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Bees are the most important pollinating insects globally, and low-temperature stress severely affects their overwintering and agricultural pollination activities. Studying the cold tolerance mechanisms of bees will help to better maintain and even expand their population numbers. This study used *Apis mellifera sinisxinyuan* and *Apis mellifera ligustica* as research subjects, collecting 10-day-old worker bees before overwintering to compare their cold tolerance differences, as well as the content of cold tolerance-related substances and gene expression levels. It was found that the *A. m. sinisxinyuan* has a lower supercooling point than the *A. m. ligustica*, with a lower freezing rate and a lower foraging temperature than the *A. m. ligustica*, indicating that the cold tolerance of the *A. m. sinisxinyuan* is significantly higher than that of the *A. m. ligustica*. At the same time, the content of trehalose and chitin in the *A. m. sinisxinyuan* was significantly higher than that in the *A. m. ligustica*. After low-temperature gradient treatment of *A. m. ligustica* worker bees, the expression changes of the AmTret1 gene and the content changes of chitin were measured, and combined with transcriptome sequencing analysis, it was found that the differential genes were enriched in the sugar metabolism pathway. The expression of AmTret1 in the *A. m. sinisxinyuan* was significantly higher, and low temperature could induce the upregulation of AmTret1 expression. The content of trehalose and chitin in bees also significantly increased after low-temperature treatment. It is speculated that AmTret1 is involved in the synthesis of chitin, thereby regulating the cold tolerance of bees. The results of this study not only help to reveal the molecular regulatory mechanisms of bee cold tolerance but also improve the mechanisms of bee sugar metabolism and substance synthesis. By clarifying the key genes for bee cold tolerance, it is expected to breed new bee strains with cold tolerance traits.