

C-9 PCR-Analyzable RFLP Markers to Identify Mitochondrial and Nuclear DNA of *Apis mellifera* (Hymenoptera : Apidae) Subspecies Groups.

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DNA markers have revealed processes involved in the expansion of the African, *Apis mellifera scutellata*, population in the America. For the regulatory control of this African bee, mitochondrial(mt) and nuclear DNA will be needed for precise genetic identification, which would be more reliable than the morphometric methods currently used. To facilitate mtDNA identification of 3 major groups of honey bee, *Apis mellifera*, subspecies : African, west European, and east European, the allele-specific amplification(ASA) are tested using competing primers, which designed from two allelic sequences at each of three polymorphic regions as follows : HincII site of Co-I, EcoRI of IsRNA, and BgiIII of Cyt-b. These competing allelic primers are differentially labeled with fluorescent dyes at their 5' terminal. The primers that match the mtDNA sequences preferentially support amplification, and the colors of the amplified products identify a different type of subspecies group. The 3 polymorphic regions of honey bee mtDNA can be amplified in a single multiplex reaction. On the other hand, several nuclear RFLP markers have been selected to discriminate African bee from European bee. For fast and economical RFLP test, we convert these RFLP markers to PCR-analyzable markers coupled with a pair of primers, which synthesized according to sequences of both flanking region of each polymorphic restriction site. A series of works like subcloning of probes, mapping of restriction site, and inverse PCR are necessary to determine the specific and polymorphic sites of restriction enzyme among subspecies groups. The amplified DNA fragments show their diagnostic size of DNA after the digestion with eligible enzymes.