

***Daphnia* uses its circadian clock for short-day recognition in environmental sex determination**

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Some organisms have developed a mechanism called environmental sex determination (ESD), which allows environmental cues, rather than sex chromosomes or genes, to determine offspring sex. ESD is advantageous to optimize sex ratios according to environmental conditions, enhancing reproductive success. However, the process by which organisms perceive and translate diverse environmental signals into offspring sex remains unclear. Here, we analyzed the environmental perception mechanism in the crustacean, *Daphnia pulex*, a seasonal (photoperiodic) ESD arthropod, capable of producing females under long days and males under short days. Through breeding experiments, we found that their circadian clock likely contributes to perception of day length. To explore this further, we created a genetically modified daphnid by knocking out the clock gene, *period*, using genome editing. Knockout disrupted the daphnid ability to sustain diel vertical migration under constant darkness, driven by the circadian clock, and leading them to produce females regardless of day length. Additionally, when exposed to an analog of juvenile hormone (JH), an endocrine factor synthesized in mothers during male production, or subjected to unfavorable conditions of high density and low food availability, these knockout daphnids produced males regardless of day length, like wild-type daphnids. Based on these findings, we propose that recognizing short days via the circadian clock is the initial step in sex determination. This recognition subsequently triggers male production by signaling the endocrine system, specifically via the JH signal. Establishment of a connection between these two processes may be the crucial element in evolution of ESD in *Daphnia*.

Short biography:



Hitoshi Miyakawa received his PhD from Hokkaido University, Japan, in March 2011. He was a researcher at the National Institute for Basic Biology (NIBB) from April 2011 to May 2015. Since June 2015, he has been an associate professor at the Center for Bioscience Research and Education (C-Bio) at Utsunomiya University. His research focuses on developmental biology, evolutionary biology, and ecology. Recently, he has been studying the molecular regulatory mechanisms and evolutionary processes of phenotypic plasticity in arthropods. He is a member of the Zoological Society of Japan (ZSJ), the Ecological Society of Japan (ESJ), the Society of Evolutionary Studies, Japan (SESJ), the

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