Wichet LEELAMANIT[1)], Rerngwit BOONYOM[1)], Sakol PANYIM[2)],
C. Chandrasekhara REDDY[3)],M. Man SINGH[4)], Takeshi HAYASHI[5)],
Hiroshi YASUE[5)], and Kazuhiro AMANO

Department of Animal Breeding and Reproduction
[1)]Department of Biochemistry, Mahidol University
[2)]Institute of Molecular Biology and Genetics, Mahidol University
[3)]Department of Zoology, Bangalore University
[4)]Research Center for Applied Science & Technology, Tribhuban University
[5)]National Institute of Agrobiological Sciences

Abstract

Molecular tools such as polymerase chain reaction (PCR)-based techniques have been widely used to analyze the genetic diversity of many social insects. Since their society has a very complicated and hierarchical structure, honeybees (*Apis*) will become a good model to study the evolution of social insects. In this report, we demonstrated a simple PCR-restriction fragment length polymorphism (PCR-RFLP) to distinguish different *Apis* species. In brief, mitochondrial DNA (mtDNA) was separately isolated from workers of five major honeybee species, *Apis mellifera* (Western honeybee), *A. cerana* (Eastern honeybee), *A. dorsata* (Giant honeybee), *A. laboriosa* (Himalayan giant honeybee), and *A. florea* (Dwarf honeybee), obtained from different geographical regions. Western and Eastern honeybees, *A. dorsata* and *A. laboriosa*, and *A. florea* were collected from Japan, Nepal, and India, respectively. The PCR technique was performed to amplify a 528-bp DNA fragment of the NADH dehydrogenase subunit 4 (ND4 according to *A. mellifera's* mtDNA sequence). Following digestion of the individual PCR products with two restriction endonucleases, *Nde*I and *Mbo*I, the digested DNA fragments were separated with agarose gel electrophoresis, stained with ethidium bromide, and visualized over UV light. The results clearly indicated that this PCR-RFLP procedure was simple but sensitive and reproducible for the identification of the genetic variability among Apis species. Therefore, this procedure will be useful to confirm other techniques for the identification of honeybees.

Keywords: *Apis*, mtDNA, PCR-RFLP