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MOLECULAR MARKER APPROACH IN HONEY BEE: A REVIEW

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ABSTRACT

The Honey bee is an important model animal for behavioral study as it is a colonial insect with complex social behavior. Many studies have characterized its fascinating behavioral repatriate, but little is known about the genetic basis of its behavior because of difficulties in maintaining selected stocks and the scarify of genetic markers. However, it is now possible to construct detailed linkage maps for insects by following the molecular markers segregation. Molecular markers analysis and genetic mapping are valuable tools for identifying chromosomal regions affecting behavioral traits. DNA markers have contributed significantly for understanding genetic basis of diversity, mapping medically and agriculturally important genes and quantitative trait loci (QTLs) in Honey bee. Molecular markers are used to infer phylogeny and biogeography of insect population and to understand modes of evolution and evolutionary trajectories. DNA markers such as mtDNA, RAPD, AFLP, microsatellite and ESTs are used as popular marker systems in honey bee genetic research. Although there are inherent advantages and disadvantages associated with each marker system, the choice of applying them depends upon the objectives of the study. The present review put light on genetic linkage mapping, quantitative trait loci analysis, behavior analysis, phylogenetic analysis, mitochondrial DNA markers, RAPD study, genetic variation and population study in honeybee.

KEYWORDS; DNA, Genetic variation, Honey bee, Molecular markers, Polymorphism, RAPD, PCR, QTL

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INTRODUCTION

After homosapiens, highly social species is the honey bee. Besides its economic importance, it has long been important for honey production, pollination of crops and behavioral studies. Yield increases of U.S. crops through honey bee pollination estimated 16.6 billion dollars[1]. Nemobiologists and behaviorists have used the honey bee as a model organism to study the molecular basis of learning. Detailed linkage mapping of the honey bee genome has been constructed for use in behavioral genetic studies.

For genetic analysis honey bee genome sequencing project (HBGP) also proposed that will benefit human health and medicine in diverse areas, including venom toxicology, allergic diseases, mental illness, infectious diseases, parasitology and gerontology. In addition, the HBGP will improve human nutrition by enabling enhanced pollination of food plants and accelerated delivery of hymenopteran parasitoids for biological control of pests[2].

Insect populations even within a species vary in morphology and behavior that attributes to their complex interaction with the environment[3]. With the development of DNA based marker system, it is observed that greater level of polymorphism could be obtained by using DNA markers than by using protein markers in many situations[4].

Over the last 18 years, DNA markers have made a significant contribution to rapid rise of molecular studies of genetic relatedness, phylogeny population dynamics or gene and gene mapping in insects[5,6,7,8,9]. Behavioral plasticity in social insects represents a complex biological phenomenon that is getting attention of molecular biologists. Honey bee is an emerging model organism that is being studied for social behavior at molecular level[10]. The unprecedented advancement in modern molecular biology, particularly in those of DNA marker technology, have already created a wealth of technical knowhow that finds useful applications of these markers especially in molecular ecology research in insects[11]. The study of insect ecology is important to understand their evaluation and diversification, and their influence on the functional and tropic links between different components of associated habitats[12,13].

In fact, they have several unique features that make them more attractive than any other genetic test system available in higher organisms. Particularly important are the following traits for genetic research:

- Male haploidy allows the study of gene expression in haplotypes. This is important for both selection and gene mapping studies.
- The rich behavioural repertoire and the social organization make it a prime system in behavioural genetics.
- The slow embryonic development offers plenty of opportunities to study gene regulation and expression during early development offers plenty of opportunities to study gene regulation and expression during early development.

These characteristics make the honeybee useful for basic genetic research. Since honeybees are of economical and ecological importance, there is obviously compelling need to understand their population behavioral and breeding genetics.

GENETIC LINKAGE MAPPING

In Honey bee, RAPD markers have been extensively used to generate genetic maps for honey bee genome. A saturated linkage map of the honey bee facilitate the characterization of complex social behavioral traits in the same way as studies of crop plants determined the number and locations of genes affecting agronomic traits[14,15,16].

Honey bee has a higher rate of meiotic recombination than any other known metazoan[17,18]. Bee display significantly more within genome variation in local recombination rate that previously reported for human, *D. melanogaster* and *C. elegans*[19]. The higher recombination rates effectively increase the

accuracy of linkage mapping and high recombination rate and the low incidence of repetitive DNA should facilitate map based cloning of genes in the honey bee[20]. Detailed linkage mapping of the honey bee genome has been constructed for use in behavioral genetic studies[17,21,22,23] and for sex determination[24].

Microsatellite based linkage maps also constructed for the genome of honey bee. A total of 541 loci have been mapped; 474 have microsatellite loci; a few additional bands produced during PCRs, one of the two rDNA loci (using ITS), the MDH locus, and three sex-linked markers (Q and FB loci and one RAPD band). Several cases of segregation distortion that appeared to result from deleterious recessiveness have been discovered and a low positive interference also detected[25].

A linkage map was constructed for the honey bee based on the segregation of 365 RAPD markers in haploid male progeny of a single female bee. Male haploid can be used as a tool to study the genetics of behavioral traits in bees because the drone transmits same genome to all of his worker progeny and haploid facilitate genetic analysis. The X locus for sex determination and genes for black body colour and malate dehydrogenase were mapped to separate linkage groups. RAPD markers were very efficient for mapping with an average of about 2.8 loci mapped for each 10 nucleotide primers that was used in PCR reaction[17].

QUANTITATIVE TRAIT LOCI ANALYSIS

Quantitative trait loci (QTL's) that influence colony level behavioral traits in bees have been mapped and most of these QTL have been confirmed in independent crosses[17,21,26,22]. QTL's that influence learning performance have been mapped based on the performance of individual drones[27].

In honey bee, social behaviors are polygenic traits and are influenced by more than one gene referred to as QTL. The two major QTLs that determine the foraging behavior in honey bee have been identified by employing RAPD markers in backcross population between bees collecting nectar and those collecting pollen. The relationship of physical to genetic distance was estimated at 52 Kb/CM, suggesting that map-based colony of genes will be feasible for honey bee species[17].

AFLP marker study was conducted to detect binary trait loci (BTLs) that influence guarding behavior of individual honey bee *Apis mellifera* L. and to locate genetic markers that are associated with BTLs on genetic maps. Two genetic maps were generated, one for each type of colony. Ten markers were associated with guarding behavior; they represented seven putative BTLs that influence honeybee guarding behavior. One of the BTLs represents a QTL that was previously detected in analysis of colony-level stinging response, others represent new loci specific to the behavior of individuals guarding the colony interance[28].

BEHAVIOR ANALYSIS

Exploiting similar procedure with molecular markers in honey bee, colony level behaviors such as stinging behavior, body size, pheromones alarm level, traits for reversal learning and hygienic behavior have also been dissected at the level of specific genomic regions[29].

AFLP markers and microsatellites have been used in dissecting the guarding and stinging behaviors in honey bee. Samples of guards, stingers, foragers and nurse bees have been taken from two backcross colonies derived from a defensive colony and a gentle colony. Results indicated that division of labor is influenced by specific QTLs. Results also showed that QTLs mapped in a population of Africanized honey bees using colony level phenotypes also influenced the expression of guarding and stinging behavior of individual bees of other populations[30].

In bumble bees AFLP markers employed to study genetic basis of ecological implications of foraging range and nest density behaviors. Obtained data suggest that even among the most common British bumble bee

species, significant differences in fundamental aspects of their ecology exist[31].

AFLP markers successfully used to map QTL governing foraging behavior in honey bee. Three quantitative trait loci (QTL) have been identified that influence a set of foraging variables, including the concentration of nectar collected and the amount of pollen and nectar brought back to the hive. This study represents the most comprehensive investigation of the genetic architecture of these foraging variables[32].

Apart from AFLP markers, use of microsatellite markers have also been very successful specially in honey bee and ants to identify genes responsible for diversity in foraging range and mating behavior[33,31], colonization[34] and Kinship relation[35].

The genetic regulation of defensive behavior is now better understood from the mapping of quantitative trait loci (QTL's) associated with the variation in defensiveness. Colony defense in other eusocial bees is less well understood, but enough information is available to provide interesting comparisons between *Apis mellifera* and other species of Apis as well as with allodapin, halictine, bombine and meliponine bees. These comparison studies illustrate the wide variety of evolutionary solutions to problems in colony defense in the Apoidea[29]. Uses of microsatellite markers successfully used specifically in honey bees and bumble bees to identify genes responsible for diversity in foraging range and mating behavior[33,31], host parasitization[36] and colonization[34].

Expressed sequence tags (ESTs) have been used as expression markers in microarray formats to predict the nursing and foraging behavior in individual honey bees. Result discovered that, individual brain messenger RNA profiles correctly predicted the behavior of bees, indicating a robust association between brain gene expressions in the individual and naturally occurring behavioral plasticity[37].

MITOCHONDRIAL DNA MARKERS AND PHYLOGENETIC ANALYSIS

Mitochondrial DNA is found to be inherited exclusively maternally in most animal system[38]. In several cases, however, these rules might have been broken since heteroplasmic individuals have been found in various organisms. Looking at the special fertilization mechanism of the egg, honey bees seem to be another candidate for a significant paternal empty DNA inheritance. *Apis mellifera* has a polyspermic mode of fertilization, with many sperms entering the egg, including the mitochondria, reach tail. Although many sperms have been found to enter the egg, only one of them fuses with the egg nucleus. The others remain as accessory sperms in the egg and usually disintegrate rapidly after the first cell division. Only rarely do they show mitotic activity yielding gynandromorphy individual[39].

Phylogenetic analysis of DNA sequence information from the mitochondrial genome (large subunit ribosomal RNA gene) of representative Apis bees suggested that advanced eusocial behavior evolved twice independently within this assemblage. Those results depart from previous hypothesis of Apis relationship by indicating a close Phylogenetic relationship between the primitively eusocial bumble bees and the stingless bees[40].

Allozyme data used to construct Phylogenetic trees for honey bee subspecies[41,42,43] and species[44], although these studies have shown that the level of genetic variation detected by allozymes in *Apis mellifera* is low.

Scientist Smith found that mtDNA of *Apis koschevnikovi* and *Apis mellifera* were both highly divergent between each of the species[45]. Garnery et al. screened mtDNA of *A. mellifera*, *A. cerana*, *A. florea* and *A. dorsata*[46]. They sequenced the intergenic region between the $tRNA^{leu}$ region and the CO-II gene, and found sequence divergence ranging from 7% to 11%. Based on the sequence data, Cornuet and Garnery present a most parsimonious phylogenetic tree, supporting the

early divergence of *A. florea*, and the close relationship of the two cave-breeding species *A. mellifera* and *A. cerana*, which is in agreement with morphometrical[47], behavioural[48] and allozyme data[44].

Phylogenetic studies utilizing mitochondrial RFLP[45] and sequences[49,50] have largely supported the 3-4 major dispersal lineages postulated by morphometric analysis. Cornuet and Garnery developed a putative evolutionary pathway of the mtDNA region sequenced by Garnery et al.[51,52], explaining the evolutionary changes through DNA duplication, elongation, and regression. They extended the sequence data 185 bp into the region of the large ribosomal unit yielding a total of 61 informative sites[47]. Based on both a neighbor joining[53] and a parsimony analysis (PAUP 3.0 software package) they obtained phylogenetic trees.

Cornuet and Garnery suggested that both cave-breeding species *A. mellifera* and *A. cerana* diverged about 5.9 million years from common ancestor[47]. Willis et al also presented a phylogenetic tree on the basis of mtDNA variability[54]. They analyzed the CO-II sequence of the wasp (Excristes roborator) as an outgroup.

Molecular phylogenetics has been shown from mitochondrial DNA sequence of honey bee subspecies (*Apis mellifera* L.). For this study a mitochondrial DNA region encompassing part of the NADH dehydrogenase subunit 2 and isoleucine transfer RNA genes were PCR amplified, cloned and sequenced for 14 morphometrically identified *Apis mellifera* subspecies and the Africanized honey bee[55].

RAPD-PCR technique was employed to review the biogeography and interspecific phylogeny of *Apis mellifera* in USA[56].

The genetic structure and phylogenetic relationships among six honey bee populations were studied using RFLP analysis on three PCR-amplified mtDNA gene segments (16s rDNA, CO I, and ND 5). The populations were sampled from various areas of Greece and Cyprus and correspond to *Apis mellifera adami, A. m. macedonica, A. m. cecropia,* and *A. m. cypria* races, based on origin. Seven different haplotypes were detected and diagnostic patterns enabled to discriminate *A. m. macedonica* from the rest of the populations[57].

The evolutionary conservation and versatility of a new set of nuclear primers for the amplification of the ribosomal internal transcribed spacer regions in insects and other invertebrates have been studied[58]. The insects studied cover the main divisions of Insecta, with one primitive wingless insect (firebrat), a primitive winged insect (damselfly) and nine other winged insects (two grasshoppers, a cockroach, an aphid, a fruit fly, an ant, a honey bee and two moths). That facilitates the intraspecific studies as well as phylogenetic analysis of closely related taxa. Several invertebrate samples were used previously in the study of Zhang & Hewitt[59].

RAPD STUDY

Understanding how genotyping structure affects the social organization, paternity analysis of worker honey bees using RAPD has been studied[60].

RAPD analysis essentially scan part of the genome containing primers site close to one another that are located in an inverted orientation, although polymorphism is treated in a dominant fashion[61,62]. The genetic differences between Africanized and European honey bees, *Apis mellifera* using RAPD-PCR has been demonstrated by Suzao et al. The RAPD markers reported here are specific to groups of honey bee subspecies (east European or African), and their representation in populations coincides with the findings of RFLP markers[63].

RAPD have been proved to be a valuable tool especially for further interpopulation studies of *Varroa Jacobsoni* collected from *Apis mellifera* L colonies in California, Texas and Germany, and specimens collected from *Apis cerena* Fab colonies in Malaysia were compared by means of RAPD[64].

Cross-section study of Larva and Pupa pattern of some RAPD products and homology of some developmentally regulated genes in *Apis mellifera* and *A. cerena* have been reported and the comparison was performed at the DNA level. In conclusion, the difference between two species at the DNA level is more obvious than that at the tissue level[65].

RAPD markers have been successfully used for the identification of three different breeds of honey bee *Apis mellifera*[66].

To determine the genetic distance between *Tetragonisea angustula* Latreille populations those are one of the most common Neotropical stingsless bees popularly known in Portuguese as jatai. 18 primers were used to generate 218 RAPD markers from 25 localities in three different Latin American countries[67].

GENETIC VARIATION AND POPULATION STUDY

The genetic variation of any species show undergoing genetic differentiation, a process which can be driven by ecological, evolutionary or historical factors[67].

Honey bee DNA probes have proved to be very useful in a study focusing on Intracolonial variability. Various techniques have been tested and have shown the great potential of molecular tools to increase our understanding of the function of the honey bee colony as a whole. In study by Oldyord et al, a DNA probe from a genomic library of the honey bee proved useful to identify different patrilines in a colony of *Apis florea*, the Asian dwarf honey bee[68]. They could identify as many as eight different RFLP patterns suggesting a mating with about eight drones. This is surprisingly high number in the light of observation by Koeniger who claimed that only a very limited number (less than five) of *Apis florea* drones mate with the queen[69]. The DNA data of Oldyord et al, do not support this view, and the estimate of eight matings may be conservative since rare patrilines may not have been detected and some drones might have had the same RFLP type[68]. Oldyord also confirmed those observations made by Page & Robinson in *Apis mellifera*, who found a variety of genetically determined species in the colony[68,70].

Another approach using molecular tools for the identification of patrilines in a colony of honey bees has been DNA fingerprinting. Moritz et al could discriminate between super, and half sister in a colony by using multilocus fingerprinting with the synthetic (GATA), oligonucleotides[71]. Similar results were obtained by Blanchetot who used M13 as a probe for DNA fingerprinting and able to identify 11 patrilines in a single colony[72].

To reveal intrapopulation variability microsatellite approach was presented by Estoup et al[73]. They isolated a series of microsatellites from a genomic library of the honey bee. One microsatellite which was composed of 13 CT tandem repeats associated with 9 GGT repeats proved to be highly variable in a population of *Apis mellifera*.

An AluI repeat element has been identified in the genome of honey bee (*Apis mellifera*), which is estimated to be in 23000 copies in bee genome. The element is 176 bp long (Accession no. X57427) and is extremely conserved among the copies. This has been used as probe in RFLP fingerprinting of different geographical populations of honey bee[74].

Eight microsatellite loci were used to investigate the genetic structure of the giant honey bee (*A. dorsata*) population in northeast India. The result suggested that *A. dorsata* aggregation are comprised of colonies that share more alleles than expected by chance and there is significant population structuring between geographical regions[75].

Comparative chromosomal investigations of three honey bee ecotypes of *Apis mellifera carnica* (the Banat, the Timok and the Syenichko-Peshterski ecotype) from Serbia were performed. G-band analyses revealed differences between honey bee ecotypes. The results point to great interecotype variability of G-band pattern of chromosomes of the carniolan honey bee in Serbia[76].

CONCLUSION

The effectiveness of biomarkers in detecting polymorphism between and among different populations, their applicability in population studies, and the establishment of genetic relationships were demonstrated in this review.

In many a time, molecular marker data help to distinguish between different species, when there is no other comprehensive way available to do so. In insects, DNA markers are used to provide raw information based on which an ecologist makes estimates of genetic diversity and gene flow between species, identified haplotypes and lineages or predicts migration and colonization history.

Honey bee plays an important role in our society and exhibit significant genetic diversity due to geographical and environmental variations. This review highlight the recent trends of applications of molecular marker in honey bee studies and explores the technological advancement in molecular marker tools that may be applied in entomological researches for better understanding of social insect ecology at molecular level. Therefore, DNA markers became the most common yardsticks for measuring genetic differences between individuals or within and between related species or population. The unprecedented advancements in modern molecular biology, particularly in those of DNA marker technology, have created a wealth of technical know-how that finds useful application in molecular ecology research in insects.

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