学 位 論 文 の 要 旨

論文題目　Studies on mouth brooding cichlids of Lake Malawi: genetic, ecological and morphological differences

（マラウイ湖産口内保育型シクリッドに関する研究：遺伝的, 生態的, 形態的差異について）

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In most recent couple of decades, East African cichlids have been studied as a classic example of fast vertebrate evolution and biological diversification. A big number of cichlid species have been identified from three great lakes of East Africa: Lakes Malawi, Tanganyika, and Victoria. Malawian haplochromine cichlids are endemic, suggesting that most of this diversification has taken place within the temporal and spatial boundaries set by the lake shores. Examination of the genetic structure of recently divergent populations or species is relevant to our understanding of the process of speciation in general. Species richness and their divergence in morphology and trophic adaptations make cichlids premier of rapid speciation. Hybridization between species or genera is considered to have rapidly boosted genetic variation, which may enable speciation and adaptive radiation.

Haplochromines are divided into 2 large groups. One is called ‘mbuna’, living on rocky shores and feeding mainly on attached algae, and another is ‘non-mbuna’, living in sandy shores or the ‘pelagic zone’ and feeding on a variety of items. Cichlid fishes have evolved incredible morphological and behavioral diversity. Within Lake Malawi, the phenomena of hybridization and retention of ancestral polymorphism explain allele sharing across species. Egg-spots, particularly on male fins, are considered to have a vital role in the courtship and spawning behavior of haplochromines. These spots contain a transparent outer ring and a bright colored yellow, orange or reddish center. In Asian countries, there may be some serious problems of genetic disturbance, particularly in bred individuals. This disturbance was mainly caused by hybridization particularly between species of genus *Aulonocara* or even between genera *Aulonocara* and *Sciaenochromis* for improvement in commercial values in view of gorgeous appearance and infection resistance.

The purpose of this study was to understand the genetic diversity and relationships among the species of these genera in relation to large groups, mbuna and non-mbuna, to examine genetic disturbance among wild and purely bred individuals and those bred in Asia including Thailand, Taiwan and Japan with sequences of the mitochondrial control regions, using haplotype network analysis, and to discuss the cause of these disturbances. This study also investigates the relationships of genetic, morphological and ecological characters of Malawian cichlids.

Chapter 1 provide the general background about the Malawian Cichlids and its importance to understand the genetic, ecological and morphological differences. It also stated the outline and purpose of this study.

Chapter 2 investigated the genetic differences within wild or bred individuals in a large group of non-mbuna Malawian cichlid genus *Aulonocara* and a related genus *Sciaenochromis.* A total of 40 individuals of 18 species or variations from genera *Aulonocara* and *Sciaenochromis* with other 13 species from 11 genera, including both mbuna and non-mbuna, as references were determined for the DNA sequence of the mitochondrial control region. In a genetic tree, constructed with Kimura-2 parameter as a distance by NJ method, many species of the genus *Aulonocara* constructed a clade with mbuna while only 2 species constructed a clade with non-mbuna. Genus *Aulonocara* created three groups among which one is created by only one species. Individuals, bred in Japan, of *A. hansbaenchi* with no genetic diversity, constructed a small congeneric clade, suggesting genetic homogeneity. In contrast, some individuals, bred in Japan, of *S. fryeri* with a high genetic diversity, suggesting genetic heterogeneity. Similarly, *Sciaenochromis fryeri* was a member of a clade by mbuna while *S.* sp. *nyassae* was a member of a clade by non-mbuna. Among *Aulonocara* species, *A. steveni* and *A*. sp. Lwanda were estimated to be relatively near to ancestors by network analysis. Some individuals of *A. hansbaenschi* and *S. fryeri*, bred in Asia, were proven to be far distant from purely bred ones. Individuals of an artificially made variation, *Aulonocara* sp. tricolor, participated in different clades, showed a high genetic diversity. These results suggest that the genera *Aulonocara* and *Sciaenochromis* might be generated by hybridization between mbuna and non-mbuna, and that there are some genetic disturbances within the species of these genera by human activities.

Chapter 3 investigated the genetic distance, diversity, and relationship of two large groups of Malawian cichlids. For this purpose, a total of 78 species from 42 genera was determined for the DNA sequence of the mitochondrial control region. The genetic tree was constructed with Kimura-2 parameter as a distance by NJ method, and it showed that mbuna created only one group whereas non-mbuna created four groups by single or multiple genera. Some genera, such as *Campsochromis* and *Labeotropheus* with no genetic diversity within genus, constructed a small congeneric clade, suggesting genetic homogeneity. In contrast, some genera, such as *Otopharynx* and *Placidochromis* with high genetic diversity, participated in multiple groups including mbuna and non-mbuna, suggesting genetic heterogeneity. Non-mbuna species showed an apparently high average genetic distance whereas mbuna species showed an apparently low average genetic distance. It was suggested that non-mbuna have attained much higher diversification than mbuna. This different diversification can be explained by a stronger ecological selection exerted in sandy areas where there are a variety of possible foods in contrast to a stronger sexual selection in rocky areas where attached algae are a main food and female's mate choice are very severe.

Chapter 4 investigated the relationship between genetic, ecological and morphological characters of cichlid fishes, in relation to two large flocks, based on different parameters by using the hierarchical clustering method. Euclidean distance was calculated between each combination of species based on the standardized means for the ecological and morphological parameters. Species diversity within groups is calculated by the Shannon–Wiener diversity index (H') which measures not only the number of species but how the abundance of the species is distributed in the community. In cluster dendrogram, non-mbuna species clusters were connected at a relatively small Euclidean distance. The diversity index (H') by the morphological characters is 0.94, which showed higher homogeneity of genetic groups in the dendrogram. On the other hand, the value by the ecological characters is 1.34, which showed higher heterogeneity in terms of genetic groups. Thus, the morphological grouping showed a better match with the genetic groupings than the ecological groupings. The clear difference between these matchings suggests a similar pattern of effects of sexual or ecological selection on morphology as well as genes.

Chapter 5 summarized the findings in this study. The result of the present dissertation provides some very important information for the cichlid divergence. The reason of widespread and extensive genetic disturbance of cichlids may be a continuous and repeated production of low cost fry with the non-purely bred parents, sometimes accompanied by a high rate of mutation. Genetic disturbances resulting from selfish artificial crossbreeding and inbreeding should be controlled. In genetic relationships, non-mbuna has attained much higher diversification by a stronger ecological selection exerted in sandy areas where there are a variety of possible foods, whereas mbuna has attained lower diversification with a stronger

sexual selection in rocky areas where attached algae are a main food. The morphological grouping showed a better match with the genetic groupings than the ecological grouping on the basis of diversity analysis. Future studies should investigate the relationships by examination of other regions of DNA. Inclusion of additional species also will help to clarify the diversification level between the two large groups and provide reliable genetic relationships and speciation history. This study will make many contributions to clarification of the cichlid evolution and divergence, and mechanisms for stable community structure.

Keywords: Cichlids, Sexual selection, Ecological Selection, Lake Malawi, Mitochondrial DNA