報告番号	※　　第　　号

主　論　文　の　要　旨

論文題目　Studies on the role of cuticular function-related genes in insecticide resistance mechanism in red flour beetles, *Tribolium castaneum*.（昆虫体内への殺虫剤の侵入抑制における表皮クチクラ関連遺伝子の機能解析）

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論　文　内　容　の　要　旨

Insect body is covered with the exoskeleton, which consists of epidermal cells and cuticular layer. The main components of insect cuticle are chitin microfibrils and cuticular proteins. Chitin is the polymer of *N*-acetylglucosamine; in chitin biosynthesis in epidermal cells, UDP-N-acetylglucosamine is incorporated into polymerized chitin by an enzyme called chitin synthase 1 (CHS1). Cuticular proteins are another components of insect cuticle. A number of cuticular proteins have been identified and characterized in model insects such as the fruit fly, mosquitoes and the red flour beetles.

In insect molting, biosynthesis of new cuticle proceeds. After ecdysis (i.e. shedding the old exoskeleton), newly synthesized cuticular layer undergoes pigmentation and sclerotization process, which is called tanning. Enzymes such as tyrosine hydroxylase (TH), dopa decarboxylase (DDC) and laccase 2 (Lac2) have been reported to be involved in the tanning process. Through the tanning process, insect integument becomes more rigid with darker color.

In general, insect epidermal cuticle has been regarded to be crucial for defense against xenobiotics such as entomopathogenic microbes and chemicals. Previous studies on the red flour beetle *Tribolium castaneum* revealed that some cuticular proteins are essential for the defense against the attack of entomopathogenic fungi *Beauveria bassiana* (Sirasoonthorn et al., 2021). However, the role of cuticle as the defense against insecticides has not yet been examined.

In this study, I examined the role of cuticular function-related genes in terms of the resistance (or defense) against topically applied insecticides, using the red flour beetle *T. castaneum*. After knockdown of a gene of interest by RNA interference (RNAi), the

beetles were topically applied with insecticides, and the short-term neurotoxic effects were evaluated. Their external morphology and the cross sections of the cuticle of abdominal sternite were also observed. In this study, neonicotinoid insecticides, which inhibit neurotransmission by acting on nicotinic acetylcholine receptor (nAChR) in synapses, were used as the test chemicals with the sublethal concentration. This thesis includes the two studies as described below.

Study 1. Functional analysis of cuticular proteins and Chitin synthase 1 (CHS1) in the insecticide resistance mechanism in *T. castaneum*.

RNAi-mediated knockdown of major adult cuticular proteins (*CPR4*, *CPR18*, and *CPR27*) was performed. By observing cross sections of abdominal sternites, it was confirmed that *CPR* knockdown caused thinner and disorganized cuticle. The knockdown of any of the *CPR* genes, either singly or in combination, resulted in enhanced susceptibility of the beetles to insecticides. These results suggested that these CPRs are indispensable in organizing the insect cuticular structure as the defense against exogenous insecticides.

Mild knockdown of *CHS1* also caused disorganized cuticle. However, it did not affect the susceptibility of the beetles to the insecticides significantly.

Study 2. Functional analysis of genes in the tanning process in the insecticide resistance mechanism in *T. castaneum*.

RNAi-mediated knockdown of genes in the tanning process (*TH*, *DDC*, and *Lac2*) was performed. Mild knockdown of *TH, DDC,* and *Lac2* genes resulted in defects in cuticle coloration as well as disorganized cuticle of the abdominal sternite. However, it did not affect the susceptibility of the beetles to the insecticides significantly.

In summary, this study revealed that major adult cuticular proteins (CPR4, CPR18, and CPR27) are indispensable for the defense against exogenous insecticides. These CPRs have been reported to be essential for the defense against the attack from entomopathogenic fungi (Sirasoonthorn et al., 2021). It was concluded that these CPRs are essential for the defensive function of the cuticle against both insecticides and microbes. Knockdown of other factors (*CHS1*, *TH, DDC,* and *Lac2*) caused disorganized cuticle, but did not have obvious effect on the susceptibility of the beetles to the insecticides. In future studies, it needs to be examined whether more severe knockdown affects the susceptibility of the beetles to the insecticides. These experiments will help to elucidate the factors that are essential for the defense of the cuticle against exogenous insecticides.