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First characterization and regulatory function of piRNAs in the Apis mellifera larval response to Ascosphaera apis invasion

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piRNAs are a class of small non-coding RNAs that play essential roles in modulating gene expression and abundant biological processes. To decode the piRNA-regulated larval response of western honeybees (Apis mellifera) to Ascosphaera apis infection, the expression pattern of piRNAs in Apis mellifera ligustica larval guts after A. apis inoculation was analyzed based on previously obtained high-quality small RNA-seq datasets, followed by structural characterization, target prediction, regulatory network investigation, and functional dissection. Here, 504, 657, and 587 piRNAs were respectively identified in the 4-, 5-, and 6-day-old larval guts after inoculation with A. apis, with 411 ones shared. These piRNAs shared a similar length distribution and first base bias with mammal piRNAs. Additionally, 96, 103, and 143 DEpiRNAs were detected in the 4-, 5-, and 6-day-old comparison groups. Targets of the DEpiRNAs were engaged in diverse pathways such as the phosphatidylinositol signaling system, inositol phosphate metabolism, and Wnt signaling pathway. These targets were involved in three energy metabolism-related pathways, eight development-associated signaling pathways, and seven immune-relevant pathways such as the Jak-STAT signaling pathway. The expression trends of five randomly selected DEpiRNAs were verified using a combination of RT-PCR and RT-qPCR. The effective overexpression and knockdown of piR-ame-945760 in A. apis-infected larval guts were achieved by feeding a specific mimic and inhibitor. Furthermore, piR-ame-945760 negatively regulated the expression of two target immune mRNAs, SOCS5 and ARF1, in the larval gut during the A. apis infection. These findings indicated that the overall expression level of piRNAs was increased and the expression pattern of piRNAs in larval guts was altered due to the A. apis infection, DEpiRNAs were putative regulators in the A. apis-response of A. m. ligustica worker larvae. Our data provide not only a platform for the functional investigation of piRNAs in honeybees, especially in bee larvae, but also a foundation for illuminating the piRNA-involved mechanisms underlying the host response to the A. apis infection.