



## Evolution of sterile caste in termites: missing link between the functions of JH and ecdysone

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Termites are a monophyletic group within cockroaches. Compared to the hymenopteran social insects, the soldier caste is regarded as the first acquired permanently sterile caste in termites. Soldiers are differentiated from workers via two molting steps, and an intermediate stage is called a presoldier. Juvenile hormone (JH) is the primary factor inducing soldier differentiation, and treatment of workers with artificial JH can generate a differentiation of presoldiers. These evidences suggest that a shift from typical hemimetabolous functions of JH and ecdysone might be required for soldier formation during termite evolution. To address this possibility, we focused on soldier differentiation of the damp-wood termite *Zootermopsis nevadensis* and nymphal molt of the woodroach *Cryptocercus punctulatus*, a member of the sister group of termites. RNA-seq analysis was performed during termite presoldier differentiation, and some candidate genes with specifically highly expression levels before the presoldier molt were observed. RNAi of these genes with JH dependent expression affected presoldier-specific morphogenesis as well as molting event; knockdown individuals were more soldier-like phenotypes than controls, but they could not molt into soldiers. It suggests that there is a novel cascade required for a two-step soldier differentiation, likely by regulating expression levels of genes involved in defensive morphogenesis and ecdysone signaling under JH. Next, based on the function analysis of ecdysone signaling, we found a gene specifically regulated by JH receptor expression only in termites. Knockdown of this gene resulted in the newly-molted individuals with normal worker phenotypes, suggesting that it possesses a crucial function on soldier morphogenesis. Based on these data, we will discuss an evolutionary scenario of sterile caste acquisition in termites within the cockroach clade.