

Evolutionary origins of the endemic ichthyofauna in Inle Lake, an ancient lake in Myanmar

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Introduction

Ancient lakes have been excellent sites for exploring speciation and community assembly processes for their unique biota evolution. The theory of island biogeography explains species diversity in isolated environments, such as islands and ancient lakes, considering the dynamics of colonization from the species pool “continent” and speciation and extinction within the “island.” According to this theory, species richness increases through colonization and in situ speciation. On the other hand, the proportion of endemic species increases through allopatric speciation under the limitation of gene flow via immigration, and also through in situ radiation under further restricted conditions. Empirical studies of community assembly in ancient lakes have mostly focused on radiation from a small number of ancestors; but the development processes of endemism under repeated colonizations are poorly understood.

Inle Lake, the only ancient lake in continental Southeast Asia (Shan Plateau, Myanmar), is one of the biodiversity hotspots. The ichthyofauna of Inle Lake is believed to have comprised widely distributed species that immigrated from the surrounding areas and endemic species that differentiated via geographic isolation, implying the assembly on a moderately isolated “island.” The aim of this thesis is to clarify the biogeographic and ecological processes by which regional endemism is formed under repeated colonizations from the surrounding areas, using the endemic ichthyofauna of Inle Lake as a model system. In Chapters 2 and 3, I first tried to clarify the origins of two endemic species with contrasting distribution patterns, i.e., an endemic species with closely related species distributed over a wide area in Southeast Asia and a species endemic to the Inle region (Inle Lake and the surrounding rivers in the adjacent areas) at the genus level. Then, in Chapter 4, the timescales and factors of the colonization and in situ diversification (i.e., within the Inle region) that could influence the development of the endemic ichthyofauna are discussed by estimating the divergence time from the closely related species and the timing of population

differentiation for almost all endemic species. Finally, in Chapter 5, I summarized the formation of the ichthyofauna in Inle Lake as revealed above in the viewpoint of island biogeographical theory with a comparison with community assembly processes in other ancient lakes.

Materials and methods

In Chapters 2 and 3, I examined the speciation and hybridization history of *Channa harcourtbutleri* (Channidae) and *Microrasbora rubescens* (Cyprinidae), respectively. Molecular phylogenetic and population genetic analyses were conducted based on mitochondrial DNA sequences and nuclear genome-wide SNPs data from the MIG-seq method, using specimens widely sampled from the distribution ranges and published data of the closely related species. In addition, inter- and intraspecific morphological differentiation were examined using geometric morphometrics and other methods.

In Chapter 4, I focused on 19 endemic species and 4 undescribed, possibly endemic species to Inle Lake, and estimated their divergence times from the closely related species based on mitogenome and genome-wide SNPs data. In addition, I estimated the genetic population structure across the distribution ranges of those species that occur also in the surrounding rivers on the Shan Plateau.

Results and discussion

In Chapter 2, I showed the importance of multiple colonizations and local adaptations to develop and maintain the genetic and morphological diversity in *Channa harcourtbutleri*. Based on genome-wide SNPs data, two genetically and morphologically differentiated lineages were found in this species, each occurring mainly in the lake and the surrounding rivers, respectively. The lacustrine lineage was inferred to have diverged from the closely related species in the early Pleistocene, while the riverine lineage arose in the late Pleistocene through re-colonization of the related species and the secondary contact. The local adaptation to different environments might have allowed the two lineages to coexist.

In Chapter 3, I showed cryptic population diversification of *Microrasbora*

rubescens and insight into its drivers. Three genetically differentiated regional lineages were found in this species, but no clear morphological differences were observed among them lineages. As this species highly depends on stagnant water environments, suggesting that the adaptation to this environment has restricted gene flow among populations through rivers for approximately 3 million years, and the morphological differentiation has been prevented due to niche conservatism.

In Chapter 4, I examined the processes and timescales of species/genetic diversity formation via colonization and in situ diversification for 23 endemic species. The estimated divergence times of those endemic species suggested the repeated colonizations of ancestral populations to Shan Plateau from the adjacent rivers (the Upper Salween and Irrawaddy rivers) during the Miocene and Pleistocene. Further, the population diversification of seven endemic species was found to have occurred in the Shan Plateau during Pliocene and Pleistocene. The extent and pattern of the diversification could possibly be explained by their ecological characteristics, such as dispersal ability and habitat.

In Chapter 5, I summarized this study and discussed the community assembly processes on biogeographic “islands” that are moderately isolated from surrounding areas. The unique ichthyofauna of the Inle region was inferred to have formed through repeated colonization and subsequent allopatric speciation. Furthermore, in situ diversification through local adaptation to different environments may have contributed to the conspicuous population differentiation and genetic diversity in some endemic species. Clarifying the ecological factors that determine the success of colonization and the coexistence of multiple species will be important for further understanding community assembly in the region.