

## LABORATORY OF BIORESOURCES



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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 the evolution of the sex determination system using medaka and relatives, adaptive significance of mate-guarding behavior, The molecular genetic basis of diversified sexually dimorphic traits in *Oryzias* species and the identification of causal genes for pigment cell mutants. In addition to these activities, our laboratory has stepped forward to lead the National BioResource Project Medaka (NBRP Medaka) from 2007.

### 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

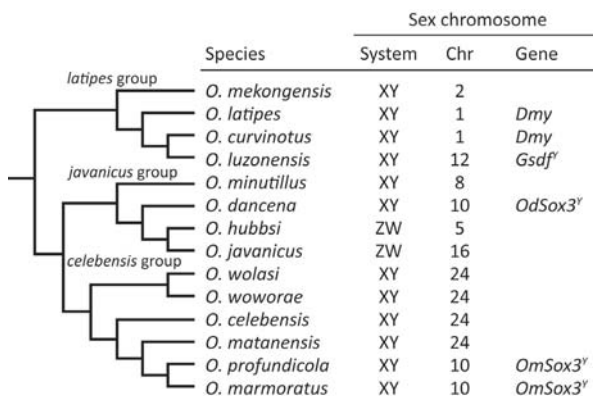


Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

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'*, 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.

### II. Identification of the causal gene of the medaka iridophore mutant, *guanineless(gu)*

The medaka has four pigment cell types: black melanophores, yellow xanthophores, white leucophores, and silvery iridophores. To date, causal genes of melanophore, xanthophore, and leucophore mutants have been elucidated, but the causal gene for the iridophore mutant remains unknown. Herein, we describe the iridophore mutant *gu*, which exhibits a strong reduction in visible iridophores throughout its larval to adult stages. The *gu* locus was previously mapped on chromosome 5, but was located near the telomeric region, making it difficult to integrate into the chromosome. We sought the causal gene of *gu* using synteny analysis with the zebrafish genome and found a strong candidate, *purine nucleoside phosphorylase 4a*. Gene targeting and a complementation test showed that *pnp4a* is the causal gene of *gu*. This result will allow the establishment of inbred medaka strains or other useful strains with see-through phenotypes, without disrupting the majority of the genetic background of each strain.

### 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 a 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 a closer proximity to the female by the far male and did not block familiarization of the far male with 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 evolutionary origin of "pair-bonding".

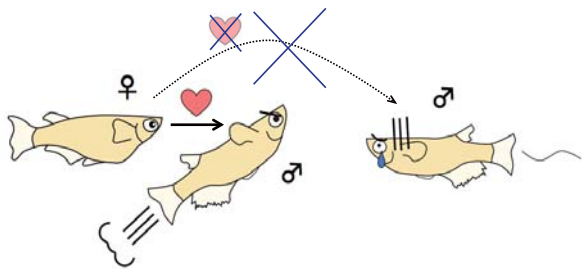


Figure 2. Persistent mate-guarding blocks familiarization between the female and the rival male, which may enhance female preference for the dominant male.

#### IV. The molecular genetic basis of diversified sexually dimorphic traits in *Oryzias* species endemic to Sulawesi, Indonesia

Sexual dimorphism is prevalent, but often differs remarkably between closely related species. However, we know little about which genetic changes can actually contribute to diversification of sexually dimorphic traits between closely related species. We therefore started to investigate the molecular genetic mechanisms underlying the diversification of sexually dimorphic body colorations in medaka (genus *Oryzias*) from Sulawesi, Indonesia. As a first step, we focused on an endemic species *Oryzias woworae*, whose males show red in the pectoral fins and blue in the lateral body. Quantitative trait loci (QTL) mapping in a  $F_2$  intercross between a male of *O. woworae* and a female of *O. celebensis*, which is a close relative without such sexually dimorphic colorations, revealed that an autosomal locus controls red pigmentation in the pectoral fins. Subsequent gene expression analysis showed that upregulation of a gene on the QTL by the *cis*-regulatory mutation(s) would be responsible for the red fins. Further analysis with targeted genome editing

technologies are underway to elucidate the molecular and developmental mechanisms underlying the red fin traits.

#### Publication List:

##### [Original papers]

- Ansai, S., Hosokawa, H., Maegawa, S., Naruse, K., Washio, Y., Sato, K., and Kinoshita, M. (2017). Deficiency of serotonin in raphe neurons and altered behavioral responses in *tryptophan hydroxylase 2*-knockout medaka (*Oryzias latipes*). *Zebrafish* 14, 495-507.
- Inoue, Y., Saga, T., Aikawa, T., Kumagai, M., Shimada, A., Kawaguchi, Y., Naruse, K., Morishita, S., Koga, A., and Takeda, H. (2017). Complete fusion of a transposon and herpesvirus created the *Teratorn* mobile element in medaka fish. *Nat. Commun.* 8, 551.
- Kimura, T., Takehana, Y., and Naruse, K. (2017). Pnp4a is the causal gene of the medaka iridophore mutant guanineless. *G3-Genes Genomes Genet.* 7, 1357-1363.
- Murakami, Y., Ansai, S., Yonemura, A., and Kinoshita, M. (2017). An efficient system for homology-dependent targeted gene integration in medaka (*Oryzias latipes*). *Zool. Letters* 3, 10.
- Sagai, T., Amano, T., Maeno, A., Kimura, T., Nakamoto, M., Takehana, Y., Naruse, K., Okada, N., Kiyonari, H., and Shiroishi, T. (2017). Evolution of *Shh* endoderm enhancers during morphological transition from ventral lungs to dorsal gas bladder. *Nat. Commun.* 8, 14300.
- Sasado, T., Kondoh, H., Furutani-Seiki, M., and Naruse, K. (2017). Mutation in *cpsf6/CFIm68* (*Cleavage and Polyadenylation Specificity Factor Subunit 6*) causes short 3'UTRs and disturbs gene expression in developing embryos, as revealed by an analysis of primordial germ cell migration using the medaka mutant *naruto*. *PLoS ONE* 12, e0172467.
- Seki, S., Kusano, K., Lee, S., Iwasaki, Y., Yagisawa, M., Ishida, M., Hiratsuka, T., Sasado, T., Naruse, K., and Yoshizaki, G. (2017). Production of the medaka derived from vitrified whole testes by germ cell transplantation. *Sci. Rep.* 7, 43185.
- Shimmura, T., Nakayama, T., Shinomiya, A., Fukamachi, S., Yasugi, M., Watanabe, E., Shimo, T., Senga, T., Nishimura, T., and Tanaka, M. (2017). Dynamic plasticity in phototransduction regulates seasonal changes in color perception. *Nat. Commun.* 8, 412.

##### [Original paper (E-publication ahead of print)]

- Nakamoto, M., Shibata, Y., Ohno, K., Usami, T., Kamei, Y., Taniguchi, Y., Todo, T., Sakamoto, T., Young, G., Swanson, P., Naruse, K., and Nagahama, Y. Ovarian aromatase loss-of-function mutant medaka undergo ovary degeneration and partial female-to-male sex reversal after puberty. *Mol. Cell Endocrinol.* 2017 July 13.

##### [Review article]

- Okuyama, T., Yokoi, S., and Takeuchi, H. (2017). Molecular basis of social competence in medaka fish. *Develop. Growth Differ.* 59, 211–218.