論文の内容の要旨

論文題目

Paleolimnological analysis of long-term ecological dynamics of cladocerans in a lake
（湖沼生態系を構成する枝角類の長期生態的動態の古陸水学的解析）

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How ecosystems form and change is one of the biggest topics addressed in ecology. Ecosystems consist of multiple hierarchy levels: genetic composition, phenotype, population, community, and ecosystem including abiotic environment. Prior studies found that evolutionary processes occur on the same time scale as ecological processes and that such contemporary evolution can reciprocally affect ecological dynamics, termed as eco-evolutionary dynamics. While interactions between different levels of ecosystems have been studied, simultaneous observations of multiple levels of natural ecosystems and integrations of these data are limited. Although long-term observations to witness responses at each level are important to understand ecosystem changes, especially to measure both genetic and phenotypic data in addition to species data like species composition and abundance of each species, they are rare and challenging. Furthermore, any ecosystem depends on the colonization of multiple species. Thus, the changes such as adaptation which occur during colonization affect later changes and structure of the ecosystem. However, observations of natural ecosystems right from their initial colonization stage are rare, owing to the difficulty related to prior prediction of initial introduction.

In my doctoral thesis, I analyzed long-term changes at multiple levels of a natural lake ecosystem, ranging from the genetic composition of some species to the ecological level including abiotic environment, since the initial stage of the lake ecosystem formation, using the paleolimnological and resurrection ecological method. Paleolimnology allows us to reconstruct past biotic and abiotic changes using lake sediment core samples and subfossils of organisms preserved in the sediment. In addition, resurrection ecology reconstructs long-term genetic and phenotypic changes by collecting living organisms at multiple time points (from past to present) using dormant stages preserved in sediments and ice cores. Combining paleolimnological and resurrection ecological analyses allow to observe the long-term changes from the genetic to ecosystem levels including environment. Paleolimnological and resurrection ecological analysis have previously revealed the long-term changes in ecosystems and how lake ecosystems, communities, and certain species, like *Daphnia*, respond to environmental changes. However, a long-term research of multiple ecological levels using same core samples and integration of paleolimnological observations is still under development. In addition, time-series observations of the early stages of several ecological processes, such as the colonization of population, are also limited in paleolimnological and resurrection ecological studies. Hence, how lake ecosystems and each ecological level respond to biotic and abiotic changes, and how each level of the ecosystem interacts in the early stages of development, are still poorly understood.

In the present study, I successfully collected lake sediment cores that included the layer indicating lake formation period in Lake Fukami-ike, Nagano prefecture, Japan. I focused on cladoceran and specifically *Daphnia pulex*, which represents a major group and species used in both paleolimnology and resurrection ecology. Japanese *D. pulex* has four lineages that are genetically distant from each other; they are originated in North America. All four lineages are the obligate parthenogenetic lineage, which can produce diapausing eggs by asexual reproduction. Hence, their diapausing eggs are genetically identical to their parent. Thus, I collected genetic information on past generation and reconstructed historical genetic dynamics and phenotypic changes.

In my doctoral thesis, I aimed to reveal the long-term changes in the natural lake ecosystem across different ecological levels, from genetic structure to community composition and the surrounding environment, using lake sediment. I also focused on the changes that occurred from the initial colonization process of the populations, which was lacking in other observation studies. I discuss how multiple ecological levels change and interact with each other during this process, and how such changes affect later ecosystem change. Based on these observations, I gathered knowledge to understand the change of multi-layered ecosystem and reciprocal interactions in natural systems.

In Chapter 2, I focused on the cladoceran community, the population dynamics of each species that constituted the cladoceran community and the related environmental changes. I reconstructed the long-term changes in the cladoceran community and environmental factors that occurred during the lake formation period using lake sediment core samples and subfossils of cladocerans. This was done to test the hypothesis regarding the transition of important control factors affecting the cladoceran community as eutrophication proceeds, which gradually proceed from lake formation and occasionally rapidly proceed by various disturbance like human activity. With these results, I revealed that the major factors controlling the cladoceran community and the relative importance of these factors were temporally changed as eutrophication proceeded. In the initial stage of the cladoceran community assemblage, the bottom-up effect strongly affected the cladoceran community as rapid eutrophication replaced initial benthic cladoceran community with a pelagic community dominated by small *Bosmina*. After rapid eutrophication occurred, the top-down effect became relatively strong due to an increase of primary production in the pelagic zone, which released the cladoceran from resource restriction and allowed predators at higher trophic levels, such as fish, to establish in the area. These results coincide with each prior study that evaluated the bottom-up and top-down effects in a lake ecosystem, separately.

In Chapters 3 and 4, I focused on the *D. pulex* population and its colonization process during early colonization. In Chapter 3, I tested the genetic dynamics using population genetic analysis based on mitochondrial DNA with diapausing eggs preserved in lake sediments. These results showed the *D. pulex* population colonized and was sustained with greatly limited haplotypes, without turnover nor recovery of genetic diversity, and that a single haplotype (Jpn2C) had dominated throughout the colonization process.

In Chapter 4, I discuss changes in the morphological phenotype related to adaptation to predation pressure during the colonization process by morphological analysis of subfossils and ephippia preserved in lake sediments.

While Chapter 3 shows that the genetic diversity of the *D. pulex* population had been limited to a single dominating haplotype, Chapter 4 shows that the morphological phenotype changed during the colonization process. Furthermore, morphological changes were adaptive to the changes in the predator community change: the body size of matured females increased and defensive traits decreased when planktivorous fish decreased and vice versa. Even when genetic diversity decreased in the early stages of colonization as traditional research suggested, biological populations were possibly able to establish and survive with adaptive phenotypic change.

In Chapter 5, I also focused on the *D. pulex* population and the mechanism maintaining genetic diversity. I tested the hypothesis that intraspecific differences in diapause strategy based on photoperiodic response can promote the maintenance of genetic diversity through the storage effect using *D. pulex* lineages originated from diapausing eggs preserved in lake sediments. I used two haplotypes of *D. pulex*, Jpn2C and Jpn1A-C2T2, belonging to distant genetic lineages. Chapter 3 shows that Jpn2C had dominated throughout the colonization process and Jpn1A-C2T2 appeared more recently without replacing Jpn2C, instead maintaining coexistence. I evaluated the competitive ability and photoperiodic response in the diapause of each lineage through laboratory experiments. These experiments showed that a competitively inferior haplotype, Jpn1A-C2T2, tended to produce diapausing eggs earlier than Jpn2C. The theoretical model analysis suggested that different photoperiod responses and interannual fluctuations of growth conditions can promote the coexistence of the two haplotypes via the storage effect, as the competitively inferior haplotype avoids competition by diapause induction. While germination timing has been investigated as an important factor for the storage effect in plants, I showed the potential importance of diapausing timing induced by photoperiodic changes in promoting the maintenance of genetic diversity. Moreover, this intraspecific difference of diapausing timing dependent on photoperiodic response could have allowed Jpn1A-C2T2 to establish after Jpn2C.

Based on the above analyses, I could simultaneously observe the multiple levels composing the natural lake ecosystem, containing from the genetic structure of *D. pulex* population to the ecosystem including abiotic environment. I found that the multiple interactions between levels that construct the natural lake ecosystem, and found that these interactions may also occur between distant ecological levels, such as between community and phenotype. I also found multiple interactions between different ecological levels during the initial stage of colonization, including interactions that related to successful colonization. These observations suggested that the interactions occurring in colonization affected ecosystem structure and later ecological changes. Furthermore, predator community affected multiple ecological levels while *D. pulex* population was influenced by many levels. These results suggest that some biological groups may facilitate cross-level interactions to occur. In Chapter 2, I report that eutrophication and predator community dynamics change cladoceran community composition and each species' population dynamics. Chapter 3 shows the possible interactions between the predator community and the genotype of the *D. pulex* population, and between the phenotype and genotype within the *D. pulex* population. Chapter 4 illustrates the interaction between the predator community and the phenotype of the *D. pulex* population, and the interaction between phenotype and population dynamics of *D. pulex*. Chapter 5 shows the interactions between the population structure, dynamics, phenotype, and genotype in the *D. pulex* population.

On the contrary, I could not observe sufficient feed-back interactions, including the "evolution to ecology feedback". To understand the feedback loops occurring in natural systems, it would be necessary to include, a more detailed taxonomic analysis of other biological communities involved, such as of fish community and phytoplankton that interact with cladocerans and *D. pulex*. Paleolimnological analysis will help us to analyze various biological communities after observation of certain species' population. The recent development in genetic analysis of sediment allows us to reconstruct the dynamics of the taxa that do not leave subfossils in sediments such as fish. To consider evolutionary responses in detail, fine genetic analyses would be effective, such as that of functional genetic regions and/or an association analysis using genome-wide Single Nucleotide Polymorphisms. These analyses on diapausing eggs preserved in lake sediment will elucidate the relative importance of plasticity and genetic evolution, and the history of selection. By integration with long-term observations like the present study, it may be possible to evaluate the "evo-to-eco feedback".

Furthermore, I considered the possibility that certain biological groups possibly facilitate cross-level interactions in ecosystem. During my research, I observed that the change in the predator community affect multiple ecological levels. Several prior studies have shown that the predator community plays a key role in controlling changes in each of the ecological levels. These results of earlier studies and my results have suggested factors that have large impacts on each level of an ecosystem may tend to influence multiple levels simultaneously, which may be caused by indirect effects on other levels. Similarly, I observed that *D. pulex* population was affected by multiple ecological levels. *Daphnia* species are well reported as species sensitive to biotic and abiotic changes: such sensitive biological groups may accumulate impacts from multiple ecological levels and mediate their effects across ecological levels. An analysis of which levels are most affected, and of which levels are more likely to affect multiple levels, may provide the much-needed data for better ecosystem management.

In addition, the present research raised a question regarding how mechanisms that maintain species diversity and those associated with intraspecific genetic diversity interact in a natural system. The storage effect, which the present research suggests as a possible mechanism to maintain genetic diversity in the *D. pulex* population, has also been shown to maintain species diversity. The diapausing egg bank of *Daphnia* could possibly serve as an effective tool to approach this question, because it allows us to reconstruct the chronology of both genetic and species diversity changes in a natural system.

Through the present study, my research suggests that interactions between ecological levels, including the interactions of distant levels, occur and may have important roles in natural systems. More specifically, during the colonization of natural populations, these interactions possibly affect colonization success, development of the community structure, and ecosystem function. In addition, my research showed that some factors, such changes in the predator community, can affect multiple biological levels at the same time. Furthermore, the present study showed the effectiveness of paleolimnological and resurrection ecological methods to observe the interactions between different ecological levels in natural systems and to provide new insights for future studies to explore such interactions, including feed-back responses and eco-evolutionary dynamics.