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Medaka is a small egg-laying "secondary" fresh water fish found in brooks and rice paddies in Eastern Asia. This species has a long history as an experimental animal, especially in Japan. Our laboratory has conducted studies on evolution of the sex determination system using medaka and relatives, identification of the causal gene of mutants for PGC migration and pigment cell development, development of human disease models, and the genetic and neural basis of sexual behavior in medaka. In addition to these activities, our laboratory is stepping forward to lead the National BioResource Project Medaka (NBRP Medaka).

I. Evolution of the sex chromosome and sex-determining genes in *Oryzias* fish

Sex chromosomes harbor a primary sex-determining signal that triggers sexual development of the organism. In mammals, *Sry* is the dominant male-determining gene located on the Y chromosome, and has evolved from the neural gene *Sox3* on the X chromosome probably through a regulatory mutation. However, independent evolution of sex chromosomes is widespread in non-mammalian vertebrates, suggesting that sex determination mechanisms are regulated by different genes and have evolved rapidly. Medaka fishes in the genus *Oryzias* have different sex chromosomes with different systems (XY and ZW), providing ideal conditions for investigating the mechanisms that lead to the rapid turnover of sex chromosomes (Figure 1). So far, different sex-determining genes, *Dmy* and $Gsdf^Y$, have been isolated from the *Oryzias* species, demonstrating that turnover of sex chromosomes is associated with the substitution of master sex-determining genes. Recently, we identified *Sox3* as a novel sex-determining gene on the XY sex chromosomes in the marine medaka *Oryzias dancena/melastgma* by positional cloning. Sex reversed phenotypes in transgenic fish and loss-of-function mutants of the Y chromosomal *Sox3* allele all point to its critical role in sex determination, suggesting that the neo-Y chromosome of *O. dancena* arose by co-option of *Sox3*. Furthermore, we found the *Sox3* gene also on the XY sex chromosomes in distantly related *Oryzias* species, *O. marmoratus* and *O. profundicola*. Fine mapping and association analysis identified the Y chromosome-specific 430-bp insertion at the *Sox3* locus, which appeared to be involved in its male determination function. The *Sox3*-dependent sex determination system in *Oryzias* species is polyphyletic, and the Y-specific insertion has not been found in *O. dancena*, suggesting that *Sox3* has evolved as the sex-determining gene independently in different lineages of *Oryzias*. These results suggest that *Sox3* might have acquired the novel male-determining function repeatedly and independently during vertebrate evolution.

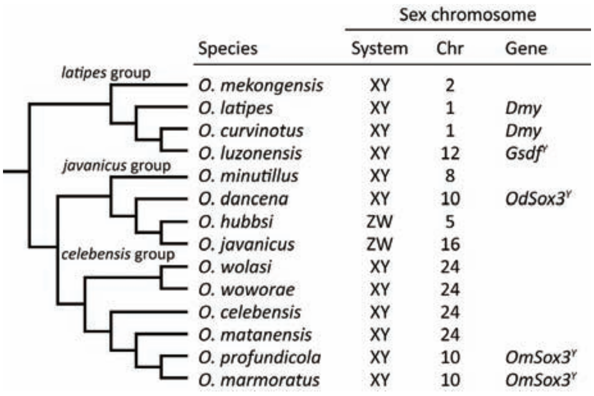

Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

II. The study of type 2 diabetes using leptin receptor knockout medaka

Leptin in mammals is a peptide hormone secreted by adipose tissue. It has been shown to play a key role in the maintenance of energy homeostasis through the regulation of food intake and a range of physiological functions. Mice with a deficiency of leptin or its receptor exhibit hyperphagia (an increase in food intake). The hyperphagia causes obesity leading to type 2 diabetes-like symptoms, which is consistent with Caucasian patients. Leptin has also been isolated from fish, including medaka, however, the amino acid sequence is poorly conserved between fish and mammals (11-30%), and fish leptins are expressed mainly in the livers. To clarify the function of leptin on fish, we generated leptin receptor knockout (LepRKO) medaka by the TILLING method. The phenotypic analyses allowed us to reveal an appetite suppressive function of leptin signaling on medaka as well as mammals, and to find new value in medaka as a novel animal model for studying type 2 diabetes. As for appetite suppressive functions; LepRKO medaka showed high expression of the mRNA of NPY (3.5-fold) and AgRP (6-fold), which are known to be orexigenic peptides, and an increase in food intake (1.7-fold). Next, as for glucose metabolism; adult mutants showed signs of diabetes, such as fasting hyperglycemia and impaired insulin secretion, which is a late-onset disorder caused by excessive feeding during post-juvenile stages. Furthermore, they showed hyperglycemia even with the same fat level in the blood, muscle, and liver as WT medaka. The symptom is consistent with those of Asian patients, not but Caucasian patients and mice with leptin signaling deficiencies. Now, we are investigating the gene expression associated with dysfunction of pancreatic tissues under various feeding conditions. This will allow us to identify the factors of diabetes that are sensitive to food intake, regardless of obesity.

III. Adaptive significance of persistent mate-guarding behavior in medaka

Males of various animals exhibit mate-guarding behavior to prevent rival males from mating with the female. Most of them, however, exhibit this behavior during only the mating period, because persistent mate-guarding is thought to have a high energy cost, which would reduce male survival rate. Previously, we reported that medaka males exhibit mate-guarding irrespective of the mating period (Yokoi et al., 2015) and it remains unknown whether there is some benefit of the persistent mate-guarding, such as enhancement of male reproductive success, in medaka fish. In addition, medaka females tend to choose visually-familiarized males as their mating partner and the adaptive significance of this female mating preference is totally unknown. Recently, we found that mate-guarding led to familiarization with the female while at the same time blocking the female's visual familiarization with other males in medaka fish. We put three fish (female, male, male) separately in a transparent three-chamber tank, which allowed the male in the center (near male) to maintain closer proximity to the female than the other male (far male). Placement of the wild-type male in the center blocked visual familiarization of the far male by the female via mate-guarding. In contrast, placement of an arginine-vasotocin receptor mutant male, which exhibits mate-guarding deficits, in the center, allowing for maintaining close proximity to the female, did not block familiarization of the far male by the female. This finding suggested that persistent mate-guarding allows males to gain familiarity with the female over their rivals, which may enhance female preference for the dominant male (Figure 2). We hope that this study helps us understand the evolutional origin of "pair-bonding".

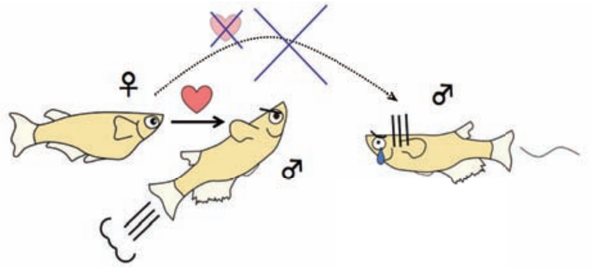

Figure 2. Persistent mate-guarding blocks familiarization between the female and the rival male, which may enhance female preference for the dominant male.

IV. National BioResource Project Medaka (NBRP Medaka) (http://www.shigen.nig.ac.jp/medaka/)

In 2007, NIBB was selected as the core facility of NBRP Medaka. Our laboratory is taking an active part in this project. With the goal of facilitating and enhancing the use of medaka as a model organism, we provide, maintain and collect living resources such as standard strains, inbred strains, and mutants in addition to frozen resources such as EST/cDNA, BAC/ Fosmid clones, and hatching enzymes, as well as integrated information on medaka. We have been providing BAC clones of medaka related species, a library screening system employing a 3D PCR strategy for evolutionary studies, and the TILLING screening system and CRISPR-Cas9 genome editing platform for promoting the reverse genetic approach. NBRP Medaka aims to establish a first rate biological resource with the highest possible levels of accessibility and ease of use.

Publication List:

〔Original papers〕

- Edeline, E., Terao, O., and Naruse, K. (2016). Empirical evidence for competition-driven semelparity in wild medaka. Popul. Ecol. *33*, 246-254.
- Isoe, Y., Konagaya, Y., Yokoi, S., Kubo, T., and Takeuchi, H. (2016). Ontogeny and sexual differences in swimming proximity to conspecifics in response to visual cues in medaka fish. Zool. Sci. *33*, 246-254.
- Kagawa, N., Honda, A., Zenno, A., Omoto, R., Imanaka, S., Takehana, Y., and Naruse, K. (2016). Arginine vasotocin neuronal development and its projection in the adult brain of the medaka. Neurosc. Lett. *613*, 47–53.
- Komine, R., Nishimaki, T., Kimura, T., Oota, H., Naruse, K., Homma, N., and Fukamachi, S. (2016). Transgenic medaka that overexpress growth hormone have a skin color that does not indicate the activation or inhibition of somatolactin-α signal. Gene *584*, 38-46.
- Takehana, Y., Sakai, M., Narita, T., Sato, T., Naruse, K., and Sakaizumi, M. (2016). Origin of the boundary populations in medaka (*Oryzias latipes* species complex). Zool. Sci. *33*, 125-131.
- Takehana, Y., Matsuda, Y., Ikuta, J., Kryukov, A.P., and Sakaizumi, M. (2016). Genetic population structure of the Japanese grass lizard, *Takydromus tachydromoides* (Reptilia: Squamata), inferred from mitochondrial cytochrome *b* variations. Curr. Herpetol. *35*, 22-32.
- Yokoi, S., Ansai, S., Kinoshita, M., Naruse, K., Kamei, Y., Young, L.J., Okuyama, T., and Takeuchi, H. (2016). Mate-guarding behavior enhances male reproductive success via familiarization with mating partners in medaka fish. Front. Zool. *13*, 21.
- Zhang, X., Guan, G., Li, M., Zhu, F., Liu, Q., Naruse, K., Herpin, A., Nagahama, Y., Li, J., and Hong, Y. (2016). Autosomal *gsdf* acts as a male sex initiator in the fish medaka. Sci. Rep. *6*, 19738.

〔Review article〕

- Naruse, K., Chisada, S., Sasado, T., and Takahana, Y. (2016). Medaka as model animal and current atatus of medaka biological resources. Res. & Knowl. *2*, 31-34.