論文の内容の要旨

森林科学 専攻

平成 16 年度博士課程　入学

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論文題目 **Reproduction ecology of pioneer ectomycorrhizal fungi, *Laccaria amethystina* and *L. laccata*, in the volcanic desert on Mount Fuji**

(富士山火山荒原における先駆的外生菌根菌ウラムラサキおよびキツネタケの繁殖生態に関する研究)

Ectomycorrhizal (ECM) symbiosis is a mutualistic plant-fungus association and ubiquitous in temperate, boreal and tropical forest ecosystems. ECM fungi are known to improve nutrient status of host trees, enhance host growth, and thus seem to be indispensable for many ecological processes in forest, especially in nutrient-deficient environments such as volcanic

deserts under primary succession.

In the volcanic desert on Mount Fuji, *Laccaria amethystina* and *L. laccata* are pioneer ECM fungal species, first colonizing on a pioneer ECM plant, *Salix reinii*. Both fungal species are also dominant in ECM fungal communities in this desert. These ECM fungi probably facilitate seedling establishment of *S. reinii* and secondarily colonizing tree species, promoting vegetation succession. The knowledge of their reproduction ecology would advance our understanding about the forest regeneration in this desert.

In the ECM fungal population, a genetically identical unit called a genet is regarded as an individual. An ECM fungal genet is composed of below ground and aboveground parts; mycelia in ECM root tips and in soil, and sporocarps. Because of the difficulty in identifying genets by morphological observations, genetic markers are usually used to identify ECM fungal genets.

Microsatellite (simple sequence repeat, SSR) markers are one of the most effective

genetic markers for identifying fungal genets, because of their high polymorphism and reproducibility. Moreover, SSRs can provide some additional genetic information, such as, genetic similarities and parentage relationships between genets, gene flow between populations, etc.

In this study, SSR genotypes of sporocarps and belowground ECM tips of *L. amethystina* and *L. laccata* were analyzed to reveal their reproduction ecology in the volcanic desert on Mount Fuji.

1. Isolation and characterization of microsatellite markers in two pioneer ectomycorrhizal fungi *Laccaria amethystina* and *L. laccata*

I employed a dual-suppression polymerase chain reaction (PCR) technique to develop SSR markers from *L. amethystina* and *L. laccata*. In *L. amethystina,* ten polymorphic SSR markers were developed. These markers showed polymorphism of two to ten alleles per locus. The observed and expected heterozygosities of these markers ranged from 0.136 to 0.545 and

0.206 to 0.877, respectively. In *L. laccata*, five polymorphic SSR markers were developed. The markers had polymorphism of three to six alleles per locus. The observed and expected heterozygosities ranged from 0.269 to 0.462 and 0.249 to 0.775, respectively. All polymorphic SSR markers developed were used for further analyses.

2. Spatio-temporal sporocarp genet dynamics of pioneer ectomycorrhizal fungi, *Laccaria amethystina* and *L. laccata*, in the volcanic desert on Mount Fuji

Since sporocarps produce haploid spores that can generate genetically different genets, the genetic structures of sporocarp populations provide important information on reproductive involvement of spores. All sporocarps of both *Laccaria* species developed in eight fine scale plots (2m x 2m for each) were collected in three consecutive years (2004, 2005 and 2006). Sporocarps were also collected from every sporocarp-forming vegetation patch within the research quadrat (5.5ha) in the proportion of one sporocarp to c.0.25m^2 in 2005 and 2006. In total, 591 genets were identified from 1,143 sporocarps of both ECM fungi by SSR

polymorphism analyses. Both fungal genets were always small in size (<1.5m in the largest distance between sporocarps belonging to the same genet in the same year). About 60% of *L. amethystina* and 75% of *L. laccata* genets found in fine scale plots were observed only in a single year, indicating most genets were renewed every year. The small size and rapid turnover of the *Laccaria* genet indicate that new genets are frequently established from haploid spores but cannot persist long. Spatial autocorrelation analyses revealed that genetically close genets were distributed in close distances. In addition, genets that shared each rare allele were always found aggregated in small areas. These results suggest that spores of both *Laccaria* species are mainly deposited in short distances.

3. Belowground genet dynamics of pioneer ectomycorrhizal fungi, *Laccaria amethystina* and *L. laccata*, in the volcanic desert on Mount Fuji.

Sporocarp populations do not necessarily correspond with belowground ECM populations, because sporocarp formation is affected by many environmental and biological

factors. To better understand reproduction ecology of both *Laccaria* species, belowground genets were analyzed. For each *Laccaria* species, 25 soil samples (5cm cubes) were collected at regular intervals from each of 10 square plots (1m x 1m) in which a sporocarp was centered, in a sporocarp season (September, 2006) and nine months after sporocarp formation (June, 2006), respectively,. Under a dissecting microscope, *Laccaria* ECM tips were collected from each soil sample and used for genet identification by SSR analyses. In the sporocarp season, the genet identical to the centered sporocarp in each plot was always detected in soil and often dominated the belowground genets in each plot. In contrast, nine months after the sporocarp formation, the genet identical to the previous year's centered sporocarp was not detected in soil in more than half plots, and instead, there were many other small genets. Majority of them shared at least one common allele with the centered sporocarp in every SSR locus. These results indicate that genets of both *Laccaria* species tend to disappear within nine months after sporocarp production and that new offspring genets are generated from the spores dispersed from the sporocarp to the

vicinity. The size and number of the genet were significantly smaller and more in June than in the sporocarp season. This suggests that most of the genets newly generated until June disappear through competition with a sporocarp-producing genet that dominated in the sporocarp season.

In conclusion, the present study demonstrates that both *L. amethystina* and *L. laccata* populations in the volcanic desert on Mount Fuji largely depend on spore-involved reproduction and rapidly establish new genets in the belowground. Such reproduction ecology of both *Laccaria* species with spore involvement and rapid establishment of new genets would secure constant ECM colonization on the host trees in this frequently disturbed desert, and thus, be the reason why they first and dominantly colonize on newly established *S. reinii* in non-ECM harsh habitat.