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

博士学位要約
Summary

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**)**		

課題設定

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. This study was to investigate the presence of the genotypic sex determinant(s) *amhy*, found in the congeneric Patagonian pejerrey (*Odontesthes hatcheri*), and genetic contribution of *amhy* and the autosomal *amha* to temperature-dependent sex determination of pejerrey.

方法論

Main experiments include rearing and sampling of pejerrey broodstock and

larvae; genotyping, expression analyses and *in vitro* cell culture.

Techniques include gene amplification, gene expression analysis by RT-PCR, qRT-PCR and *in situ* hybridization, molecular cloning, cell culture, transient transfection and luciferase reporter gene assay

実験

To examine the presence of *amhy* in pejerrey, genomic DNA from 24 broodstock fish was extracted, subjected to amplification by PCR using *amhy*/*amha*-specific primers, and then sequenced. Larvae derived from a cross between an $amhy^{-}/^{-}$ female and an $amhy^{+}/^{-}$ male were reared at 25°C (MixPT) for up to 14 weeks after hatching (wah) and sampled every two weeks for the analyses of *amhy* and *amha* mRNA expression (RT-PCR and ISH) in relation to gonadal sex differentiation. The remaining larvae were sampled at the end of 14 wah for the determination of sex ratios by light histology. To investigate 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 progeny from an XY male and XX female was reared at 17, 25, or 29ºC (female-, mixed-sex-, and male-promoting temperatures, respectively) during the critical period of thermolabile sex determination of pejerrey and beyond until 14 weeks after hatching (wah). Larvae were sampled weekly until 10 wah and the fins and trunks were used for *amhy*-based genotyping and real-time PCR expression analyses of *amhy*, *amha*, *amhrII*, and *cyp19a1*, respectively. All remaining larvae from each temperature were sampled at 14 wah for histological

determination of sex ratios. In addition, a luciferase reporter assay with the presumptive promoters (~3kb 5' upstream fragment) of both *amh* paralogues was performed to investigate the regulation of these two genes by cortisol and 11-Ketotestosterone *in vitro*. The glucocorticoid receptor expression plasmid was co-transfected with luciferase reporter plasmids containing *amhy* or *amha* promoter into endothelial progenitor cells. Transcriptional activity was measured 48 hours post-transfection in cells exposed to different cortisol doses.

結果

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.

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.

結論・考察

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. Future studies will focus on the interactions between *amhy* and *amha* by producing transgenic pejerrey and estrogenic regulation of *amhy* and *amha* as to unravel the molecular mechanisms of low temperature-induced feminization.