

報告番号	第 号
------	-----

主論文の要旨

Variation of body weight and seasonal reproduction in populations from different latitudes: genetic analyses using wild-derived medaka fish

論文題目

(異なる緯度に由来する集団における体重と季節繁殖の変異：野生由来メダカを利用した遺伝学的解析)

氏名

IMAEDA YASSUMOTO Tamiris

論文内容の要旨

Seasonal changes in the environment are more robust and dramatic in higher latitudes. Therefore, latitudinal variation in various physiological functions has been widely predicted. In this study, I use forward genetic approaches to search for the genetic mechanisms responsible for different body weights and suppression of reproduction under short days in medaka fish from different latitudes.

Body weight is a complex trait that depends on many variables, and is of economical, medical and ecological interests. To better understand the genetic basis of body weight, I compared two wild medaka stocks: one from higher latitudes and greater body weight, and the other from lower latitudes and lower body weight. Those populations were crossed and quantitative trait locus (QTL) analysis was performed using 126 F₂ individuals. A significant QTL was found on chromosome 4. Inside that region, there were 163 protein-coding genes, and a search in literature revealed that 12 of them hold some connection to body weight or growth. Among them, eight had some amino acid substitution between the two analyzed medaka populations, and the substitution on the gene *sned1* was predicted by PROVEAN (Protein Variation Effect Analyzer) to affect the protein function. The QTL found in that analysis was estimated to explain 14% of the variance between the two populations. That result may help to uncover what loci are responsible for the differences of growth and body weight in populations from different latitudes.

A similar approach was made in the investigation of suppression of reproduction under short days. In higher latitudes, seasonal breeders suppress reproduction in autumn and avoid producing offspring in the harsh winter conditions. In lower latitudes, however, the favorable season for reproduction tends to be longer.

Therefore, suppression of reproduction under short days varies with latitude, and may determine whether an animal will be a seasonal or a non-seasonal breeder. In previous research in our research group, QTL analysis was performed with F₂ generation of the cross between Kiyosu medaka, from higher latitude and responsive to short days, and Miyazaki medaka, from lower latitude and non-responsive to short days. A significant QTL with 349 genes was found. The goal was to unveil the genetic mechanism that determines whether a population will be a seasonal or a non-seasonal breeder. In this current study, I selected 32 candidate genes within the QTL region accordingly to known connections to reproduction or circadian rhythms, and analyzed the SNPs between the two populations. Two of those genes, *pebp4* and *disp3*, had an amino acid substitution in the protein that was predicted to affect function. Moreover, the substitution in *disp3* is inside a sterol-sensing domain, which may increase the chances of affecting phenotypes related to reproduction. In addition to the SNPs in the QTL regions, RNA-Seq was performed to obtain information on differently expressed genes (DEGs) between the two populations, as this could be another way to generate different phenotypes. Kiyosu and Miyazaki adults were exposed to short days (SD) (10L 14D) or long days (LD) (14L 10D) for 14 days, and sampled at zeitgeber time (ZT) 0 or ZT10, and their brain region containing the hypothalamus and pituitary was sampled for RNA-sequencing. Genes with a fold change higher than 1.5 transcripts per million (TPM) and FDR p-value lower than 0.05 were considered DEGs. To validate physiological conditions of two populations, I performed a gene ontology (GO) analysis. Top enriched GO terms for DEGs between Kiyosu and Miyazaki under short day condition highlighted the sensory perception of light, steroid metabolic process, and steroid hormone biosynthesis, supporting the differences in the reproductive status of those two populations. When focusing on the DEGs inside the QTL region, five of them were listed as having some relationship with reproduction. One of them, *disp3*, is particularly interesting because, as aforementioned, an amino acid substitution was observed between Kiyosu and Miyazaki.

Another gene that was considered a strong candidate was *klf9*. That gene is located close to the QTL peak and has an amino acid substitution between the two populations. It also has connections both to circadian rhythms and to reproduction. Therefore, it was further investigated. The strategy I chose was an investigation of loss of function using the genome-editing tool CRISPR/Cas9 to create *klf9* KO medaka. *klf9* KO medaka were able to mate and produce healthy offspring, which means *klf9* is not essential for reproduction in both males and females. To test whether *klf9* is essential for the response to short days, a SD-response experiment was performed with 6-months old medaka. However, there was no significant

difference in gonadal regression after two weeks in short days in comparison to wild type females, which suggests that *klf9* is not essential for the response to short days. Those results are still under confirmation. Additionally, as *klf9* was hypothesized to have a role in stress response due to responsiveness to glucocorticoids, I performed a “novel tank” behavioral experiment to investigate the difference between *klf9* KO and wild type in anxiety-like behavior. In this test, *klf9* KO females displayed significantly higher anxiety-like behavior than wild-type females. The result opens the possibility of an interesting investigation on the role of *klf9* in modulation of stress-response in medaka.

In this study, I was interested in the genetic mechanism of phenotypes of populations from different latitudes. A QTL for body weight was found, with genes related to body weight and growth, and I can conclude it explains part of the variance between the two populations. Regarding the suppression of reproduction by short days, I found interesting candidate genes inside the QTL, and also learnt about their differences in expression levels. Those analyses provided a list of genes that, so far, I conclude are the most promising candidates for the response to short days. Although results for *klf9* were negative, further investigations on the other candidate genes may hopefully lead us to unveil the genetic mechanism that determines whether a population will be a seasonal or a non-seasonal breeder.