杨小舟蛾性信息素结合蛋白基因鉴定及功能研究

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摘要：【目的】鉴定杨小舟蛾性信息素结合蛋白基因 *PBPs*，明确杨小舟蛾 PBPs 对其性信息素活性成分的结合特性，为杨小舟蛾性信息素识别机制研究奠定基础，也可为利用化学生态学手段进行杨小舟蛾综合防控提供分子靶标。【方法】基于触角转录组测序，挖掘杨小舟蛾嗅觉相关基因；通过生物信息学和分子生物学手段对各类嗅觉相关基因进行序列分析和构建系统发育树，探究杨小舟蛾与其他鳞翅目昆虫化感基因的进化关系；采用原核表达和荧光竞争结合法研究 PBPs 重组蛋白与杨小舟蛾信息素活性成分的结合特性。【结果】（1）共挖掘到 156 个候选嗅觉基因：37 个 *OBP*、23 个 *CSP*、2 个 *SNMP*、41 个 *OR*、5 个 *GR*、13 个 *IR*、35 个 *CXE*。其中包括 3 个 *PBP* 基因，其氨基酸序列均具有 6 个保守半胱氨酸结构位点，且与其他鳞翅目昆虫的 PBP 基因聚为一支。杨小舟蛾的嗅觉基因通常与其他舟蛾科昆虫的嗅觉基因以极高的支持率聚为同一分支。（2）*PBP* 组织表达谱显示 *PBP1，2，3* 均在成虫触角中显著高表达，且雄虫触角中的表达量显著高于雌虫。（3）通过原核表达和蛋白质纯化获得 PBP1，2，3 重组蛋白，荧光竞争结合实验结果表明，三者与荧光探针 1-NPN 的解离常数分别为：PBP1 $K_{1\text{-}NPN}$=8.73uM，PBP2 $K_{1\text{-}NPN}$=8.19uM，PBP3 $K_{1\text{-}NPN}$=9.40uM，且 3 种 PBP 都能结合杨小舟蛾性信息素活性成分 Z13,E15-18:Ald（PBP1 *Ki*= 0.68uM，PBP2 *Ki*=0.54uM，PBP3 *Ki*= 0.70uM）。【结论】（1）本研究共挖掘出 156 个杨小舟蛾嗅觉基因，鉴定出 3 个杨小舟蛾信息素结合蛋白 PBP1，2，3。系统发育分析结果表明昆虫的嗅觉基因在相近物种间高度保守；（2）杨小舟蛾 PBP1，2，3 与其信息素活性成分 Z13,E15-18:Ald 具有极强的结合力，表明该昆虫识别性信息素过程有多种结合蛋白共同参与。

关键词：杨小舟蛾；嗅觉基因；系统发育分析；信息素结合蛋白

Identification and Functional Analysis of pheromone binding proteins in *Micromelalopha sieversi*（Lepidoptera：Notodontidae）

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Abstract:【Objective】This study aims to identify olfactory genes of *M.sieversi* and the binding properties of pheromone-binding proteins to the sex pheromone active components of *M.sieversi* which could lay a foundation for the further study of its sex pheromone recognition mechanism, and also provide molecular targets for the comprehensive control of the pest by chemical ecological methods. 【Method】The olfactory genes were identified based on antennal transcriptome sequencing. Then we applied some bioinformatic analysis and molecular biology experiments. Various kinds olfactory genes were captured and phylogenetic trees were constructed to explore the evolutionary relationship of chemosensory genes among *M.sieversi* and other lepidopteran insects. The binding properties of PBPs recombinant protein and pheromone active components were studied by prokaryotic expression and fluorescent competitive binding method. 【Result】 (1) A total of 156

candidate olfactory genes were identified: 37 *OBP*, 23 *CSP,* 2 *SNMP*, 41 *OR*, 5 *GR*, 13 *IR* and 35 *CXE* genes. Among them, three PBP genes with 6 conservative cysteine sites in their amino acid sequence, and they all clustered with PBP genes from other lepidopteran insects. The olfactory genes bootstrap value. (2) PBP1, 2,3 were significantly highly expressed in adults antennae, and the expression level in male antennae were significantly higher than those in female antennae. (3) PBP1, 2,3 recombinant proteins were obtained by prokaryotic expression and protein purification. The results of fluorescence competitive binding experiments showed that the dissociation constants of the three PBPs and fluorescent probe 1-NPN were as follows: PBP1 $K_{1\text{-}NPN}$=8.73uM, PBP2 $K_{1\text{-}NPN}$=8.19uM, PBP3 $K_{1\text{-}NPN}$=9.40uM, and all the three PBPs can bind the sex pheromone active ingredient Z13,E15-18:Ald strongly (*PBP1* Ki= 0.68uM, *PBP2* Ki=0.54uM, *PBP3* Ki= 0.70uM).【Conclusion】(1) A total of 156 olfactory genes were identified including 3 pheromone-binding proteins in this study. Phylogenetic analysis showed that the olfactory genes of insects were highly conserved among related species. (2) All three PBPs showed strong binding abilities to Z13,E15-18:Ald indicating that a variety of binding proteins were involved in the process of pheromone recognition in this pest.

Key words: *Micromelalopha sieversi*; olfactory genes; phylogenetic analysis; pheromone binding protein.