博士論文（要約）

Communities and population genetic structures of ectomycorrhizal fungi in ice-age relict forests of *Pinus pumila* over Japan

（ハイマツ氷期遺存林における外生菌根菌の群集・集団遺伝構造）

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1. Background

Recent human activities have rapidly altered the environment of the earth, causing some serious issues such as global warming and habitat fragmentation. These human-induced changes have large impacts on wildlife from genetic to landscape levels. But available knowledge is largely biased toward macro-organisms such as plants and animals, and we know very little about how the current environmental changes would affect micro-organisms despite their importance in various ecosystem processes.

Ectomycorrhizal (ECM) fungi are dominant soil microbes in forest ecosystems. They colonize tree roots symbiotically and enhance water and nutrient acquisitions of the host trees. Thus, ECM fungi often determine host tree performance and survival, playing the keystone roles in the forest ecosystems. Many previous studies have documented communities and population structures of ECM fungi in boreal, temperate and tropical forests, even in relation to environmental changes. In contrast, we know almost nothing about ECM fungi in alpine ecosystems, which is expected to be affected by the current global warming most severely. Here I examined ECM fungi in alpine relict forests to infer how the global warming and habitat fragmentation affect community composition and population genetic structures of alpine ECM fungi.

Chapter 1 provides the background of the thesis. Chapter 2 examines how ECM fungal communities develop along host growth in alpine forests. Two new *Rhizopogon* species that are dominant in seedling establishment stages are described after morphological and phylogenetic analyses with other known *Rhizopogon* species in Chapter 3. Then, the effect of climate, especially temperature, on alpine ECM fungal communities in resident tree roots (Chapter 4) and soil spore banks (Chapter 5) are evaluated. In Chapter 6, population genetic structures are compared between host trees and ECM fungi, and between ECM fungi with wind and animal dependent spore dispersals by SSR analyses. Chapter 7 summarizes key findings of this study and provides overall discussions.

2. Materials and Methods

Ice-age relict forests dominated by *Pinus pumila* have been confined to alpine regions of higher mountains having different climates since the end of the last glacial period, representing an ideal opportunity to evaluate the effect of climate change on ECM fungi over long periods. Moreover, the mono-dominant forests are advantageous for evaluating abiotic factors in structuring ECM fungal communities because of the absence of different host effects. Nine study sites were established in mature *P. pumila* forests on different mountains, covering the entire distribution range of *P. pumila* in Japan. Two soil cores (5 × 5 × 10 cm each) were separately collected from surface litter and mineral subsoil layers at 30 points per site. In addition, 30 seedlings established at open grounds were randomly collected with their root systems and surrounding soils. Spore banks were surveyed using the bioassay method, where pine seedlings were planted in the soil samples so that fungal spores preserved in the soil would germinate and colonize the seedling roots.

ECM root tips in the soil samples and on field or bioassay seedlings were classified into morphotypes, from each of which three root tips were subjected to DNA extraction separately. Ribosomal DNA ITS sequences obtained by PCR and direct sequencing were clustered into operational taxonomic units based on ≥97% similarity threshold. Taxonomic identify of each OTU ("species" hereafter) was assigned based on Blast search results in INSD. Frequency of individual ECM fungal species was used to analyze community change along host developmental stages. The relative importance of geographic distance, climates and soil conditions in structuring ECM fungal communities in mature forests and spore banks were quantified using redundancy analyses.

For population genetic analyses, DNA extracts confirmed to be *Rhizopogon alpinus* and *Suillus spraguei* in the above community analyses were selected. These two species are phylogenetically close, belonging to the monophyletic Suilloid group. Spores of *S. spraguei* are dispersed from its above-ground sporocarps by wind. But *R. alpinus* produces hypogeous sporocarps, from which spores are dispersed by feces of animals eating the sporocarps. To compare their population genetic structures with those of the host, 30 *P. pumila* leaf samples were collected at each study site and subjected to DNA extraction. These *R. alpinus*, *S. spraguei*, and *P. pumila* samples were genotyped by 7, 8, and 13 SSR markers, respectively, including newly developed 14 markers in total. Genetic diversity within individual populations, genetic differentiation among populations, and inbreeding coefficients were analyzed by calculating population genetic parameters. Population genetic structures were also examined by assignment tests and compared among the three species.

3. Results and discussion

ECM fungal species detected showed a clear successional pattern along host developmental stages. Seedlings of *P. pumila* were colonized by fewer ECM fungal species, where *Rhizopogon* and *Suillus* predominated. These fungi may facilitate seedling establishment of *P. pumila,* playing keystone roles in the alpine ecosystem. With the host growth, species richness and diversity of ECM fungi increased. Mature *P. pumila* trees were associated with diverse fungal species, such as Russulaceae, Thelephoraceae, and Cortinariaceae especially in the litter layer.

Based on morphological and molecular phylogenetic analyses of *Rhizopogon* sporocarp specimens collected in this study, two new species, *Rhizopogon alpinus* sp. nov. and *Rhizopogon nitidus* sp. nov. were described. Especially, the former species was confirmed in all of the research sites except Hokkaido island, which is located above the biogeographical boundary known as Blakiston's line.

From the entire research sites, 218 ECM fungal species were confirmed to inhabit ice-age relict *P. pumila* forests. A majority of ECM fungal species were Holarctic components, distributed in Europe and North America. Fewer species were shared with temperate forests in Japan despite close geographical distances. These results indicate that many ECM fungi migrated from Siberia during the ice-ages and have been confined to alpine regions in Japan after the last glacier period, as demonstrated in the host *P. pumila*. However, some species, especially *Rhizopogon* species including the newly described ones, were not confirmed in other geographical regions, suggesting they may have evolved in the alpine regions of Japan probably because of their limited gene flow abilities.

Community analyses found that ECM fungal composition was explained more by climate variables, especially by summer temperatures, than distance and soil variables. In addition, ECM fungal communities observed at lower temperature *P. pumila* sites were composed of species distributed at lower temperature regions in the world, suggesting the strong effects of temperature on ECM fungal communities. It should be noted here that these temperature effects on ECM fungal communities were not accompanied by above-ground vegetational changes. While we know that the global warming is affecting above-ground plant and animal communities, its impact on below-ground ECM fungi may be much earlier and severer.

Population genetic analyses found contrasting structures among ECM fungi and the host *P. pumila*. Populations of *R. alpinus* were clearly differentiated among nine sites, indicating no gene flow would have occurred between mountains over long periods. In contrast, no significant genetic differentiation was observed among *S. spraguei* populations, indicating its panmictic spore dispersal by wind across mountains. The extent of genetic differentiation among *P. pumila* populations was in the middle of the two ECM fungi, probably because of its mixed gene flow mechanisms using wind dispersed pollen and animal dispersed seeds. Because fungal spores are inherently small and abundant, and often thought to travel longer distances than plant seeds and be less affected by habitat fragmentation. However, as shown in our results, it is not necessarily true for hypogeous fungal species that depend on animals for spore dispersal.

4. Conclusions

This study generated the most comprehensive data set of alpine ECM fungal communities that have ever been documented. The dominance of Holarctic fungal components and the strong temperature effect on community composition indicate that alpine ECM fungal communities are composed of cold adapted species. With the global warming, these fungi must migrate higher elevations, although such habitats are not necessarily available on lower mountains. This will not a big problem for ECM fungi having wind dispersal spores, because they can migrate across mountains as demonstrated in *S. spraguei* population genetic results. However, local extinction could be a serious problem for hypogeous ECM fungal taxa, because their spores are unlikely to be dispersed between mountains as shown in *R. alpinus*. Currently, *R. alpinus* was found from most *P. pumila* forests examined and showed low inbreeding coefficients. But if the global warming continues further, *R. alpinus* populations will inevitably become smaller and fragmented on isolated mountains, eventually caught in the extinction vortex. Given the possible keystone roles of *R. alpinus* in *P. pumila* forest regeneration, its extinction may drastically change the entire ecosystem and threaten many plant and animal species inhabiting the forests.