## Division of Seasonal Biology (Adjunct)

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Animals living outside the tropics adapt various physiology and behavior to seasonal changes in the environment. For example, animals restrict breeding to specific seasons to maximize survival of their offspring in temperate zones. As animals use changes in day length and temperature as seasonal cues, these phenomena are referred to as photoperiodism and thermoperiodism, respectively. We use comparative approaches to understand these mechanisms. Medaka fish provide an excellent model to study these mechanisms because of their rapid and robust seasonal responses. In this division, we are trying to uncover the underlying mechanisms of seasonal adaptation.

## I. Underlying mechanism that defines the critical photoperiod

It is well established that the circadian clock (i.e., an internal biological clock with a period of approximately 24 hrs) is somehow involved in seasonal time measurement.

However, it remains unknown how the circadian clock measures day length. It has been reported that Medaka populations that were caught at higher latitudes have more sophisticated responses to day length (Sawara and Egami, 1977). For example, Medaka fish caught in Hokkaido have a longer critical day length (i.e., duration of photoperiod required to cause a response) than those caught in Okinawa. To uncover the underlying mechanism of seasonal time measurement, we are currently performing a forward genetic analysis in Medaka populations collected from various latitudes all over Japan.

### 1-1 Variation in critical photoperiod with latitude in Medaka fish

To perform a forward genetic analysis, we have obtained 11 populations including wild populations, closed colonies, and inbred strains from all over Japan. We have examined the effects of changing day length to determine the critical day lengths that will cause seasonal responses in the gonad. In winter, fish were subjected to 10, 11, 12, 13, and 14 h day lengths with warm temperatures. Then gonadal development was examined to determine the critical day length.

As a result, we found differences in the critical day length among Medaka populations. That is, Medaka from higher latitudes required longer day lengths while those from lower latitudes required shorter day lengths.

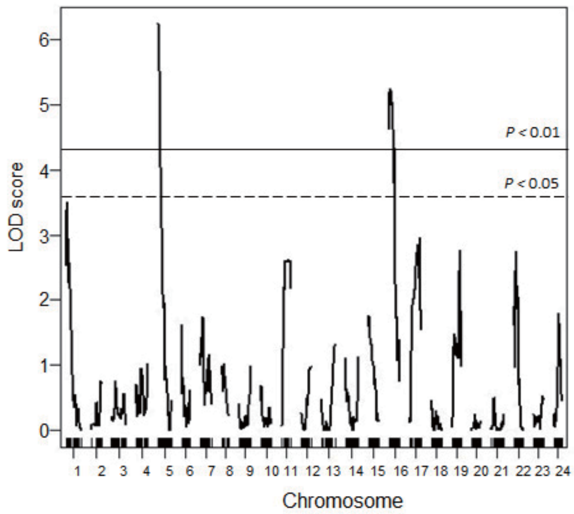


Figure 1. Result of QTL analysis for critical day length.

### 1-2 Quantitative trait loci (QTL) analysis of critical day length

To identify the genes regulating critical day length, quantitative trait loci (QTL) analysis was conducted using $F_2$ medaka derived from crosses between Northern and Southern populations. As a result, we identified significant QTLs using Restriction-site Associated DNA (RAD) markers (Figure 1). We have also performed whole genome re-sequencing using various Medaka strains that show different critical photoperiods, and identified potential candidate genes that define the critical day length.

## II. Mechanism that determines seasonal breeders and non-seasonal breeders

Animals that reproduce year-round (e.g., human beings and laboratory mice) are so-called non-seasonal breeders. In contrast most animals living outside of tropical zones reproduce only during a particular period of the year. Therefore, they are called seasonal breeders. However, the underlying mechanism that determines seasonal breeders and non-seasonal breeders remains unknown. To uncover this mechanism, we performed a forward genetic approach.

### 2-1 Geographic variations in the responses to short day stimulus

When we transferred Medaka fish from summer conditions to winter conditions, we noticed that Medaka from lower latitudes do not regress their gonads even under short day conditions. Accordingly, we next examined the responses to short day conditions using 20 populations derived from various latitudes. As a result, populations from higher latitudes showed gonadal regression, while populations from lower latitudes did not regress their gonads (Figure 2).

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2017. The former title is indicated by an asterisk (*).

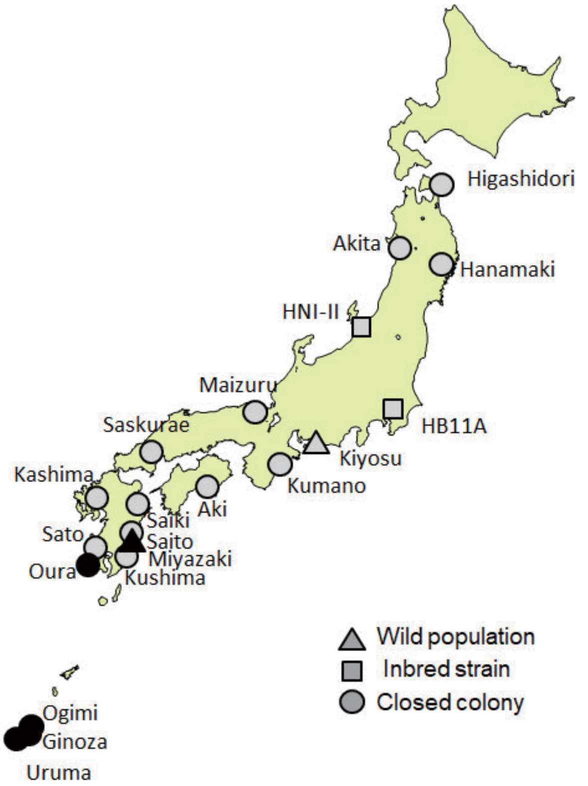


Figure 2. Medaka from lower latitudes (Solid black symbols) do not regress their gonads even under short day conditions.

### 2-2 QTL analysis of genes determining seasonal breeders and non-seasonal breeders

To identify genes that determine seasonal breeders and non-seasonal breeders, we performed QTL analysis using $F_2$ generations and identified a significant QTL that determines seasonal breeders and non-seasonal breeders. We are also performing a genome-wide association study to identify responsible genes.

## III. Transcriptome analysis of seasonality in Medaka fish

In addition to the forward genetic approach, we have performed genome-wide transcriptome analysis of brain, eye, and liver of Medaka fish to understand the underlying mechanism of seasonal adaptation.

### 3-1. Seasonal changes in behaviors

Medaka kept under winter conditions stayed at the bottom of the tank, whereas medaka kept under summer conditions swam all over the tank. In general, fish avoid strong light stimulus (i.e., negative phototaxis). Summer medaka avoided white light stimulus, while winter medaka failed to show this negative phototaxis, suggesting that medaka are less sensitive to light under winter conditions, compared to summer conditions. When color preference was examined using three-dimensional computer graphics (3D-CG), summer medaka exhibited a preference for virtual fish with nuptial coloration, while winter medaka exhibited no such preference. This observation implies that the medaka's color perception is influenced by seasons.

### 3-2. Seasonal changes in phototransduction pathway

The temporal pattern of gene expression in medaka eyes associated with changes in seasons was examined by microarrays. This analysis identified summer induced genes that include various opsin genes and genes involved in downstream phototransduction pathways (Figure 3). We next examined the functional significance of these genes' expression using LWS (long wavelength sensitive) opsin-null fish. As a result, LWS opsin-null fish showed reduced negative phototaxis to white light compared to wild type fish under summer conditions, suggesting that summer-induced LWS opsin is critical for the emergence of negative phototaxis. In addition, LWS opsin-null fish showed weaker preference for virtual fish with nuptial coloration under summer conditions, suggesting that summer-induced LWS opsin is crucial for the emergence of mate preference observed in summer.

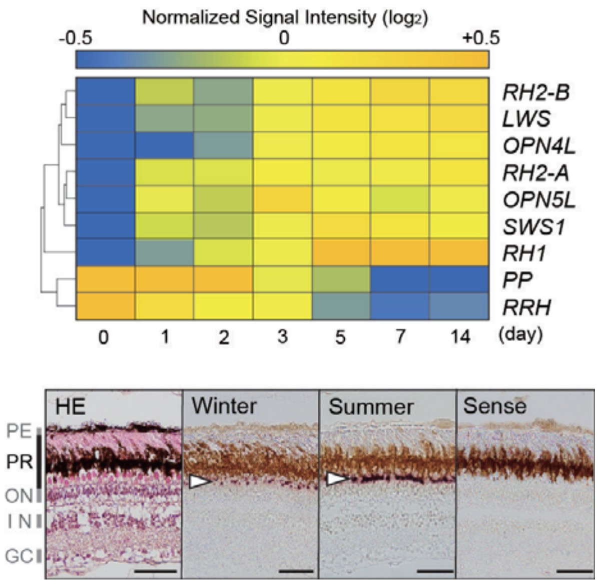


Figure 3. Genome-wide expression analysis reveals dynamic seasonal changes in opsin gene expression within the eye. Top: Results of microarray analysis. Bottom: Expression of LWS opsin within the retina.

## Publication List:

〔Original paper〕


- Shimmura, T., Nakayama, T., Shinomiya, A., Fukamachi, S., Yasugi, M, Watanabe, E., Shimo, T., Senga, T., Nishimura, T., Tanaka, M., Kamei, Y., Naruse, K., and Yoshimura, T. (2017). Dynamic plasticity in phototransduction regulates seasonal changes in color perception. Nat. Commun. *8*, 412.


〔Review articles〕


- Ikegami, K., and Yoshimura, T. (2017). Molecular mechanism regulating seasonality. In: Biological Timekeeping: Clocks, Rhythms and Behaviour. 589-605.
- Tamai, K.T., and Yoshimura, T. (2017). Molecular and neuroendocrine mechanisms of avian seasonal reproduction. Adv. Exp. Med. Biol. *1001*, 125-136.