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論文内容の要旨

Summary of thesis contents

Sex-determination systems can be divided into two groups: genotypic sex determination (GSD) and environmental sex determination (ESD). ESD is an adaptive life-history strategy that allows control of offspring sex in response to environmental cues in order to optimize fitness. Microcrustacean daphnids produce female offspring by parthenogenesis under favorable conditions, whereas in response to various unfavorable external stimuli (*e.g.*, short day-length, low temperature, and oligotrophy), they produce male offspring. However, the molecular basis of ESD remains largely unknown. Although treatment of juvenile hormone (JH), which is an endocrine factor regulating the common fundamental biological processes such as metamorphosis, molting, and reproduction in insects and crustaceans, has been reported to induce male production in daphnids, the role of JH as a sex-determining factor remains elusive due to the lack of a suitable model system for its study.

In this thesis, I established such a system for ESD studies in *Daphnia pulex*. I found that WTN6 strain switches from producing females to producing males in response to short day conditions, while the MFP strain only produces females, irrespective of day-length. Asking whether JH has a novel physiological role as a sex-determining factor in *D. pulex*, I demonstrated that a JH biosynthesis inhibitor suppressed male production in WTN6 strain reared under the male-inducible (short day) conditions. Moreover, I showed that juvenile hormone acid *O*-methyltransferase (JHAMT), a critical enzyme of JH biosynthesis, displays methyl farnesoate (MF: innate JH in crustaceans)-generating activity by catalyzing farnesoic acid, implying that MF is an innate JH in *D. pulex* as well as other known crustaceans. Finally, I found that expression of the *JHAMT* gene activated significantly just prior to the MF-sensitive period (40-60 h after ovulation) for male production in the WTN6 strain,

but not in the MFP strain, when maintained under male-inducible conditions. These results suggest that MF synthesis regulated by JHAMT is necessary for male offspring production in *D. pulex*.

In order to elucidate up- and downstream events of MF signaling during sex determination processes, I compared the transcriptomes of WTN6 strain reared under the long-day (female-producing), short-day (male-producing) and MF-treated (male-producing) conditions. I found that expression level of genes involved in the ionotropic glutamate receptors, known to mediate vast majority of excitatory neurotransmitting processes in various organisms, were significantly varied in WTN6 strain reared under short day conditions but not in MF-treated condition. Administration of specific agonists and antagonists, especially for the *N*-methyl-D-aspartic acid (NMDA) receptor, strongly increased or decreased the proportion of male-producing mothers, respectively, implying that NMDA receptor(s) act as an upstream regulator of MF signaling in *D. pulex*. In addition, I also identified candidate genes responsible for downstream factors of MF signaling (*e.g.*, protein kinase C pathway and hemoglobin-related genes). I identified several candidate genes regulating ESD which strongly suggests that they may be essential factors for male offspring production in *D. pulex*.

The gene *doublesex* (*dsx*) is known as a key factor regulating sexual development in insects. Previous study revealed that *Daphnia magna* has two *dsx* genes (*dsx1* and *dsx2*), and *dsx1* expression during embryonic stages is responsible for the male trait development. The *D. magna dsx* genes have been thought to have arisen by a cladoceran-specific duplication; therefore, it was needed to investigate the evolutionary conservation of sex-specific expression of them and to further assess their functions in the ESD. I searched for *dsx* genes in four closely related cladoceran species and identified the orthologs of both *dsx* genes from *D. pulex*, *D. galeata* and

Ceriodaphnia dubia, yet only a single *dsx* gene was found from *Moina macrocopa*. Molecular phylogenic analysis using arthropod *dsx* suggested that the duplication event of *dsx* gene likely occurred prior to the divergence of these cladoceran species, since the giant tiger prawn *Penaeus monodon* (a more basal species than cladocerans in arthropod) was rooted ancestrally to both DSX1 and DSX2 clade of cladocerans. Therefore, this result suggested that *M. macrocopa* lost *dsx2* gene secondarily. Furthermore, all *dsx* genes of five cladoceran species examined had similar amino acid structure containing highly conserved DM and oligomerization domains, and exhibited male-biased expression patterns, indicating that these genes may have similar functions for ESD in cladoceran species.

My findings provide novel insights into the genetic underpinnings of ESD and they begin to shed light on the physiological function and its regulatory mechanisms of MF as a male-fate determiner and *dsx1* gene as a key factor for male traits development in daphnids.