Molecular studies on the genotypic and temperature-dependent sex determination of pejerrey Odontesthes bonariensis

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

専　攻 Major	応用生命科学	氏　名 Name	張燕
論文題目 Title	Molecular studies on the genotypic and temperature-dependent sex determination of pejerrey *Odontesthes bonariensis* **(**ペヘレイ*Odontesthes bonariensis*の遺伝的・温度依存的性決定の分子機構に関する研究**)**		

Pejerrey *Odontesthes bonariensis* is an excellent species for the study of temperature-dependent sex determination (TSD) in teleosts. In this species, sex ratios reach 100% female or 100% male at the environmentally relevant temperatures of 17°C (female producing temperature, FPT) and 29°C (male producing temperature, MPT) respectively, when the thermal exposure occurs between hatching and the onset of histological differentiation of the gonads (around 5 weeks post-hatch). At intermediate temperatures (around 24-26°C; mixed sex-producing temperatures, MixPT), a large variation in sex ratios (e.g. 20-80%) is observed between progenies from different parents at a given temperature. These observations suggest a potential genetic involvement in the sex determination of pejerrey. In the context of the recent discovery of the presence of a sex determining gene *amhy* (male-specific duplication of the autosomal anti-Müllerian hormone gene) in the congeneric species *O. hatcheri* (Patagonian pejerrey), the purpose of this study was to determine the presence of this sex determining gene and its involvement in the variable sex ratios observed at intermediate temperatures.

In order to study the genetic contribution of *amhy* in the process of gonadal sex determination/differentiation in pejerrey, this thesis was divided into three chapters as follows. I first verified the presence of *amhy* and its paralogue, the autosomal *amha*, in this species. I then investigated the transcriptional profiles of *amhy* and *amha* at feminizing and masculinizing temperatures during early larval development with the aim of evaluating their relationship with TSD and testis formation. The last chapter of my thesis focused on the regulation of *amhy* and *amha in vitro* by cortisol and 11-Ketotestosterone (11-KT) using *amhy* and *amha* presumptive promoters.

First, an *amhy* homologue was successfully isolated and cloned from wild and laboratory-reared pejerrey. Screening of wild and laboratory-reared pejerrey for *amhy* revealed a high, although not complete, linkage with phenotypic sex. The sex ratio in an *amhy*+/*amhy*- full sibling progeny reared during the thermolabile period of sex determination at an intermediate temperature of 25ºC was 68.7% male: 31.3% female; all *amhy*+/- fish developed as males whereas about 2/3 and 1/3 of the *amhy*-/- were female and male, respectively. At 25°C, transcription of *amhy* in *amhy*+/- animals persisted in larvae throughout the period of sex determination and histological gonadal differentiation. The autosomal *amha* was expressed in the gonads of all *amhy*+/- but only in part of the *amhy*-/- animals and seemed to be related to maleness in the latter. These observations suggest that both *amhy* and *amha* are important for testicular differentiation in pejerrey. These findings also represent the first clear genomic evidence that genetic and environmental sex determinants can coexist in species with marked TSD such as the pejerrey.

The second chapter examined the relative contribution of *amhy* and *amha* to the TSD process of pejerrey. XY and XX larvae derived from a XX mother and a XY father were reared at 17ºC

(female-promoting temperature, FPT) and 29ºC (male-promoting temperature, MPT) during the critical period of thermolabile sex determination and used for transcriptional analyses of *amhy* and *amha* by qRT-PCR. In addition, I analyzed the expression profiles of ovarian type aromatase *cyp19a1a* (critical for female development) and *amh* type II receptor *amhrII* (critical for male development), at the FPT and MPT respectively. Histological analyses at the end of experiment revealed that the MPT yielded a single-sex male population and that the FPT yielded a mixed sex population whereby 59% of the XY fish developed as males and the remaining as females. *amhy* mRNAs were abundant in XY larvae from both the FPT and MPT groups at the beginning of the sex determination period and then declined. *amha* expression was highly correlated with maleness. During the sex determination period, *amha* was upregulated in a few of the XY larvae at the FPT and in both genotypes at the MPT. *cyp19a1a* expression was found to be inversely proportional to temperature in XX fish whereas in XY genotypes a dimorphic distribution of *cyp19a1a* was observed at the 17ºC. *amhrII* expression did not differ between XX and XY fish although it was higher at 25ºC and 29ºC than that at 17ºC in both genotypes. Thus, these results suggest that *amhy* expression is temperature-independent while *amha* and *amhrII* expression were temperature-dependent. This indicates that temperature may modulate *amha* expression though *amh* receptors which then induce masculinization in pejerrey.

A previous study has shown that the stress-related hormone cortisol promotes 11-KT production during high temperature-induced masculinization of pejerrey. The 3rd chapter focused on how the two *amh* paralogues of pejerrey interact with stress and sex steroid axis during gonadal differentiation. A luciferase reporter assay was performed with the presumptive promoters (~3kb 5' upstream fragment) of both *amh* paralogues. The glucocorticoid receptor expression plasmid was first co-transfected with luciferase reporter plasmids containing *amhy* or *amha* promoter into endothelial progenitor cells (EPCs). Transcriptional activity was then measured 48 hours post transfection in cells exposed to different cortisol and 11-KT doses. Transcriptional activity analyses showed that the *amhy* promoter did not respond to any cortisol or 11-KT doses. On the other hand, both cortisol and 11-KT activated the *amha* promoter. The transcriptional activity of *amha* promoter revealed a cortisol dose-dependent manner, which suggests high water temperature induces *amha* expression by elevating cortisol and androgen levels in pejerrey.

This study is the first to show evidence of the co-existence of GSD and TSD in pejerrey. Although *amhy* is considered the genotypic sex determinant, the autosomal *amha* may also be involved in testis formation in pejerrey. In addition, my study also reveals the significance of cortisol and androgen signaling, especially at high temperatures, as transcriptional regulators for the *amha* gene during the process of masculinization.