Electronics Engineering Co., Tokyo, Japan) was employed to identify *S. grevillei* genets.

Results

Species-specific and polymorphic DNA marker

We first examined whether the five SSR markers were species-specific to *Suillus grevillei* in PCR amplification of sporocarp DNA from *S. grevillei* and the other six ECM fungal species that produced sporocarps in the study area, and of DNA from the host tree, *Larix kaempferi*. No fragments were amplified by any of the primer pairs when DNA of *S. luteus*, *Boletus fraternus*, *B. pulverulentus*, *Russula cyanoxantha*, and *R. mariae* was used as the template (Table 1). Fragments amplified from *S. laricinus*, which is a close relative of *S. grevillei*, by SG-1 to SG-4 were indistinguishable from those amplified from *S. grevillei*, while SG-5 did not amplify DNA from *S. laricinus*. None of the primer pairs amplified fragments from *L. kaempferi* DNA. Moreover, with the SG-5 marker, the 72 *S. grevillei* sporocarps representing 60 genets identified by ISSR markers could be divided into 49 SSR groups. Sporocarps comprising individual genets were classified into the same SSR group and most SSR groups were consistent with genets. Though SG-5 marker did not distinguish all of the *S. grevillei* genets in the stand, it was confirmed that the marker can distinguish those in the areas surrounding the horizontal plot and sections (within a 10 m diameter) made in this study, by the polymorphic analysis of the sporocarps collected in these areas in 1997, 1998 and 1999. These results showed that the SG-5 marker was not only species-specific to *S. grevillei* but also highly polymorphic.

Table 1 PCR amplification results for five SSR markers when DNA of *Suillus grevillei*, the other six ECM fungal species found in the study area, and the host tree, *Larix kaempferi*, was used as the template.

Species	SG-1	SG-2	SG-3	SG-4	SG-5
Suillus grevillei	+	+	+	+	+
Suillus laricinus	+	+	+	+	−
Suillus luteus	−	−	−	−	−
Boletus pulverulentus	−	−	−	−	−
Boletus fraternus	−	−	−	−	−
Russula cyanoxantha	−	−	−	−	−
Russula mariae	−	−	−	−	−
Larix kaempferi	−	−	−	−	−
Groups identified from 72 *S. grevillei* sporocarps	2	8	9	48	49

+ : Positive amplification of fragments of the expected size by PCR
− : No amplification

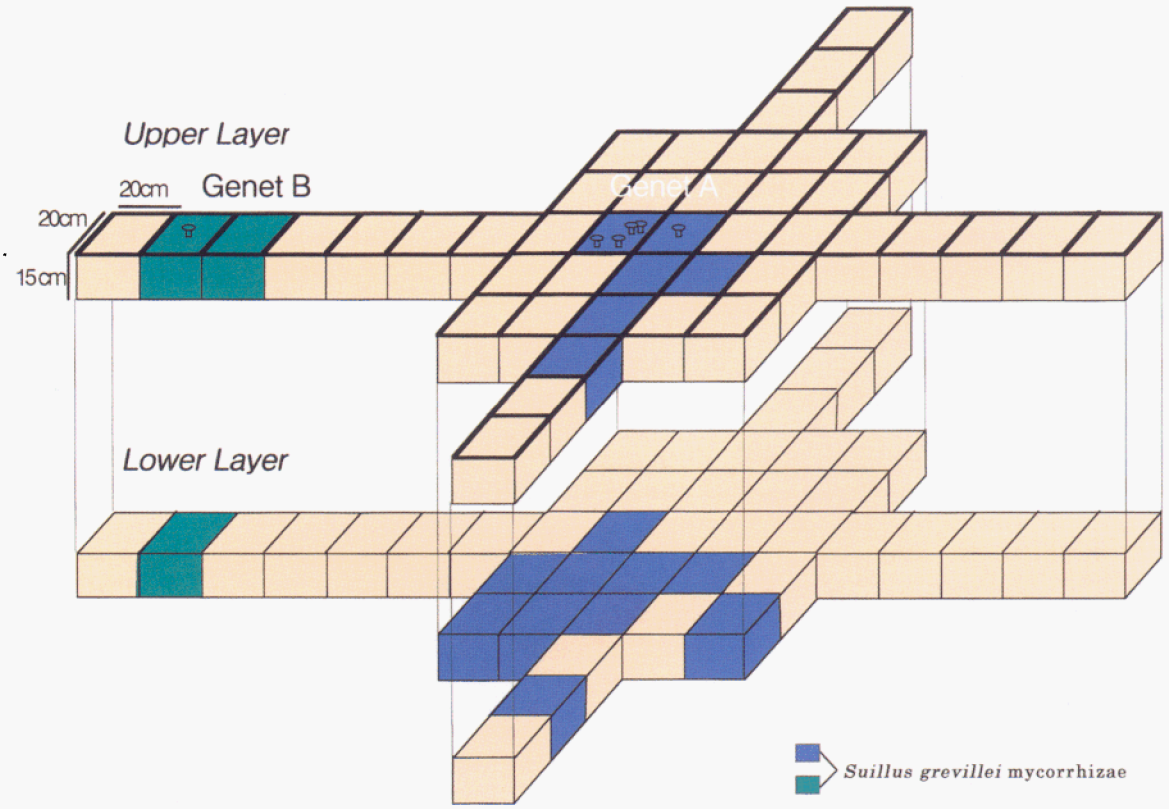


Fig. 2. The horizontal distribution of sporocarps, ectomycorrhizae, and mycelia of *Suillus grevillei* genets in two soil layers. Ectomycorrhizae and mycelia of genets A and B were identified in the blue and green squares, respectively. Note that the *S. grevillei* ectomycorrhizae and mycelia present match the genet of the sporocarp growing above.

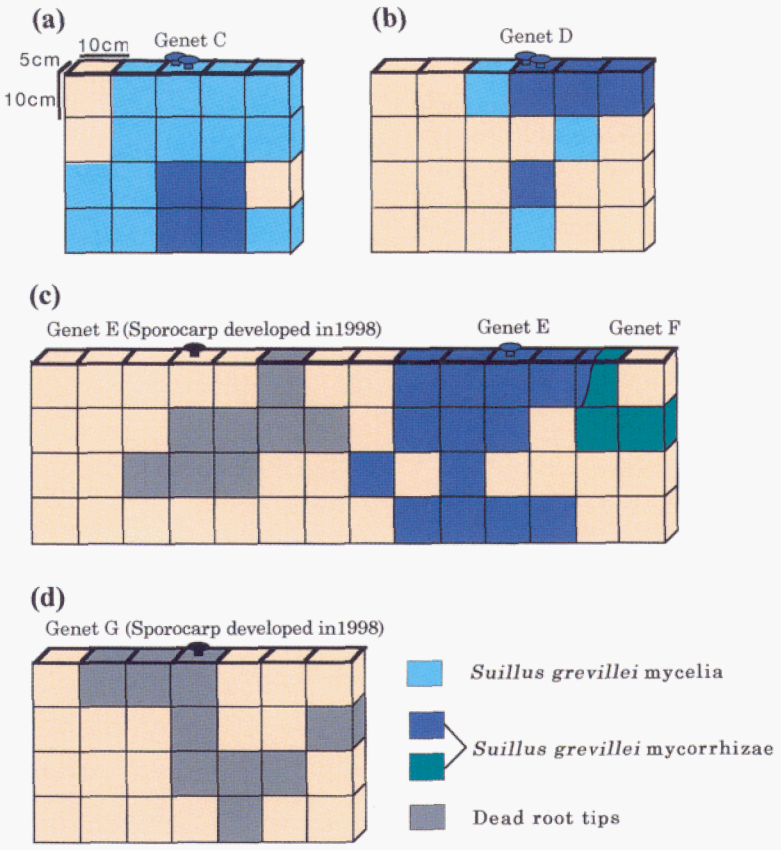


Fig. 3. The vertical distribution of sporocarps, ectomycorrhizae, and mycelia of *Suillus grevillei* genets in longitudinal soil sections. (a) The distribution of the subterranean parts of genet C. (b) The distribution of the subterranean parts of genet D. (c) The distributions of genets E and F are shown, as well as the dead root tips beneath where sporocarps of genet E emerged the previous year (1998). (d) The distribution of dead root tips under the location where a sporocarp of genet G emerged the previous year. The subterranean parts of genets were identified in the blue or green squares. Dead root tips were found in the dark-gray squares. Color and black sporocarps indicate the positions of sporocarps that emerged in the sampling year (1999) and the previous year (1998), respectively. Note the absence of *S. grevillei* ectomycorrhizae and mycelia under the position of sporocarps that developed in the previous year.

The morphological types of Suillus grevillei *mycorrhizae*

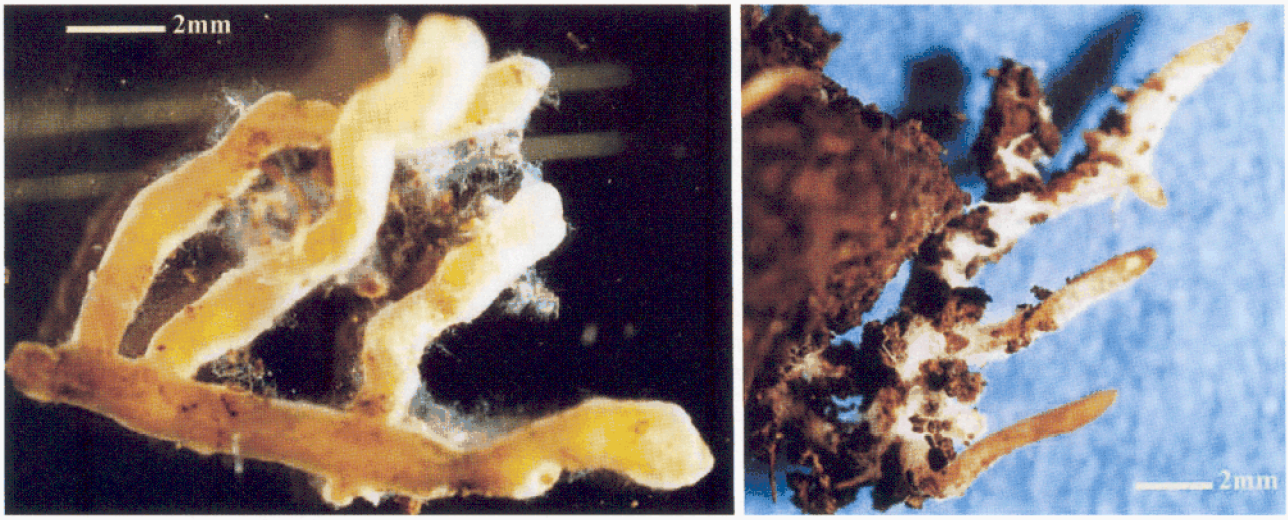
Five major morphological types of ectomycorrhizae were found on *Larix kaempferi* roots in the sampled soil blocks. PCR using DNA extracted from two of the ectomycorrhiza types amplified the same SG-5 fragments as from nearby *S. grevillei* sporocarps (Fig. 4), but none from the DNA of the three other types. Therefore, we presumed that the former two types of ectomycorrhizae included *S. grevillei* mycorrhizae and that the morphological differences in the *S. grevillei* mycorrhizae reflected differences in their developmental stages.

Horizontal distribution of subterranean parts

The horizontal distributions of ectomycorrhizae and mycelia under sporocarps of two *Suillus grevillei* genets (genet A and B) were mapped using SG-5 (Fig. 2). Although ectomycorrhizae were found in almost all of the soil blocks, *S. grevillei* ectomycorrhizae and mycelia were found only in some blocks under or near the sporocarps of either genet. Many aggregates of type 2 ECM root tips identified as genet A using SG-5 were distributed in a narrow area covering about 400 cm^2, and most were partially dried out. A few fresh ECM root tips of genet A, mostly type 1, were scattered in or around the aggregates. The sporocarps emerged at the periphery of the genet. The ectomycorrhizae and mycelia of genet A occupied less than half of the sampled blocks. Interestingly, the other samples were dominated by the ectomycorrhizae of other unknown fungal species, with ITS bands different from that of *S. grevillei*.

Distribution of Suillus grevillei *subterranean parts on the longitudinal soil sections*

Longitudinal soil sections were made under the sporocarps of *Suillus grevillei* genets C and D. White mycelia were densely distributed over the surface of both sections. Using SG-5, most of the mycelia in the samples from the section under sporocarps of genet C were found to be the same genet as the sporocarps; however, all of the mycelia in samples from the section under sporocarps of genet D were from other fungal species. Ectomycorrhizae belonging to



Type 1 Type 2

Fig. 4. The two morphotypes of *Suillus grevillei* mycorrhizae identified using the SG-5 marker. Type 1 is irregularly branched, with a white, cottony mantle and bent root tips. Type 2 is also irregularly branched with a white, cottony mantle, but its surface is reflective and its root tips are straight.

genets C and D were also identified; most of these were partially dried, like those of genet A. Although the two genets both produced two sporocarps, genet C had a broader distribution of mycelia and higher density of ectomycorrhizae than genet D (Fig. 3a, 3b). In addition, the mycorrhizae of genet C aggregated at depths from 20 to 40 cm, while those of genet D were mostly distributed near the ground.

Another longitudinal section was dug under the two *Suillus grevillei* sporocarps of genet E (Fig. 3c). One of these sporocarps emerged in 1998 and the other in 1999. White mycelia were distributed sparsely in this section. Only the mycelia and ectomycorrhizae on the surface directly under the sporocarp that developed in 1999 were identified as genet E. The right end of this section was dominated by ectomycorrhizae of genet F, which developed sporocarps nearby in 1998. On the left, beneath the location of the sporocarp that developed in 1998, ECM roots of another tree species and several fresh *Larix* mycorrhizae of species other than *S. grevillei* were found in some blocks, while other blocks contained many dead ECM root tips.

Another longitudinal section, beneath where a genet G sporocarp developed in 1998, contained evenly-distributed mycelia on the surface and several fresh ectomycorrhizae in four squares, but none of these were *Suillus grevillei* mycelia or ectomycorrhizae. As in the case of genet E, aggregates of dead ECM root tips were also found (Fig. 3d).

Discussion

In this study, we analyzed polymorphisms in subterranean ectomycorrhizae and mycelia of *Suillus grevillei* genets and identified subterranean ECM genets. Several studies related to fungal identification in ectomycorrhizae beneath sporocarps have been reported. Using a somatic incompatibility test, Dahlberg and Stenlid (1994) demonstrated that fungal isolates from ectomycorrhizal aggregates in two 5 m^2 areas are compatible with *S. bovinus* sporocarps that emerge above ground there. More recently, via ITS-RFLP analyses, the same ECM fungal species as the sporocarps that developed above the ground were identified in the subterranean ECM communities (Gardes and Bruns 1996, Matsuda and Hijii 1999). These studies have revealed some ecological aspects of ECM subterranean communities or populations. However, methods applied in these studies seem to have a low resolution for subterranean genet analyses. A species-specific and highly polymorphic DNA marker is necessary to identify subterranean parts of target ECM genets.

Though some *Suillus grevillei* genets identified by ISSR marker could not be distinguished from each other by the SG-5 marker used in the present study, most genets were consistent with SG-5 groups. Especially, the genets in the areas surrounding the horizontal plot and sections (within 10 m in diameter) were completely distinguished by SG-5. This indicates that SG-5 is sensitive enough to identify those subterranean genets. This marker was found from only five *S. grevillei* SSR markers, moreover, the other four also showed specificity to *S. grevillei* and its close relative, *S. laricinus*. The high frequency of species-specific markers among SSR markers suggests that screening SSR loci may be the best approach to finding suitable markers for identification of subterranean ECM genets. It would reveal new aspects of ECM fungal ecology to investigate the subterranean genets of other ECM species using species-specific and polymorphic DNA markers that would surely be isolated from SSR loci, like SG-5.

The genet identification of subterranean ectomycorrhizae and mycelia showed that the development of sporocarps was correlated with nearby aggregates of subterranean parts

of the genet. However, the number of sporocarps was not always consistent with the size of the subterranean part of the genet, and sporocarps were not always centered over the subterranean parts. What is more, some genets, for example, genet F in the present study, did not develop sporocarps. These observations indicate that sporocarp development is not correlated quantitatively, and might reflect the larger dependence of sporocarp formation on environmental conditions.

No *Suillus grevillei* mycelia or ectomycorrhizae were detected in sections below where sporocarps had emerged the year before (Figs. 3c and 3d). Since the *S. grevillei* ectomycorrhizae and mycelia detected in the soil under the sporocarps were the same genet as the sporocarp in every case in this study, there likely were *S. grevillei* ectomycorrhizae and mycelia under the sporocarps the year before, i. e., in 1998. The aggregates of dead ECM root tips found below the site of the emergence of the sporocarps in 1998 are therefore likely the remnants of ECM aggregates, including those of *S. grevillei*. The observation that most of the *S. grevillei* ECM aggregates under this year's sporocarps were beginning to die supports this speculation. Therefore, the *S. grevillei* mycelia and most ectomycorrhizae are rather labile, and wither within one year. In fact, Downes *et al.* (1992) found that most mycorrhizae of *Tylospora fibrillosa* and *Paxillus involutus* formed with *Picea sitchensis* become darkened and wrinkled after they are formed for 60 days. Many studies also indicated that the life span of individual ectomycorrhizal tips is not so long, being usually less than several months (Alexander and Fairley 1983, Rygiewicz *et al.* 1997).

The change in the location of the subterranean parts of a genet from one year to the next, as shown in Fig. 2c, suggests that *Suillus grevillei* genets change location rather than enlarge their habitat year after year. We have already reported that sporocarps of individual *S. grevillei* genets usually emerge in close but different positions year by year (Zhou *et al.* 2000). The positions of emerging sporocarps belonging to individual genets were found to change annually also in other ECM fungal species (De la Bastide *et al.* 1994, Dahlberg 1999). Therefore, these changes could be interpreted as a migration of the subterranean parts from year to year. The process by which the subterranean parts of a *S. grevillei* genet migrate is still unknown. It will be interesting to follow the spatiotemporal change in *S. grevillei* mycorrhizal development more closely.

ECM fungi reproduce by mycelial development and spore dispersal from sporocarps. A balance between the two reproductive patterns in a population has been inferred from genet size, based on the assumption that small and large genets correspond to reproduction by spore dispersal and mycelial development, respectively (Dahlberg and Stenlid 1995, Anderson *et al.* 1998, Zhou *et al.* 1999, Gherbi *et al.* 1999). However, the rapid alteration of subterranean genets that we observed suggests that reproduction via mycelia development does not always result in an increase in genet size year after year.

In conclusion, our results emphasize the qualitative, but not quantitative, correlation of *Suillus grevillei* sporocarp emergence with the subterranean development of ECM aggregates of the same genet, and the rapid alteration of subterranean genets. The rapid alteration of subterranean genets should lead to reevaluation of the widely held assumption that a smaller genet size indicates a bias towards sexual reproduction.

We thank the staff of Fuji University Forest of the University of Tokyo for help in sampling. This research was supported in part by a grant from PROBRAIN and Grants-in-Aid (Nos. 09NP0901 and 10460064) from the Ministry of Education, Science, Sports and Culture of Japan.

References

Alexander, I.J. and Fairley, R.I. 1983. Effects of N fertilization on populations of fine roots and mycorrhizas in spruce humus. Plant Soil **71**: 49-53.

Anderson, I.C., Chambers, S.M. and Cairney, J.W.G. 1998. Use of molecular methods to estimate the size and distribution of mycelial individuals of the ectomycorrhizal basidiomycete, *Pisolithus tinctorius*. Mycol. Res. **102**: 295-300.

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

Bretagn, S., Costa, J.M., Besmond, C. and Carsique, R. 1997. Microsatellite polymorphism in the promoter sequence of the elongation factor 3 gene of *Candida albicans* as the basis for a typing system. J. Clin. Micro. **35**: 1777-1780.

Dahlberg, A and 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. and Stenlid, J. 1995. Spatiotemporal patterns in ectomycorrhizal populations. Canad. J. Bot. **73**: 1222-1230.

Dahlberg, A. 1997. Population ecology of *Suillus variegatus* in old Swedish Scots pine forests. Mycol. Res. **101**: 47-54.

Dahlberg, A. 1999. Somatic incompatibility in mycorrhizae. *In* A. Varma and B. Hock, eds., Mycorrhiza 2nd, Springer-Verlag, Berlin, pp. 111-132.

De la Bastide, P.Y., Kropp, B.R. and Piche, Y. 1994. Spatial distribution and temporal persistence of discrete genotypes of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) Orton. New Phytol. **127**: 547-556.

Downes, G.M., Alexander, I.J. and Cairney, J.W.G. 1992. A study of spruce (*Picea stichensis* (Bong) Carr.) ectomycorrhizas. I. Morphological and cellular changes in mycorrhizas formed by *Tylospora fibrillosa* (Burt) Donk and *Paxillus involutus* (Batsch ex Fr.) Fr. New Phytol. **122**: 141-152.

Dural, D.M., Harniman, S.M.K, Berch, S.M. and Goodman, D.M. 1996. Glossary. *In* D.M. Goodman, D.M. Durall, J.A. Trofymow and S.M. Berch, eds., Concise descriptions of North American ectomycorrhizae, Mycologue Publications, Sidney.

Gardes, M. and Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. Molec. Ecol. **2**: 113-118.

Gardes, M. and Bruns, T.D. 1996. Community structure of ECM fungi in a *Pinus muricata* forest: above- and below-ground views. Canad. J. Bot. **74**: 1572-1583.

Gherbi, H., Delarelle, C., Selosse, M.A. and Martin, F. 1999. High genetic diversity in a population of the ectomycorrhizal basidiomycete *Laccaria amethystina* in a 150-year-old beech forest. Molec. Ecol. **8**: 2003-2013.

Harley, J.L. and Smith, S.E. 1983. Mycorrhizal symbiosis. Academic Press, New York.

Matsuda, Y. and Hijii, N. 1999. Characterization and identification of *Strobilomyces confusus* ectomycorrhizas on Momi fir by RFLP analysis of the PCR-amplified ITS region of the rDNA. J. For. Res. **4**: 145-150.

Neu, C., Kaemmer, D., Kahl, G., Fischer, D. and Weising, K. 1999. Polymorphic microsatellite markers for the banana pathogen *Mycosphaerella fijiensis*. Molec. Ecol. **8**: 523-525.

Rygiewicz, P.T., Johnson, M.G., Ganio, L.M., Tingey, D.T. and Storm, M.J. 1997. Lifetime and temporal occurrence of ectomycorrhizae on ponderosa pine (*Pinus ponderosa* Laws.) seedlings grown under varied atmospheric CO_2 and nitrogen levels. Plant Soil **189**: 275-287.

Smith, S.E. and Read, D.J. 1997. Mycorrhizal symbiosis, 2nd eds. Academic Press Ltd, London.

White, T.J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M.A. Innis, D.H. Gelfand, J.J. Sninsky and T.J. White, eds., PCR protocols: a guide to methods and applications, Academic Press, New York, pp. 315-322.

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

Zhou, Z., Miwa, M. and Hogetsu, T. 2000. Genet distribution of *Suillus grevillei* populations in two *Larix kaempferi* stands over two years. J. Plant Res. **113**: 365-374.

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

(Received October 13, 2000; accepted March 15, 2001)