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Research and Bioresources (NO.18)

## The Evolution of Sex-Determining Genes from Diversity in Medaka

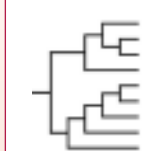
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### Introduction

Approximately 30 species of medaka are widely distributed in Asia, ranging from India to Japan, including the Japanese medaka (killifish, *Oryzias latipes*). The National Bioresource Project (NBRP) Medaka has preserved 13 species and 20 strains out of these 30 existing species. We previously demonstrated that the sex chromosomes of *Oryzias* fishes are extremely diversified. Here, we elucidate part of the diversification mechanism of sex chromosomes by identifying sex-determining genes in the Indian medaka (*O. dancena*), which is widely distributed from East India to the Malay Peninsula. Here, I introduce the content of our research.

### Sex Determination and Sex-Determining Genes in Vertebrates

In most vertebrates, males and females are determined by sex chromosomes. For example, in mammals, if individuals possess XX and XY sex chromosomes, they become males and females, respectively. The male specific Y chromosome contains the *Sry* gene, which determines sex, with differentiation into a male being initiated by the functioning of this gene. However, the *Sry* gene does not exist in other vertebrates, with the chromosome that functions as the sex chromosome differing across taxa. Sex determination is highly diversified in fish species. The sex chromosomes of *Oryzias* fishes are extremely diverse. A sex-determining gene, *Dmy*, was identified in the Japanese medaka, which was the second case in vertebrates. However, the *Dmy* gene only exists in two medaka species, with other related species possessing different sex chromosomes (Fig.1).



Species	Sex determination	Sex chromosome	Sex-determining gene
<i>Oryzias latipes</i>	XY	1	<i>Dmy</i>
<i>O. curvinotus</i>	XY	1	<i>Dmy</i>
<i>O. luzonensis</i>	XY	12	<i>Gsdf</i> <sup>r</sup>
<i>O. mekongensis</i>	XY	?	--
<i>O. hubbsi</i>	ZW	5	--
<i>O. javaicus</i>	ZW	16	--
<i>O. dancena</i>	XY	10	<i>Sox3</i> <sup>Y</sup>
<i>O. minutillus</i>	XY	8	--
<i>O. celebensis</i>	?	?	--

Fig. 1. Phyletic relationships and the sex-determining mechanism in *Oryzias* fishes. Sex chromosomes in *Oryzias* fishes differ according to species. The *Dmy* gene found in *Oryzias latipes* does not exist in other related species, except for *O. curvinotus*.

This fact indicates that sex chromosomes and sex-determining genes have changed many times within a short period. Thus, we aimed to elucidate the change in the sex-determining mechanism at the molecular level. Specifically, we searched for the sex-determining gene in the Indian medaka (*O. dancena*), which did not possess the *Dmy* gene.

### Identification of the Sex-Determining Gene in the Indian medaka

Previous studies have revealed that the sex determination of the Indian medaka is the XY type, and that its sex chromosome is homologous with an autosome (the 10th chromosome). We used positional cloning to identify the sex-determining gene in the Indian medaka. First, we created a detailed genetic linkage map near the sex-determining locus. We also created a physical map near the sex-determining locus by using a bacterial artificial chromosome (BAC) genomic library of the Indian medaka, from which we determined the entire base sequence of this region. As a result, we were able to narrow down the sex-determining region (SD region) to 140 kb on the X chromosome and 310 kb on the Y chromosome (Fig. 2).

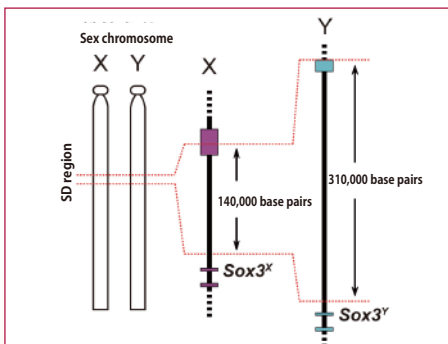


Fig. 2. Sex chromosomes (X and Y) and the SD region in the Indian medaka. The SD region that is involved in sex determination consists of 140,000 base pairs on the X chromosome and 310,000 base pairs on the Y chromosome. The *Sox3* gene exists near the SD region. The *Sox3* gene exists on both the X (*Sox3*<sup>X</sup>) and Y (*Sox3*<sup>Y</sup>) chromosomes, with both chromosomes having the same amino acid sequence.

The SD region is filled with repeated sequences including transposons, with no known gene that encode proteins being found in this region. However, the *Sox3* gene was found in a region adjacent to the SD region. When part of the Y-chromosome sequence, including the *Sox3* gene and the SD region, was experimentally introduced to an XX individual, the sex of the

individual changed to male. When the *Sox3* gene on the Y chromosome was knocked out using genome editing techniques in an XY individual, the sex of the individual changed to female. These experiments indicate that the *Sox3* gene is a sex-determining gene in the Indian medaka, and that the expression control region (enhancer) of the *Sox3* gene exists in the SD region. In other words, the acquisition of the enhancer may generate a new Y chromosome.

### Sox3 Gene, an Ancestor of the Sry Gene

The *Sox3* gene is highly homogeneous with *Sry*, a sex-determining gene in mammals, and it encodes a transcription factor. Both genes (*Sox3* and *Sry*) may have an ancestral allelic relationship, because both genes exist on the same locus. However, since the *Sry* gene is of extremely old origin, its past allelic relationship is unknown. In addition, the relationship between the *Sox3* gene and sex determination had not been reported in vertebrates other than mammals. Our work may directly prove that a new sex-determining gene could be generated by functional differentiation between *Sox3* alleles, showing that the same *Sox3* gene has independently evolved as a sex-determining gene in mammals and in *Oryzias* fishes.

### Diversification Mechanism of Sex-Determining Gene

In *Oryzias* fishes, two male-determining genes have been identified other than *Sox3* in the Indian medaka. One is the *Dmy* gene that is found on the Y chromosome in the Japanese medaka (*O. latipes*) and the other is the *Gsdf* gene that is found on the Y chromosome in the Luzon medaka (*O. luzonensis*) (Fig. 3).

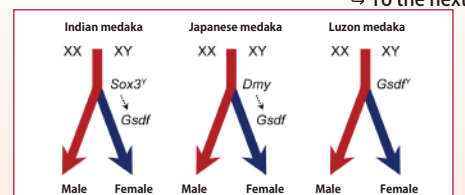


Fig. 3. Diversification of sex-determining genes in *Oryzias* fishes. Three species from the genus *Oryzias* (i.e., the Indian medaka, the Japanese medaka, and the Luzon medaka) possess different male-determining genes (*Sox3*<sup>X</sup>, *Dmy*, and *Gsdf*<sup>r</sup>). However, the *Gsdf* gene is highly expressed in XY individuals of all three species. In the Indian medaka, the *Sox3*<sup>X</sup> gene is assumed to control the expression of the *Gsdf* gene (which was located below the *Sox3*<sup>Y</sup> gene), resulting in the generation of a new sex-determination mechanism.

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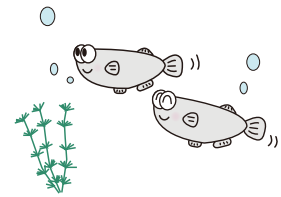
Although the *Dmy* gene only exists in two medaka species, the *Gsdf* gene is expressed in the testis of many fish species. In the Japanese medaka, the *Gsdf* gene is thought to be downstream of the *Dmy* gene. Here, we revealed that the *Gsdf* gene is also highly expressed in the gonad of the Indian medaka, and that its expression is dependent on the expression of the *Sox3* gene on the Y chromosome. These results indicate that the sex-determining cascade below the *Gsdf* gene has been interspecifically preserved, and that a new sex-determining gene may have been generated through the mutation of the *Gsdf* gene or the acquisition of transcriptional regulatory elements for the *Gsdf* gene (*Sox3* and *Dmy*).

### Conclusion

Following the discovery of the sex-determining gene in the Indian medaka, the diversification mechanism of a sex-determining gene and the preservative property of a downstream gene have been elucidated. However, many questions remain, such as whether the *Gsdf* gene is a common master gene in sex determination and how did the alternation between the XY type and ZW type occur. In the future, we intend to elucidate both the diversity and generality in the sex-determining mechanism by obtaining new information about other species in the genus *Oryzias*.

### Reference

Takehana *et al.*, Co-option of *Sox3* as the male-determining factor on the Y chromosome in the fish *Oryzias dancena*. *Nature Communications* 5: 4157 (2014)



## Construction of Linux virtual environment in Windows

Some time has passed since the use of next-generation sequencers became commonplace. Nevertheless, having a Linux environment is still a requisite for analyzing sequence data, because analysis tools often cannot be installed in a Windows environment. In this article, I shall introduce a method for installing a Linux virtual machine on Windows using VMware Player, a tool that is free for non-commercial use.

### Installing VMware

1. Download the "VMware Player for Windows" installer from the download page<sup>1</sup> (Fig.1).
2. Double-click on the installer to launch it once it has downloaded, and follow the instructions to complete the installation.



Fig. 1. Downloading VMware

### Obtaining a Linux distribution

While there are several Linux distributions available, we will use Bio-Linux 8, a distribution based on Ubuntu 14.04 that comes pre-installed with several useful analysis tools. Download the ISO file from the Bio-Linux download page<sup>2</sup> (Fig.2).

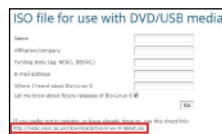


Fig. 2. Downloading Bio-Linux

### Installing Bio-Linux on VMware

1. Launch VMware Player and click on [Create a New Virtual Machine].
2. Select "Installer disc image file (iso)," click on [Browse], and select the ISO file downloaded during the previous step. Then, click [Next].
3. Select "Linux" as the guest OS and "Ubuntu 64 bit" as the version. Proceed with the installation wizard by choosing other settings pertaining to your computer's environment.
4. After the wizard has completed the installation, the new virtual machine will be displayed (Fig.3). Select the virtual machine, and click on [Play Virtual Machine] to launch it.



Fig. 3. VMware startup screen



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5. In the screen that is displayed after launching the virtual machine, click on [Install Bio-Linux] to begin installing Bio-Linux (Fig.4).
6. Follow the instructions from the installation wizard to complete the installation.

Note: Because Bio-Linux is developed in English, make sure to select English as the language during the installation.

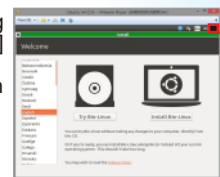


Fig. 4. Bio-Linux installation screen

### What you can do with Bio-Linux

Bio-Linux comes pre-installed with several tools. Double-click on "Bio-Linux Documentation" located on the desktop, and double-click on "Bioinformatics Docs" to view detailed explanations for the available tools (Fig.5).

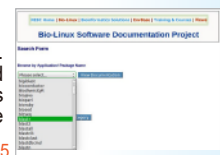


Fig. 5. Documentation for Bio-Linux tools, etc.

### Starting up and shutting down the virtual machine

In order to shut down the virtual machine, click on the cog icon in the top right-hand corner of the Linux desktop (Fig.4-A) and select [Shut Down ...]. In order to start the virtual machine, click on the created virtual machine in the VMware Player window (Fig.3) and click on [Play Virtual Machine].

### Configuring the virtual machine

You can configure various settings, such as those related to the memory and processor, by selecting the virtual machine on the VMware Player screen and clicking [Edit virtual machine settings].

In this edition, I have explained how to install Bio-Linux. In the next edition, I will introduce the steps required for performing analyses using some of the tools that come preinstalled with Bio-Linux. (Shunsuke Maeda)

<sup>1</sup> [https://my.vmware.com/en/web/vmware/free#desktop\\_end\\_user\\_computing/vmware\\_player/6\\_0](https://my.vmware.com/en/web/vmware/free#desktop_end_user_computing/vmware_player/6_0)  
<sup>2</sup> <http://environmentalomics.org/bio-linux-download/>

## Database of this Month

### RRC(Research Resource Circulation)



DB name : RRC(Research Resource Circulation)  
 URL : <http://rrc.nbrp.jp/>  
 Language: Japanese and English  
 Original contents:  
 • Information about research papers and patents obtained using NBRP resources  
 Features:  
 • Information about a paper can be registered.  
 • A research paper can be registered only with PM ID.  
 • From a research paper, information about the related resource can be accessed.  
 • RRC has been registered in PubMed LinkOut.  
 Cooperative DB: Each site of the NBRP, NCBI PubMed  
 DB construction group: NBRP Information  
 Management organization: Genetic Resource Center, NIG  
 Year of first DB publication: 2007 Year of last DB update: 2014

- Number of registered papers : 14,801
  - Number of registered patents : 862
- (As of October 2014)

Comment from a developer : RRC is a database and a registration site for accessing information about research papers and patents obtained using the NBRP resources. In 2013, the RRC became a provider of the PubMed LinkOut, and a resource order page can be accessed from PubMed Abstract through the RRC. The NBRP is in the midst of developing a function whereby information about a research paper registered in the RRC is automatically added to the detailed page of each resource. Thus, a virtuous cycle of resources and research activities can be realized.

When a research paper or a patent obtained using the NBRP resources is published, please register it with the RRC.

### Contact Address

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 1111 Yata, Mishima-shi, Shizuoka 411-8540, Japan  
 Tel.: 055-981-6885 (Yamazaki)  
 E-mail : [brnews@shigen.info](mailto:brnews@shigen.info)

### Editor's Note

I am able to vaguely understand that the mechanism of determining the sex of an organism is diversified, but it is interesting that such diversity is observed in medaka and its related species, even though sex determination is genetically controlled. From the perspective of animal evolution, it is quite interesting that sex-determining genes similar to those found in mammals have been found in fishes belonging to the genus *Oryzias*, with this research being the first to document this phenomenon. The fact that approximately half of all medaka species, including the Japanese medaka, can be obtained from the NBRP collection is advantageous to medaka researchers. I look forward to seeing the future development of this research (Y. Y.).

### BioResource Information

(NBRP) [www.nbrp.jp/](http://www.nbrp.jp/)  
 (SHIGEN) [www.shigen.nig.ac.jp/](http://www.shigen.nig.ac.jp/)  
 (WGR) [www.shigen.nig.ac.jp/wgr/](http://www.shigen.nig.ac.jp/wgr/)  
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## BioResource Now!

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