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Antennal carboxylesterases in a moth, structural and functional diversity

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Pheromone-degrading enzymes (PDEs) are supposed to be involved in the signal inactivation step within the olfactory sensilla of insects by quickly degrading pheromone molecules. Because esters are widespread insect pheromone components, PDEs belonging to the carboxylesterase (CCE) family have been the most studied. However, only two CCEs were both identified at the molecular level and functionally characterized as PDEs until recently. In the pest moth *Spodoptera littoralis*, we have identified an unsuspected diversity of antennal CCEs, with a total number of 30 genes. Two CCEs, enriched in antennae and belonging to distinct clades, were shown to present different substrate specificities toward pheromone and plant compounds. A same CCE was also shown to efficiently degrade both pheromone and plant components. Our results suggest that the structural evolution of antennal CCEs reflects their functional diversity and that a complex set of CCE-mediated reactions take place is the olfactory organs of moths.

Keywords: lepidoptera, noctuidae, olfaction, carboxylesterase, odorant-degrading enzymes, pheromone

Abbreviations: OBP, odorant-binding protein; ODE, odorant-degrading enzyme; OR, olfactory receptor; PDE, pheromone-degrading enzyme

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Olfaction play a fundamental role in many insect species to locate and choose mates, food sources, hosts and oviposition sites essential for their survival. Intensive research has led to the identification of a diversity of genes involved in the sequential step of odorant reception within the olfactory organ, i.e. the antennae, which bear the olfactory sensilla. This process includes the binding and transport of odorant molecules by odorant-binding proteins (OBPs) throughout the sensillum lymph, their recognition by odorant receptors (ORs) and their inactivation through their degradation by specific enzymes called Odorant-Degrading Enzymes (ODEs).[1] The first studies on odorant degradation were focused on male moth pheromonal system because of its high sensitivity and selectivity. While flying to calling females, male moths can react to the loss of the odorant trail in less than 0.5 sec,[2] suggesting that they are able to reset their sensory system in few milliseconds. Rapid degradation of pheromone in male antennae is thus believed to play an important role in signal termination. The correlation between the various enzymatic activities found in insect antennae[3] and the chemical structures of their pheromone components has led to the hypothesis that each insect species possess specialized Pheromone-Degrading Enzymes (PDEs).[4]

Among putative PDEs, the carboxylesterases (CCEs; EC 3.1.1.1) have been the most studied, but few molecular and functional data are still available on antennal CCEs. Through molecular cloning, one antennal esterase gene has been identified in the noctuid moths *Mamestra brassicae*, *Spodoptera littoralis* and *Sesamia nonagrioides*, in the beetle *Popilia japonica* (PjapPDE) and in the bee *Apis mellifera*.[5-8] Three CCEs have been cloned in the wild silkmoth *Antheraea polyphemus*,[9,10] whereas five genes have been identified in the tortricid *Epiphyas postvittana* by transcriptomic analysis.[11] All these CCEs were predicted as extracellular and could thus potentially interact with the odorant molecules within the sensillum lymph, in the vicinity of the ORs. Functional data were only available for two male antennal specific CCEs, ApolPDE from *A. polyphemus*[9,12] and PjapPDE.[7] Their rapid kinetics toward pheromones strongly

suggests that enzymatic inactivation could play a role in the dynamic of signal termination.

In the moth *Spodoptera littoralis*, a worldwide crop pest, the sex pheromone blend is a mix of esters, suggesting the involvement of CCEs in pheromone degradation.[13] To investigate the structural and functional diversity of *S. littoralis* antennal CCEs, we have developed a transcriptomic approach on male antennae.[14] Phylogenetic analysis of the identified sequences coupled to the study of their tissue-related distribution has allowed us to precise their putative function. We have then selected two CCEs restricted to the antennae to test their catalytic properties toward pheromones but also toward plant components. Indeed, the ability of CCEs to hydrolyze other odorants than pheromones was surprisingly not studied, despite that esters are widespread among odorants emitted by plants.[15]

Our results show that the antennae of this species expressed a surprising diversity of CCEs for such a specialized tissue. Twenty CCEs[6,16] were first identified and ten additional sequences were isolated more recently (Maïbèche-Coisne et al.,[5] pers. com., GenBank acc. numbers HQ122611 to HQ122620), leading to a final number of 30 genes. This is very high, especially when compared with the whole repertoires of CCEs identified in other species through genome analysis (from 24 in the bee to 69 in the silkworm *B. mori*).[17,18] Phylogenetic analysis revealed that *S. littoralis* antennal sequences are distributed among the three classes of CCEs already defined.[17] Eight sequences clustered within the first class, which contains predominantly intracellular active enzymes. Most of them are supposed to have digestive or detoxification functions, based on their expression in insect midgut, or have been implicated in insecticide resistance.[19] Two sequences clustered within the third class, which brought together proteins implicated in neuro/developmental functions,[20] such as acetylcholinesterases and other non-catalytic proteins. All the others sequences from *S. littoralis* antennae belong to the second class, which contains mostly secreted and generally catalytically active enzymes. For a few, functional data suggest a role in hormone and pheromone processing.[19] This class includes noteworthy ApolPDE and PjapPDE, as well as Juvenile-Hormone (JH) esterases involved in JH catabolism.

We have functionally characterized two CCEs, SlCXE10 and SlCXE7, belonging to the first and second class, respectively.[21,22] In situ hybridization revealed that these two genes were associated with long and short olfactory sensilla, tuned to pheromones and plant odorants, respectively. We showed that SlCXE10 was able to efficiently hydrolyze a green leaf ester emitted by host plants, but not the pheromone components. The intracellular location of SlCXE10 suggests also that the CCE-based metabolism of odorant is not restricted to the sensillum lymph and could occur within the cells. SlCXE7 is predicted as extracellular and presented different substrate specificities as it was able to efficiently hydrolyze the two sex pheromone components, but also the plant odor. This result demonstrates that a same CCE could have a dual function depending of its sensillar expression: acting as a PDE and reducing plant's odorant background noise within the pheromone-sensitive sensilla, or acting as an ODE within the sensilla tuned to this plant volatile. Interestingly, for both genes, the transcript levels were induced by male exposure to the odorants, showing that odorant molecules could modulate CCE expression, allowing the insect to adapt its antennal catabolism to the fluctuations of its odorant environment.

These studies revealed that the antennae are a hot-spot for esterase activities and suggest that the structural diversity of antennal CCEs could partially reflect their substrate specificities. As CCEs are known to play a crucial role in insecticide metabolism in non-olfactory organs, participating in insecticide resistance, we will now investigate if some antennal CCEs from *S. littoralis* may participate in olfactory neuron protection by xenobiotic detoxication.

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