DIVISION OF SPECIATION MECHANISM (ADJUNCT)

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During the long evolutionary history of vertebrates, they 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. Therefore, speciation is one of the important factors 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, especially on a molecular level. The aim of our group's research is to propose the processes and mechanism of the speciation of vertebrates using a molecular approach. To accomplish the aim of our research, we choose the East African cichlid fishes as a model animal for the study of speciation (Figure 1).

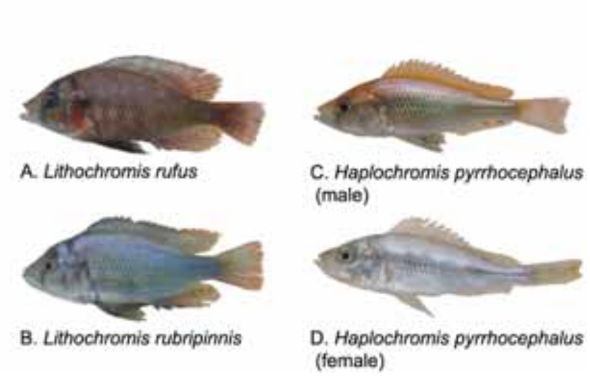

Figure 1. Cichlid fishes in Lake Victoria. These were caught in the field by the expedition of our group.

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

Although cichlid fishes are broadly distributed in tropical region throughout the world, our group focuses on the species that are endemic to three great lakes and their drainages in East Africa. There are three lakes, Lakes Tanganyika, Malawi and Victoria, and, in total, 1,000 or more cichlid fishes live in the lakes. It was reported that Lake Tanganyika was established 12 million years ago, that Lake Malawi was established 2 million years ago, and that Lake Victoria was established 12,000 years ago. Thus, it is thought that the cichlids evolved in each lake after the lakes formed. Endemic cichlids of Lake Victoria diversified to 700 or more species from a small number of ancestors during its short history. This fact 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, 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 elucidation of their evolutionary history, most of selectively-neutral polymorphic alleles (presence/absence of retroposons at orthologous site in the genome) are retained both within and among species of this lake. Namely, 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 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 (speciation).

II. Field research in Lake Victoria

Lacustrine environment of Lake Victoria highly diverged according to the difference of area such as turbidity, depth and bottom. 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 habitat, our group and Dr. N. Okada's laboratory (Tokyo Inst. Tech.) conducted field investigation around Mwanza Gulf in the southern shore of Lake Victoria since 2004, August (Figure 2). In 2005, this investigation was conducted twice. The first period started in August, 2004, and finished at the beginning of February 2005. In this first research period, our group sent K. Takahashi to collect approximately 4,000 individuals of Victorian cichlids from various ecological habitats. The second research was conducted over the course of three months (from the beginning of June 2005 to the end of August 2005). In this second research period, our group collected a further 1,400 specimens. At present, our group has almost 130 species of Victorian cichlids in our laboratory.

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

Varied body coloration of cichlids is one of the examples for their phenotypic diversity. Cichlids are known to depend mostly on 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 the turbidity and depth of lake water. In a collaborative work with the Dr. N. Okada's laboratory in Tokyo Institute of Technology, our group proposed that 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 the actual research in the field, our group focuses on the evolution of opsin genes for visual system in several Victorian cichlid species. *Lithchormis rufus* and *L. rubripinnis* (Figure 1A and B) inhabit shallow water near by the shoreline only in Mwanza Gulf (Figure 2, panel B), and we found geographical clines of nuptial coloration on their male. We are carrying out the analysis of the opsin gene family of these species to detect the genetic variations of chromatic vision as a result of adaptation for each male color among the populations. We also collected *Haplochromis pyrrhocephalus,* which is broadly distributed in the lake (Figure 1C and D), from several localities and depths. The light environment for this species is considerably different among the populations. To elucidate the adaptation of vision system for various light conditions, we are analyzing the six types of the opsin gene family. A more extensive analysis on molecular evolution of opsin genes in Victorian cichlids is in progress in our division.

Publication List:

Original papers

Kijimoto, T., Watanabe, M., Fujimura, K., Nakazawa, M., Murakami, Y., Kuratani, S., Kohara, Y., Gojobori, T., and Okada, N. (2005). cimp1, a novel astacin family metalloproteinase gene from East African cichlids, is differentially expressed between species during growth. Mol. Biol. Evol. *22*, 1649-1660.

Sasaki, T., Nikaido, M., Hamilton, H., Goto, M., Kato, H., Kanda, N., Pastene, L.A., Cao, Y., Fordyce, R.E., Hasegawa, M., and Okada, N. (2005). Mitochondrial phylogenetics and evolution of mysticete whales. Syst. Biol. *54*, 77-90.

Sugawara, T., Terai, Y., Imai, H., Turner, G.F., Koblmuller, S., Sturmbauer, C., Shichida, Y., and Okada, N. (2005). Parallelism of amino acid changes at the RH1 affecting spectral sensitivity among deep-water cichlids from Lakes Tanganyika and Malawi. Proc. Natl. Acad. Sci. USA *102*, 5448-5453.

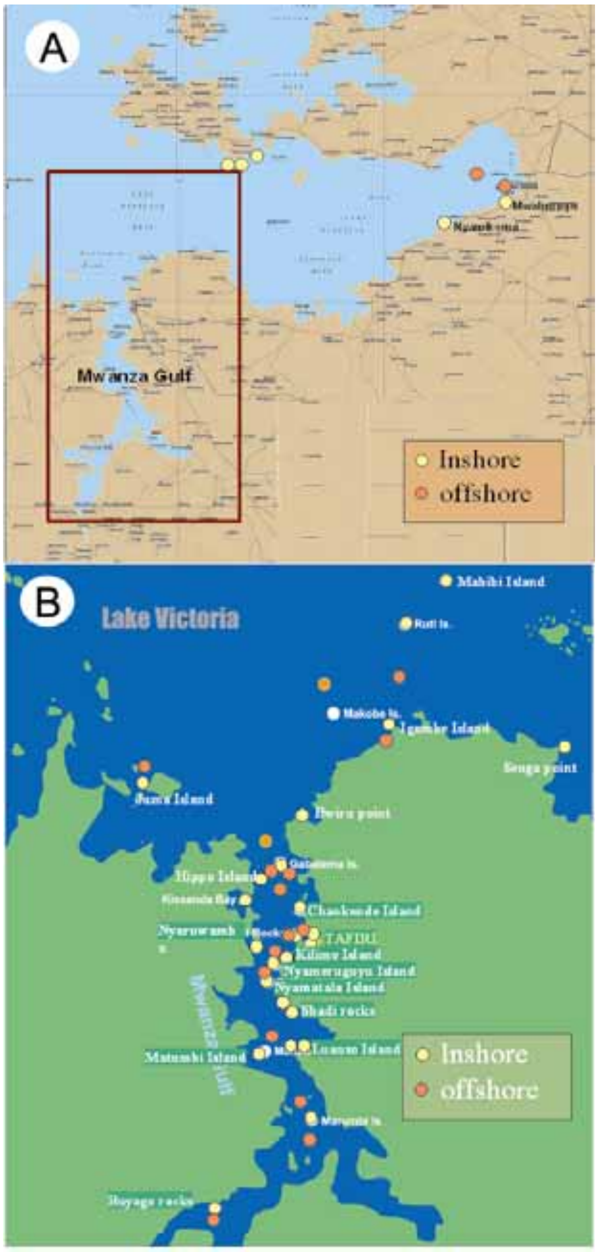

Figure 2. Localities of collection of cichlids in Lake Victoria. Panel A: Sampling localities in southern shore of Lake Victoria. The area surrounded by a brown rectangle in this panel corresponds to the region shown as a magnified map of panel B. Panel B: Sampling localities in Mwanza gulf. Sampling was conducted by angling and trawling and by using gill nets. Sampling was conducted in collaboration with Tanzania Fisheries Research Institute (TAFIRI).