Transcriptional regulation underlying the development of sexual differences in teleost fishes

博士論文（要約）

Transcriptional regulation underlying the development of sexual differences in teleost fishes

（硬骨魚の性差形成における転写制御機構の解明）

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Introduction

Almost all vertebrates reproduce sexually and exhibit morphological, physiological and behavioral differences between female and male. Sexual differences reflect selective pressure and are associated with survival and reproductive success of each sex (Ellegren and Parsch 2007; Williams and Carroll 2009). Since females and males share most of genomic content, sexual differentiation mainly relies on transcriptional regulation. Sexual differences in gene expression have been observed not only in genes on sex chromosomes but also on autosomal chromosomes in many vertebrates (Kang et al. 2011). Epigenetic modifications, such as DNA methylation, are considered to be candidate regulatory mechanisms for sexual differences in gene expression. Previous studies found significant correlation between DNA methylation and sexual differences in gene expression (Shao et al. 2014; Xu et al. 2014). In vertebrates, DNA methylation primarily occurs at cytosine-guanine dinucleotides (CpG), and especially, DNA methylation around the promoter sites influences sexual differences in gene expression (Navarro-Martín et al. 2011; Matsumoto et al. 2013) by directly or indirectly suppressing gene expression (Jones and Takai 2001; Bird 2002; Klose and Bird 2006).

Teleost fishes display divergent and flexible sexual developmental systems, which is triggered by genetic and/or environmental factors (Kobayashi et al. 2012). Sex differences in gene expression have also been reported in many teleost fishes, and thus, transcriptional regulation through DNA methylation might play an essential role in sexual differences in gene expression (Navarro-Martín et al. 2011; Shao et al. 2014). However, how DNA methylation affects sex difference in gene expression is poorly understood in teleost fishes. Moreover, in teleost fishes, hermaphroditism is found at least 27 families across 7 orders (Mitcheson and Liu 2008). Interestingly, some coral reef fishes are able to change their sex rapidly during the course of life according to social circumstance (Godwin 2009). Socially controlled sex change is observed in many different fish lineages and involves protogyny (from female to male), protandry (from male to female) and bi-directional sex change (both direction serially) (Kuwamura and Nakamura 1998; Reavis and Grober 1999; Kobayashi et al. 2012). During sex changes, sexual differences are reconstructed rapidly using completely identical genomes. Although sex change depending on social status is assumed to be initiated by brain response, little is known about change in brain during sex change. Since gonochoristic fishes exhibit differential gene expression in brain (Hiraki et al. 2012; Sharma et al. 2014), transcriptional regulation is probably involved in initiation of sex change and emergence of sex specific behavior.

In this thesis, I focused two aspects of the transcriptional regulation underlying sexual differences in teleost fishes; epigenetic regulations of sexual difference and changes in gene expressions

during sex changes. In chapter 1, I examined the effect of CpG on sexual differences in gene expression and conducted genomic analysis using *Poecilia reticulata* which has remarkable sexual dimorphism. In chapters 2 and 3, I investigate changes in gene expression in brain tissues during rapid sex change using *Trimma okinawae* showing bi-directional sex change. In chapter 2, I focused on the sex hormone receptor genes and examined the association of the gene expression levels in brain with behavioral sex change. In chapter 3, transcriptome analysis was conducted to understand the change trends of gene expression during behavioral sex change at whole transcriptomic levels.

Chapter 1

Sexual differences in gene expression have been observed not only in genes on sex chromosomes but also on autosomal chromosomes in many vertebrates. Epigenetic modifications, such as DNA methylation, play a crucial role in sexual differences in gene expression. Previous studies found significant correlation between DNA methylation and sex-biased gene expression via transcriptome and methylome analyses. In particular, variable levels of DNA methylation at promoter regions influence sex-biased gene expression and sex differentiation in vertebrates by directly (blocking transcription factors from binding to promoter regions) or indirectly (recruiting proteins related to chromatin formation) suppressing gene expression. In this chapter, I examined the effect of CpG, which is primary target of DNA methylation, on sexual differences in gene expression using *P. reticulata*. Sliding window and GAM analysis revealed that genes with sexual difference in expression had different feature of CpG densities from genes lacking sexual difference. Especially, male upregulated genes with intermediate CpG density downstream of TSS exhibited greater sexual difference in gene expression. This trend implies that DNA methylation on downstream of TSS regulates male typical gene expression and contributes to notable sexual differences in morphology of *P. reticulata*. These results suggest that transcriptional regulations through DNA methylation on CpGs play crucial roles in sexual differences in teleost fishes.

Chapter 2

Socially controlled sex-change occurs rapidly following social status change. Especially, behavioral sex-change begins within minutes and is assumed to occur independently of the gonad change. *T. okinawae* is an appropriate model organism for exploring the mechanism in rapid behavioral sex change, since the sex change of both directions can be manipulated and observed in aquariums. During sex changes, sexual

differences are reconstructed rapidly using completely identical genomes. Since gonochoristic fishes exhibit differential gene expression in brain, transcriptional regulation is probably involved in initiation of sex change and emergence of sex specific behavior. Although sex hormones and these receptors are known as key regulators for sexual differences in physiological and behavioral traits, the involvement of sex hormone receptors in rapid sex-change is still unclear. In chapter 2, I obtained the partial coding sequences of two androgen receptor genes (*ara* and *arb*) and three estrogen receptor genes (*era*, *erb1* and *erb2*) and quantified the gene expression levels before and after behavioral sex-change in brain of *T. okinawae*. In this thesis, I defined individuals after an hour from the initiation of sex-changes as male-like female and female-like male. The expression levels were measured by real-time quantitative PCR and compared among four groups. The results showed that the expression levels of *erb1* and *erb2* in male brains were higher than those in female brains, and the expression levels of *arb*, *erb1* and *erb2* significantly decreased during male-to-female within an hour after the onset of behavioral sex change (Fig. 1). Contrary, no significant change in the expression of five receptor genes were observed during female-to-male sex change. These results suggest that the expression changes in sex hormone receptor genes were related to rapid behavioral sex changes in *T. okinawae*, but the effects differed depending on the directions of the sex change.

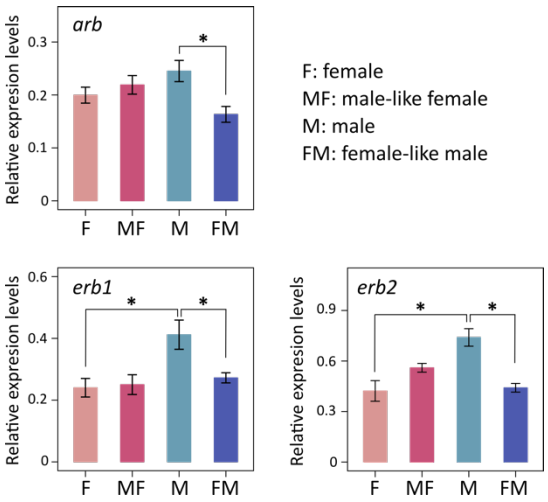

Figure. 1 Gene expression levels of *arb*, *erb1* and *erb2*. All values are normalized to *b-actin* and plotted the mean with standard error. Significant differences (P <0.05) are marked by asterisk as determined by tukey HSD test.

Chapter 3

In chapter 3, in addition to sex hormone receptor genes, I tried to detect differentially expressed genes (DEGs) between before and after behavioral sex change by RNA-seq using the brain of *T. okinawae*. Recent studies performed transcriptomic analyses in protogyny and protandry fishes and showed that few genes were detected as sex-biased expression genes. However, it is unclear how the expression levels of genes in the brain change during behavioral sex changes. To examine gene expression changes during behavioral sex changes at a whole transcriptome level, I performed RNA sequencing on Illumina HiSeq 4000 platform and constructed *de novo* transcriptome assembly in brain of *T. okinawae*. Then, I analyzed the gene expression changes before

and after behavioral sex-change and identified 86, 65 and 170 DEGs between males and females, between female and male-like female, and between male and female-like male, respectively. During female-to-male sex change (i.e., male-like female), the expression profile of DEGs rapidly transitioned to those of male brain (Fig. 2). On the other hands, during male-to-female sex change (i.e., female-like male), the expression profile of DEGs completely differed from both of male and female. These findings, along with the results in chapter 2, suggest that the transcriptional regulations involved in behavioral sex change were different depending on the directions of the sex change. The results also showed that gene expression levels of many ribosomal protein genes are up-regulated in fish behaving as female, which suggests those genes are involved in rapid emergence of female like behavior.

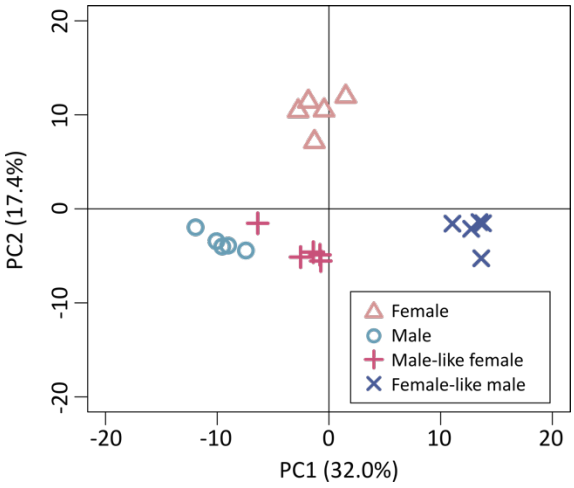

Figure. 2 Result of principle component analysis using only differentially expressed genes. Each plot indicates the value of an individuals of female, male, male-like female or female-like male. Numbers in parentheses indicate the proportion of the variance explained by the principal components.

Discussion

Teleost fishes, which consist of over 30,000 species, are highly diverse group among vertebrates (Nelson 2006). They exhibit diverse and flexible sexual system including hermaphroditism resulted from extremely high sexual plasticity unlike other vertebrates (Munakata and Kobayashi 2010; Godwin 2010; Piferrer et al. 2012; Kobayashi et al. 2013). While sexual systems which retain high plasticity are of great interest to evolutionary ecologists and ichthyologists, the mechanism has been largely unknown compare to birds and mammals. My analysis using genome and transcriptome datasets of *P. reticulata* demonstrated that genes with sexual difference in gene expression had different feature of CpG densities compared with genes without sexual difference. Since male and female share almost all genomic contents, epigenetic regulations including DNA methylation should play essential roles on sexual differences. Furthermore, sexual differences in gene expression also have been documented in sex change fish despite the fact that they have completely identical genome. during sex change of *T. okinawae.* A series of results in this thesis highlighted the importance of transcriptional regulation in the construction and maintenance of sexual difference of teleost fishes. Recently,

development and improvement of next generation sequencer has been remarkable, and therefore we are able to obtain massive information, such as genome, transcriptome and methylome even in non-model species. Further comprehensive analyses using closely related species are expected to clarify the underlying mechanism of sexual differentiation in teleost fishes.