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 groupís research is to propose 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 (Figure 1).

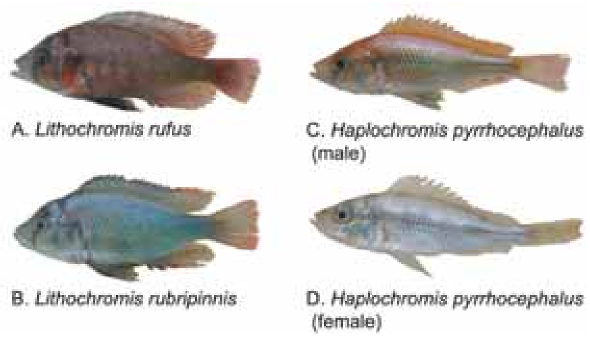

Figure 1. Cichlid fishes in Lake Victoria. These were caught in the field by our group.

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 ñ Tanganyika, Malawi, and Victoria - and their drainages in East Africa. One thousand or more species of cichlid fishes live in the lakes. It is believed 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. 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 site 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 (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. 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. Okadaís laboratory (Tokyo Inst. Tech.) began conducting field investigations around Mwanza Gulf on the southern shore of Lake Victoria in August, 2004 (Figure 2). In 2006, two investigations were conducted, and we collected approximately 3,000 individual Victorian cichlids from various ecological habitats. These expeditions increased the number of species of these cichlids available for our study to approximately 150 (rough estimation). Pictures were taken of each individual, immediately after collection, to record their live colors. 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

Varied body coloration of cichlids is one of the examples for 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 femaleís 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 the lake water. In a collaborative work with Dr. N. Okadaís 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 2, panel B), and we found geographical clines of nuptial coloration on their male. We are carrying out an 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 the vision system to various light conditions, we are analyzing the six types of the opsin gene family. A more extensive analysis of the molecular evolution of opsin genes in Victorian cichlids is in progress in our division.

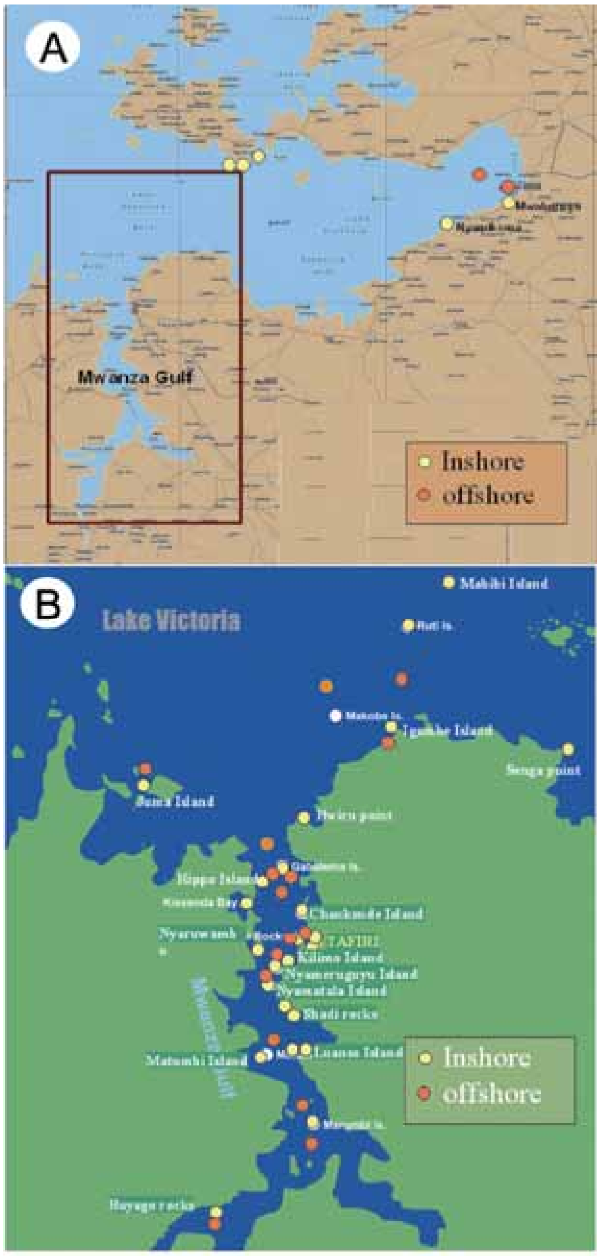

Figure 2. Localities of collection of cichlids in Lake Victoria. Panel A: Sampling localities in southern shore of Lake Victoria. The area surrounded by a brown rectangle in this panel corresponds to the region shown as a magnified map of panel B. Panel B: Sampling localities in Mwanza gulf. Sampling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

Publication List:

Original papers

Kobayashi, N., Watanabe, M., Kijimoto, T., Fujimura, K., Nakazawa, M., Ikeo, K., Kohara, Y., Gojobori, T., and Okada, N. (2006). *Magp4* gene may contribute to the diversification of cichlid morphs and their speciation. Gene *373*, 126-133.

Nikaido, M., Hamilton, H., Makino, H., Sasaki, T., Takahashi, K., Goto, M., Kanda, N., Pastene, L.A., and Okada, N. (2006). Baleen whale phylogeny and a past extensive radiation event revealed by SINE insertion analysis. Mol. Biol. Evol. *23*, 866-873.

Sasaki, T., Nikaido, M., Wada, S., Yamada, T.K., Cao, Y., Hasegawa, M., and Okada, N. (2006). *Balaenoptera omurai* is a newly discovered baleen whale that represents an ancient evolutionary lineage. Mol. Phylogenet. Evol. *41*, 40-52.

Terai, Y., Seehausen, O., Sasaki, T., Takahashi, K., Mizoiri, S., Sugawara, T., Sato, T., Watanabe, M., Konijnendijk, N., Mrosso, H.D. J., Tachida, H., Imai, H., Shichida, Y., and Okada, N. (2006). Divergent selection on opsins drives incipient speciation in Lake Victoria cichlids. PLoS Biol. *4*, e433.