Patterns of inheritance with RAPD molecular markers reveal novel types of polymorphism in the honey bee

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Summary. The polymerase chain reaction (PCR) was used to generate random amplified polymorphic DNA (RAPD) from honey bee DNA samples in order to follow the patterns of inheritance of RAPD markers in a haplodiploid insect. The genomic DNA samples from two parental bees, a haploid drone and a diploid queen, were screened for polymorphism with 68 different ten-nucleotide primers of random sequence. Parents were scored for the presence or absence of individual bands. An average of 6.3 bands and 1.3 polymorphisms for presence/absence were observed per primer between the parents. Thirteen of these primers were used to determine the inheritance of RAPD marker alleles in the resulting progeny and in haploid drones from a daughter queen. Four types of polymorphisms were observed. Polymorphisms for band presence/absence as well as for band brightness were inherited as dominant markers, meeting Mendelian expectations in haploid and diploid progeny. Polymorphisms for fragment-length were also observed. These segregated in a near 1:1 ratio in drone progeny. The last type of polymorphism was manifested as a diploid-specific band. Mixing of amplification products after PCR showed that the diploid-specific band was the result of heteroduplex formation from the DNA of alternate alleles in heterozygotes. In two of the four cases of heteroduplex formation, the alternative alleles were manifested as small fragment-length polymorphisms, resulting in co-dominant markers. This is the first demonstration that a proportion of RAPD markers are not inherited in a dominant fashion.

Key words: Random amplified polymorphic DNA (RAPD) – Apis mellifera – Genetic markers

Introduction

Much has been learned about honey bee (Apis mellifera L.) biology and behavior, yet little is known of its genome organization. Linkage tests with visible mutants of honey bee have demonstrated linkage between only three pairs of loci (Tucker 1986). The difficulty in finding linkage lies in the deficiency of suitable genetic markers. In order to have markers to construct a linkage map, we are investigating the inheritance of random amplified polymorphic DNA (RAPD) in the honey bee.

Other methods for finding variation at the level of DNA sequences have already been useful for honey bee genetics. Restriction fragment length polymorphisms (RFLPs) have provided genetic markers for population studies that document introgression between Africanized honey bees and European races in South America (Hall 1990; Sheppard et al. 1991). However, the RFLP technique is limited because individual insects contain insufficient quantities of DNA for extensive analysis. RFLP analysis using specific oligonucleotide repeats as probes can generate a “DNA fingerprint” and can be used to assess the relatedness of individual bees in colonies (Blanchetot 1991; Moritz et al. 1991). However, the banding patterns produced by these probes are complex due to the existence of many loci with related sequences. Genetic analysis of the banding pattern is further complicated because some of the loci are hypervariable. The complex patterns can be simplified only by cloning individual loci for use as probes (Wong et al. 1987). An alternative approach for obtaining genetic markers involves the polymerase chain reaction (PCR). Nanogram quantities of genomic DNA used as a template for PCR with ten-nucleotide primers of random sequence can generate RAPD markers in higher plants, humans, fungi and bacteria (Williams et al. 1990). RAPD markers have been
used as dominant markers for genome mapping in plants (Williams et al. 1990, 1992) and for the identification of plant disease-resistance genes (Martin et al. 1991; Michelmore et al. 1991). In mapping experiments RAPD markers have been shown to sample repetitive and single-copy sequences dispersed throughout the genome (Williams et al. 1992).

The honey bee is especially amenable for analysis with RAPD markers because it is haplodiploid. Honey bee females are diploid, but the male (drone) is haploid, the result of parthenogenetic development of an unfertilized egg (arrhenotoky). Moreover, due to an abortive meiosis, the sperm of a drone are all genetically identical (except for mutations) and hence all of a drone's progeny share this genome. Thus analysis of haploids can provide: (1) complete information for heterozygous loci in the parental queen that is not confounded by dominance effects and (2) clarification of the banding patterns in diploid siblings. This is a report on the frequency of polymorphism and some novel patterns of inheritance observed for RAPD markers in the honey bee.

Materials and methods

A virgin honey bee queen (referred to as the parental queen) was crossed to a single haploid male (the parental drone). DNA aliquots from these parents, from haploid male (drone) and diploid female (worker) progeny, and from one virgin queen daughter and her drone progeny were used individually as templates to generate RAPD markers. The frequency of polymorphism between the parents was observed and the mode of inheritance in haploid and diploid progeny was determined.

Honey bee samples and DNA extraction

A single virgin queen was raised (see Laidlaw 1979) and then instrumentally inseminated with the semen of a single, presumably unrelated, drone (Laidlaw 1977). Twenty haploid drone and 12 diploid worker progeny were subsequently collected. In addition, a daughter queen was raised and 94 of her drone progeny were also collected. All bees were quickly frozen on dry ice and stored at -70°C prior to DNA extraction. Total genomic DNA was isolated from individual bees by a modification of the method of Saghai-Maroof et al. (1984). Bees were ground with plastic pestles in microcentrifuge tubes that contained a CTAB extraction buffer (1% hexadecyltriethyl ammonium bromide, 0.75 M NaCl, 50 mM Tris, pH 8, 10 mM EDTA and 100 ng/ml of Proteinase K). Samples were incubated at 60°C for 2 h and extracted with phenol/chloroform and then with chloroform. The DNA was precipitated with two volumes of ethanol and the samples were centrifuged at 4000 g and 4°C. After resuspension in 10 mM Tris, 1 mM EDTA, the DNA was then quantified with a fluorometer (Hoeffer) and diluted to 5 ng/μl in 10 mM Tris, pH 7.6, 0.3 mM EDTA.

Polymerase chain reaction and electrophoresis

Target DNA sequences were amplified by PCR in a Perkin Elmer Cetus (480) thermal cycler. A single ten-nucleotide oligomer of random sequence (Operon Technologies Inc., Alameda Calif.) containing at least 50% G-C served as a primer for each reaction. Reaction conditions were as reported by Williams et al. (1990) except that only 5 ng of honey bee DNA was used per reaction. Reactions were incubated for 45 cycles consisting of 1 min at 94°C, 1 min at 35°C, a 2 min temperature transition to 72°C and 2 min at 72°C.

Amplification products were resolved on either 1.9% agarose gels (0.9% Ultra-pure agarose, BRL, and 1% NuSieve agarose, Hoefer) or on 0.6% Ultra-pure agarose with 1% Syngergel (Diversified Biotech, Newton Centre, Mass.). Gels were run in 0.75 x TBE buffer at 4 V/cm for 5 h 30 min and stained with ethidium bromide. In some cases amplification products were mixed in order to determine whether the observed diploid-specific bands were due to the reannealing of two homologous DNA strands that also contained a non-homologous region (heteroduplex formation). Samples were amplified separately as usual, then EDTA (5 mM) was added to the amplification products in order to inhibit residual Taq polymerase activity. The amplified DNA from two individual bees was mixed, heated to 94°C and allowed to reanneal at room temperature.

Results and discussion

Frequency of polymorphism

Many RAPD markers were polymorphic for band presence/absence between the two original parents. The 68 different random decamers that were used for PCR with honey bee DNA generated 432 scoreable bands for an average of 6.3 bands per primer. Ninety of these bands were polymorphic for band presence/absence between the two parents, an average of 1.3 polymorphic loci per primer. However, other types of polymorphisms which could not be detected in the parents were revealed in the inheritance study. Altogether, polymorphisms of four types were found: band presence/absence, band brightness, polymorphism, fragment-length polymorphism, and heteroduplex-band polymorphism. These types of markers occurred 20, six, eight and four times, respectively, for an average of about three polymorphisms per primer.

Inheritance of RAPD markers

Thirteen primers were used to follow the inheritance of polymorphic markers. Analysis included DNA from the two original parents, along with five drones derived from the parental queen and five of her worker progeny. The inheritance of some of the markers was studied in more detail with a larger sample of 20 drones and 12 workers. In addition, a daughter queen and 94 of her drones were analyzed with nine of the primers. All polymorphic markers for which the daughter queen was heterozygous segregated in ratios that were not significantly different from 1:1 (for all 24 loci; P > 0.05, chi-squared test).

Four types of RAPD marker polymorphisms were observed in the progeny (Table 1). The first two types, band presence/absence polymorphism and a novel type of polymorphism, band brightness, segregated as dominant markers. A 1:1 segregation ratio of these polymorphisms is expected in the haploid drones of a heterozygous queen because drones are equivalent to