Production of mKast mutatnt drones and heterozygote mutant workers by genome editing using CRISPR/Cas9

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The European honeybee (*Apis mellifera* L.) is a social insect, and the molecular and neural bases underlying its behaviors have extensively been investigated. Especially, based on the assumption that the mushroom bodies (MBs), a higher order center of the insect brain, are related to regulation of social behaviors, genes preferentially expressed in the MBs in honeybee brain have been identified. However, the function of these genes in regulating social behaviors remain to be elucidated, because effective gene manipulation methods have not been available until recently in the honeybee. We recently established a basic technique to produce mutant honeybee drone using CRISPR/Cas9 [Kohno *et al*., *Zool. Sci.* (2017)]. Here, we applied CRISPR/Cas9 to produce *mKast* mutant honeybees. *mKast* is expressed in an adult brain-selective manner and, in the MBs, it is preferentially expressed in a certain subtype (middle-type) of Kenyon cells (KCs) that consist MBs. We have successfully produced mosaic queens (F0) from fertilized eggs injected with sgRNA and Cas9 mRNA targeting *mKast* as well as *mKast* null mutant drones (F1) derived from the mosaic queens. Immunoblot analysis using anti-mKast antibody revealed that mKast expression is completely lost in mutant drone heads. *In situ* hybridization of *jhdk*, which is differentially expressed among KC subtypes in the honeybee MBs, revealed that there are all KC subtypes in the mutant drone brains, suggesting that mKast is dispensable for differentiation of KC subtypes. During the process to produce homozygous mutant workers (F3), we also found that *mKast* mutant drones undergo normal sexual maturation, and successfully produced heterozygous mutant workers (F2) by artificial insemination of wild-type queens with the sperm from mutant drones. These results indicate that *mKast* is dispensable for normal development, sexual maturation, and differentiation of KC subtypes, at least in drone.