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Identification and Characterization of Novel Genes Involved in Quinone Synthesis in the Odoriferous Defensive Stink Glands of the Red Flour Beetle, Tribolium castaneum

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Abstract : The defense strategy is very common in the insect world. Defensive substances play a wide variety of functions for beetles, such as repellents, toxicants, insecticides, and antimicrobics. Beetles react to predators, invaders, and parasitic microbes with the release of toxic and repellent substances. Defensive substances are directed against a large array of potential target organisms or may function for boiling bombardment or as surfactants. Usually, Coleoptera biosynthesize and store their defensive compounds in a complex secretory organ, known as odoriferous defensive stink glands. The red flour beetle, Tribolium castaneum (Coleoptera: Tenebrionidae), uses these glands to produce antimicrobial p-benzoquinones and 1-alkenes. In the past, the morphology of stink gland has been studied in detail in tenebrionid beetles; however, very little is known about the genes that are involved in the production of gland secretion. In this study, we studied a subset of genes that are essential for the benzoquinone production in red flour beetle. In the first phase, we selected 74 potential candidate genes from a genome-wide RNA interference (RNAi) knockdown screen named 'iBeetle.' All these 74 candidate genes were functionally characterized by RNAi-mediated gene knockdown. Therefore, they were selected for a subsequent gas chromatography-mass spectrometry (GC-MS) analysis of secretion volatiles in respective RNAi knockdown glands. 33 of them were observed to alter the phenotype of stink gland. In the GC-MS analysis, 7 candidate genes were noted to display a strongly altered gland, in terms of secretion color and chemical composition, upon knockdown, showing their key role in the biosynthesis of gland secretion. Morphologically altered stink glands were found for odorant receptor and protein kinase superfamily. Subsequent GC-MS analysis of secretion volatiles revealed reduced benzoquinone levels in LIM domain, PDZ domain, PBP/GOBP family knockdowns and a complete lack of benzoquinones in the knockdown of sulfatase-modifying factor enzyme 1, sulfate transporter family. Based on stink gland transcriptome data, we analyzed the function of sulfatase-modifying factor enzyme 1 and sulfate transporter family via RNAi-mediated gene knockdowns, GC-MS, in situ hybridization, and enzymatic activity assays. Morphologically altered stink glands were noted in knockdown of both these genes. Furthermore, GC-MS analysis of secretion volatiles showed a complete lack of benzoquinones in the knockdown of these two genes. In situ hybridization showed that these two genes are expressed around the vesicle of certain subgroup of secretory stink gland cells. Enzymatic activity assays on stink gland tissue showed that these genes are involved in p-benzoquinone biosynthesis. These results suggest that sulfatase-modifying factor enzyme 1 and sulfate transporter family play a role specifically in benzoquinone biosynthesis in red flour beetles.

Keywords : Red Flour Beetle, defensive stink gland, benzoquinones, sulfate transporter, sulfatase-modifying factor enzyme 1
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