DIVISION OF SPECIATION MECHANISM (ADJUNCT)

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During their long evolutionary history, vertebrates acquired extensive diversity in such areas as morphology, ecology and behavior. It is believed that many organisms inhabiting the earth at present are derived from an ancestral species and became diversified in the evolutionary process with speciation. Speciation, therefore, is an important factor of diversification. How, then, does speciation occur? Although various theoretical models have been proposed with respect to speciation, its mechanism has been difficult to clarify so far, particularly on a molecular level. The aim of our group's research is to propose and clarify the processes and mechanism of the speciation of vertebrates using a molecular approach. To accomplish this aim, we chose the East African cichlid fishes as the model animals for our study of speciation (Figures 1 and 2).

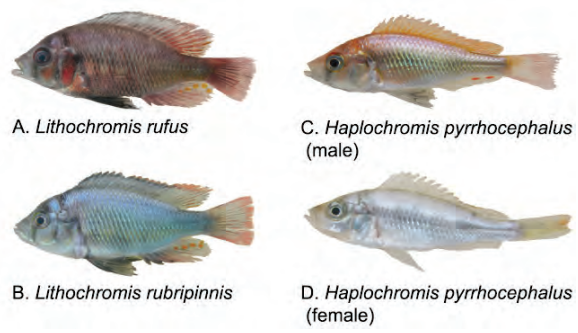

Figure 1. Cichlid fishes in Lake Victoria. These were caught in the field by our group.

Figure 2. Live color of male *Pundamilia nyererei*

Ⅰ. Intention of our research focusing on cichlids in Lake Victoria

Although cichlid fishes are broadly distributed in tropical regions throughout the world, our group focuses on the species that are endemic to three great lakes – Tanganyika, Malawi, and Victoria - and their drainages in East Africa. One thousand or more species of cichlid fishes inhabit these lakes. It is believed that Lake Tanganyika was established 12 million years ago, Lake Malawi 2 million years ago, and Lake Victoria 12,000 years ago. Thus, it is thought that the cichlids evolved in each lake after the lakes had formed. The endemic cichlids of Lake Victoria diversified to 700 or more species from a small number of ancestors during its short history. This means that explosive adaptive radiation of the cichlid species occurred in this lake.

To date, our group has accomplished certain results in molecular phylogenetic studies of the East African cichlids and other vertebrate animals; for example, the elucidation of the evolutionary history of baleen whales. From the results of our phylogenetic analysis of Lake Victoria cichlids, using insertions of retroposons (SINEs: short interspersed elements) as markers for the elucidation of their evolutionary history, we have seen that most of the selectively-neutral polymorphic alleles (presence/absence of retroposons at orthologous sites in the genome) are retained both within and among the species of this lake, which is to say that polymorphisms among Lake Victoria cichlids are trans-specific. Such homogeneous genome within/among the species of Victorian cichlids provide us with the following criterion for the elucidation of the mechanism of speciation: if we can characterize a certain allele that is uniquely fixed at a certain locus in natural populations of a certain species, we can assume that this gene may possibly be related to positive selection, which may be a significant factor promoting speciation.

Ⅱ. Field research in Lake Victoria

The lacustrine environment of Lake Victoria is highly diverse, thanks to area differences such as turbidity, depth, and bottom type. Depending on such variable habitats, cichlids also show phenotypic diversity adapting to respective habitats. To obtain ecological data and natural fish samples of Victorian cichlids adapting to various habitats, our group and Dr. N. Okada's laboratory (Tokyo Inst. Tech.) have been conducting field expeditions around Mwanza Gulf on the southern shore of Lake Victoria since 2004 (Figure 3). Thousands of fish individuals have been collected so far at various ecological habitats, and the number of species of these cichlids available for our study reached approximately 150 (rough estimation). Pictures were taken of each individual, immediately after collection, to record their live colors (Figure 2). For the purpose of genetic analyses, fin clips from representative individuals were preserved in ethanol.

Ⅲ. Analysis of candidate genes for elucidation of speciation and diversification

Cichlids' varied body colorations are one of the examples of their phenotypic diversity. Cichlids are known to depend mostly on a visual system when they choose their mating partner, and such color variations are considered to affect the female's choice. Therefore, it could be considered that the body colors of males play an important role for recognition by the visual system of females during the course of reproduction. In addition, the visual system of cichlids must have been affected by environmental differences in their habitat such as turbidity and depth of the lake water. In a collaborative work with Dr. N. Okada's laboratory at the Tokyo Institute of Technology, our group proposed that the RH1 gene, which is one of the groups of opsin genes, evolved in parallel with the depth of their habitat among cichlid species in Lakes Tanganyika and Malawi. Based on actual research in the field, our group focused on the evolution of opsin genes for the visual system in several Victorian cichlid species. *Lithchormis rufus* and *L. rubripinnis* (Figure 1A and B) inhabit shallow water near the shoreline only in Mwanza Gulf (Figure 3, panel B), and we found geographical clines of nuptial coloration on the males. We are carrying out an analysis of the opsin gene family of these species to detect genetic variations of chromatic vision that seem to be related to adaptation for such specific male colors. Another interesting issue concerning the evolution of the opsin gene family can be seen for the species *Haplochromis pyrrhocephalus*, which is broadly distributed in the lake (Figure 1C and D). The light environment for this species is considerably different among populations. To elucidate the mode of adaptation of their vision system to such various light conditions, we are analyzing the six types of their opsin gene family. A more extensive analysis of the molecular evolution of opsin genes in Victorian cichlids is in progress in our division.

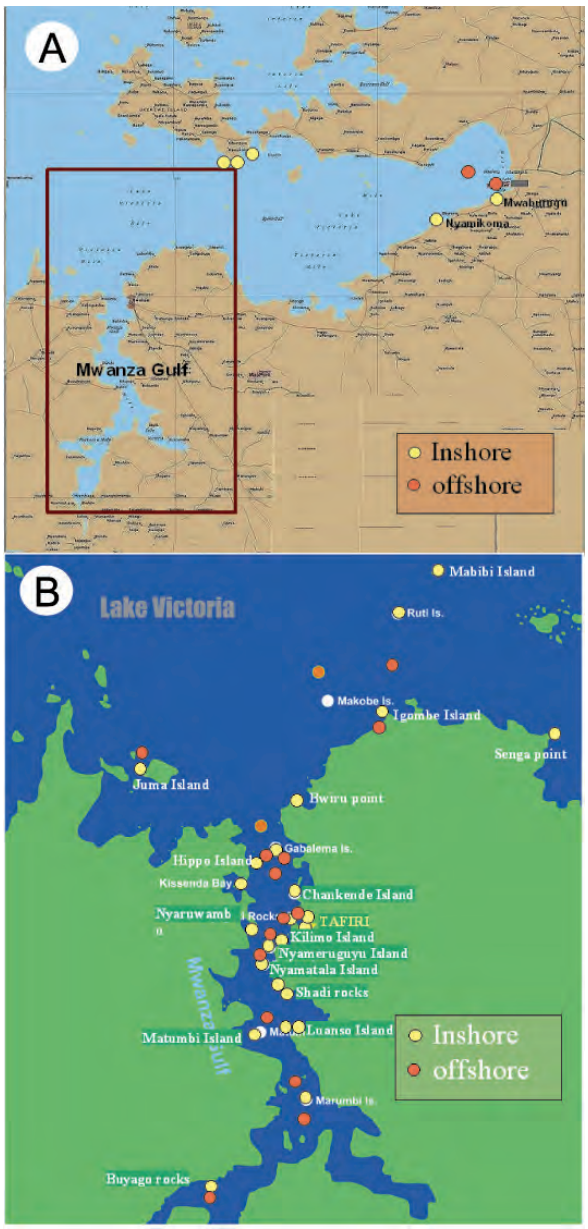

Figure 3. Localities of collection of cichlids in Lake Victoria since 2004. Panel A: Sampling localities in southern shore of Lake Victoria. The area surrounded by a brown rectangle in this panel corresponds to the region shown as a magnified map of panel B. Panel B: Sampling localities in Mwanza gulf. Sampling was conducted by angling and trawling and by using gill nets. Sampling was conducted in collaboration with Tanzania Fisheries Research Institute (TAFIRI).

Publication List

〔Original papers〕

- Fujimura, K., and Okada, N. (2007). Development of the embryo, larva and early juvenile of Nile tilapia *Oreochromis niloticus* (Pisces: Cichlidae). Developmental staging system. Develop. Growth Differ. *49*, 301-324.
- Nikaido, M., Piskurek, O., and Okada, N. (2007). Toothed whale monophyly reassessed by SINE insertion analysis: The absence of lineage sorting effects suggests a small population of a common ancestral species. Mol. Phylogenet. Evol. *43*, 216-224.