Genetic diversity among queen bee, worker bees and larvae in terms of retrotransposon movements

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Abstract

Objective

Honeybee (*Apis mellifera* L.) is a model organism, contributing significant effect on global ecology by pollination and examining due to its social behaviour.

Methods

In this study, barley-specific *Sukkula* and *Nikita* retrotransposons were analysed using IRAP (Inter-Retrotransposon Amplification Polymorphism) marker technique, and the relationships between retrotransposon movements and development were also investigated in three different colonies of the Caucasian bee (*Apis mellifera caucasica*). Furthermore, transposon sequences belonging to *Apis mellifera*, *Bombus terrestris*, *Triticum turgidum* and *Hordeum vulgare* were also examined to figure out evolutionary relationships.

Results

For this purpose, a queen bee, five worker bees, and five larvae from each colony were studied. Both retrotransposons were found in all samples in three colonies with different polymorphism ratios (0-100% for *Nikita* and 0-67% for *Sukkula*). We also determined polymorphisms in queen-worker (0-83% for *Nikita*, 0-63% for *Sukkula*), queen-larvae (0-83% for *Nikita*, 0-43% for *Sukkula*) and worker-larvae comparisons (0-100% for *Nikita*, 0-63% for *Sukkula*) in colonies. Moreover, close relationships among transposons found in plant and insect genomes as a result of *in silico* evaluations to verify experimental results.

Conclusion

This work could be one of the first studies to analyse plant-specific retrotransposons' movements in honeybee genome. Results are expected to understand evolutionary relationships in terms of horizontal transfer of transposons among kingdoms.

Introduction

The vast majority of all known animal species are members of the Arthropoda phylum, and the class Insecta is the most significant class of Arthropods. Almost 80% of animal species defined in the world are found in the class Insecta (Stork et al. 2015; Zhang 2013). Honeybee (*A. mellifera* L.) is an economically and ecologically critical social insect for human beings. In the beehive, female bees (queen and worker) originate from fertilized eggs and males (drone) from unfertilized eggs. If larvae are specially fed with royal jelly, it turns into queen bees and produces thousands of eggs in a day while if not feed with royal jelly, they become sterile workers. Therefore, the bee is a very suitable model organism to study the relationship between nutrition (environment) and genetic structure (The Honeybee Genome Sequencing Consortium 2006).

Transposons, mobile genetic elements, are divided into two groups including Class I (RNA transposons or retrotransposons) and Class II (DNA transposons). They move via copy-paste and cut-paste mechanism, respectively. They constitute different portions of animal genomes (3-60%). This ratio is very high in plant genomes (up to 90%) (Canapa et al. 2015). It is reported that the genome size of the honeybee is 236 Mb, and approximately 3% of the genome consist of transposons. Although there are many retrotransposons in the honeybee genome, there are little pieces of evidence for active transposable elements (The Honeybee Genome Sequencing Consortium 2006).

In this study, we aimed to investigate the presence and movements of barley-specific *Nikita* and *Sukkula* retrotransposons by using IRAP marker technique and evaluation the polymorphisms in different colonies at different developmental stages in honeybee genome for the first time. Moreover, we obtained transposon sequences belonging to different genomes from NCBI to figure out the evolutionary relationships.

Materials And Methods

Honeybee materials

In this study, three colonies of *Apis mellifera caucasica* were selected from the beekeeping unit of Department of Animal Sciences of Faculty of Agriculture in Ondokuz Mayıs University. Genomic DNA of a total of 33 honeybee samples consisting of 11 samples from each colony (1 queen, 5 workers, and 5 larvae) were isolated according to Evans et al. (2013). The concentration and quality of genomic DNA were determined using a spectrophotometer and 1% (w/v) agarose gel.

IRAP analyses

Barley-specific *Nikita* and *Sukkula* retrotransposons' movements were analysed by IRAP molecular marker technique. Primer sequences were 5'ACCCCTCTAGGCGACATCC3' for *Nikita* and 3'GGAACGTCGGCATCGGGCTG5' for *Sukkula* (Leigh et al. 2003). IRAP-PCR was carried out in 25 µL of the reaction mixture containing 6.5 µL of nuclease-free dH₂O, 12.5 µL of MasterMix (DreamTaq Green PCR Master Mix, Thermo Scientific™), 2 µL of 10 µM/µL primer (0.8 µM/µL), 4 µL of 20 ng/µL (3.2 ng/µL) template genomic DNA. Final concentrations were indicated in parentheses. The amplification conditions were as follows: one initial denaturation step (95°C for 3 min) followed by 35 cycles of denaturation (95°C for 30 s), annealing (54°C for 30 s) and extension (72°C for 1 min). The reaction was completed by a final extension step (72°C for 5 min). PCR products were resolved on 1% agarose gel (w/v) in 1X TBE (Tris-Boric acid-EDTA) at 120 V for 120 min. Then, agarose gel photographed on a UV transilluminator and band profiles belonging to samples were evaluated.

Calculations of polymorphism

Band profiles of queen bees, worker bees, and larvae were examined one by one for each sample, and monomorphic and polymorphic bands were determined. Polymorphism rates among samples were calculated using the Jaccard similarity index. Jaccard's similarity index could be calculated using the formula: $N_{AB}/(N_{AB} + N_A + N_B)$; where N_{AB} is the number of bands shared by two samples, N_A represents amplified fragments in sample A, and N_B represents amplified fragments in sample B (Jaccard 1908).

Evolutionary relationships among transposons

Transposon sequences of related species *Apis mellifera* and *Bombus terrestris* in addition to *Nikita* and *Sukkula* sequences belonging to *Triticum turgidum* and *Hordeum vulgare* were examined to evaluate evolutionary relationships among these species and verify our IRAP results. For this purpose, sequences were retrieved from NCBI (www.ncbi.nlm.nih.gov) and a phylogenetic tree was constructed by using these sequences via MEGA X (Kumar et al. 2018) with following parameters: neighbour-joining (NJ) method (Saitou and Nei 1987) with the p-distance model (Nei and Kumar 2000), and even bootstrap test (1000 replicates) (Felsenstein 1985).

Results

Nikita showed higher polymorphism than *Sukkula*

Barley-specific *Nikita* retrotransposon was identified in queen bees, worker bees, and larvae. As a result of IRAP-PCR, samples displayed a band profile in the range of 200-1500 bp (Fig. 1).

Nikita retrotransposon showed a very high percentage of polymorphism (0-100%) in all samples. Polymorphism levels are 0-71% among the members of the first colony, 0-60% among the members of the second colony and 0-100% among the members of the third colony. Polymorphism rates also detected among queens (25-33%), workers (0-100%) and larvae (0-83%) in three colonies (Table 1)

Moreover, polymorphism rates were also detected within colonies. Polymorphism rates of queen-workers and queen-larvae were the same in the first colony (0-71%). Polymorphism ratio was also determined as 14-71% for workers-larvae. In the second colony, there was 0-50% polymorphisms for queen-workers, 0-57% for queen-larvae. However, this rate increased by 67% for workers-larvae. The most polymorphic patterns were determined in the third colony. Polymorphism rates for queen-worker and queen-larvae were found to be the same but higher (0-83%) compared to other colonies. Besides, 0-100% of polymorphism rates were determined as a result of comparison between workers and larvae.

Similar to *Nikita*, barley-specific *Sukkula* retrotransposon was also identified in the queen, workers and larvae, ranging from 250 to 1500 (Fig. 2). When compared to *Nikita*, *Sukkula* indicated lower polymorphism percentages among samples.

In general, polymorphism rates among individuals of all colonies for *Sukkula* retrotransposon were determined in the range of 0-67%. However, polymorphism rates among samples in the first colony were 0-43% while 0-63% for the second and third colonies. When compared to queens, workers and larvae in three colonies, 14-57%, 20-50% and 0-43% ratios were observed, respectively (Table 2).

Table 2
Sukkula retrotransposon polymorphism ratios

FIRST COLONY												SECOND COLONY												
		Q1	W1	W2	W3	W4	W5	L1	L2	L3	L4	L5	Q2	W1	W2	W3	W4	W5	L1	L2	L3	L4	L5	
FIRST COLONY	Q1	0																						
	W1	20	0																					
	W2	20	0	0																				
	W3	20	0	0	0																			
	W4	20	0	0	0	0																		
	W5	20	0	0	0	0	0																	
	L1	43	29	29	29	29	29	0																
	L2	43	29	29	29	29	29	0	0															
	L3	43	29	29	29	29	29	0	0	0														
	L4	43	29	29	29	29	29	0	0	0	0													
	L5	43	29	29	29	29	29	0	0	0	0	0												
SECOND COLONY	Q2	57	43	43	43	43	43	14	14	14	14	14	0											
	W1	20	0	0	0	0	0	29	29	29	29	29	43	0										
	W2	67	50	50	50	50	50	63	63	63	63	63	57	50	0									
	W3	20	0	0	0	0	0	29	29	29	29	29	43	0	50	0								
	W4	40	20	20	20	20	20	43	43	43	43	43	33	20	40	20	0							
	W5	40	20	20	20	20	20	43	43	43	43	43	33	20	40	20	0	0						
	L1	20	0	0	0	0	0	29	29	29	29	29	43	0	50	0	20	20	0					
	L2	43	29	29	29	29	29	0	0	0	0	0	0	14	29	63	29	43	43	29	0			
	L3	43	29	29	29	29	29	0	0	0	0	0	0	14	29	63	29	43	43	29	0	0		
	L4	43	29	29	29	29	29	0	0	0	0	0	0	14	29	63	29	43	43	29	0	0	0	
	L5	43	29	29	29	29	29	0	0	0	0	0	0	14	29	63	29	43	43	29	0	0	0	0
THIRD COLONY	Q3	43	29	29	29	29	29	0	0	0	0	0	14	29	63	29	43	43	29	0	0	0	0	
	W1	40	20	20	20	20	20	43	43	43	43	43	33	20	40	20	0	0	20	43	43	43	43	
	W2	40	20	20	20	20	20	43	43	43	43	43	33	20	40	20	0	0	20	43	43	43	43	
	W3	67	50	50	50	50	50	63	63	63	63	63	57	50	0	50	40	40	50	63	63	63	63	
	W4	50	33	33	33	33	33	50	50	50	50	50	63	33	20	33	50	50	33	50	50	50	50	
	W5	40	20	20	20	20	20	43	43	43	43	43	33	20	40	20	0	0	20	43	43	43	43	
	L1	43	29	29	29	29	29	0	0	0	0	0	14	29	63	29	43	43	29	0	0	0	0	
	L2	50	33	33	33	33	33	29	29	29	29	29	17	33	50	33	20	20	33	29	29	29	29	
	L3	50	38	38	38	38	38	13	13	13	13	13	25	38	50	38	50	50	38	13	13	13	13	
	L4	20	0	0	0	0	0	29	29	29	29	29	43	0	50	0	20	20	0	29	29	29	29	
	L5	57	43	43	43	43	43	38	38	38	38	38	29	43	33	43	33	33	43	38	38	38	38	

The highest polymorphism rates were identified for queen-larvae (43%) in the first colony. Also, there was 20% polymorphism for queen-workers and 29% for worker-larvae. In the second colony, the lowest polymorphism rate was observed for queen-larvae comparison (14%). On the other hand, higher polymorphism was detected for queen-workers (33-57%) and workers-larvae (0-63%). These rates were 0-38% for queen-larvae, 43-63% for queen-workers and 20-63% for workers-larvae in the third colony.

In silico analyses showed similarities among retrotransposons in insect and plant genomes

A phylogenetic tree was constructed by using 53 different sequences (Fig. 3). All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 105 positions in the final dataset. We observed that all sequences were clustered into two groups. Most of *A. mellifera*

sequences were found in the first group. Moreover, *A. mellifera* and *B. terrestris* sequences were found in one clade while *Sukkula* sequences formed a second clade. In the second group, similar to the first one, *A. mellifera*, *B. terrestris* and *Sukkula* sequences were observed. In addition, *Nikita* sequences were also determined in this group.

Discussion

Transposable elements can be inherited via vertical transfer which is genetic transfer from ancestral to descendant species (Markova and Mason-Gamer 2015; Wallau et al. 2016). On the other hand, many evidence also showed that these sequences move horizontally (Zhang et al. 2020). Phylogenetic relatedness among species is an important factor for horizontal transfer of transposons (HTT). There are many studies in which a retrotransposon specific to a particular plant is detected in a different plant. In one of these studies, *Nikita* and *Sukkula* retrotransposons were studied in the aniseed. Polymorphism was not found in both retrotransposons but their presence in the aniseed genome was determined (Marakli 2018). In another study, these two retrotransposons were identified in two varieties of black pine (*Pinus nigra* var. *pyramidata* and *Seneriana*). *Sukkula* retrotransposon showed 0-76% polymorphism in *pyramidata* variety while no polymorphism was detected in *Seneriana* variety. Similarly, *Nikita* polymorphism (0-56%) was detected only in *pyramidata* variety (Marakli et al. 2019).

In addition to closely related species, HTT could also observe in distantly related taxa (Gao et al., 2018; Metzger et al., 2018; Zhang et al., 2020). Concordant with this opinion, we identified barley-specific retrotransposons in chicken genome for the first time in our previous study (Mercan et al. 2021). In this presented study, barley-specific *Nikita* and *Sukkula* movements were also analysed by using IRAP marker technique in bees with different growth capabilities due to feeding in different periods. Higher polymorphism ratios were determined in bee genome (0-100% for *Nikita* and 0-67% for *Sukkula*) among queen, workers and larvae. Moreover, similar sequences were identified among transposons in *Apis mellifera*, *Bombus terrestris*, *Triticum turgidum* and *Hordeum vulgare* genomes. These results could be supported by Meyerowitz (2002), reporting a common ancestor for plants and animals.

In honeybee genome, there are very few transposons including 15 partial sequences of a *Copia* family sequence with highly distorted copies, 6 partial sequences matching the coding *BEL 12* element of *Anopheles gambiae*, and 3 highly degraded copies of a *DIRS* retrotransposon (Eiglmeier et al. 2005; Goodwin et al. 2004). Moreover, 11 LTR and 7 non-LTR retrotransposon residues in *Drosophila* were also found in honeybee genome (Kaminker et al. 2002). Other study was carried out by Gillespie et al. (2006). They suggested that ribosomal DNA units of honeybees contain active non-LTR retrotransposons of the *R2* family. They also reported that although active *R2* non-LTR retrotransposons were identified, *R1* line was not found in honeybee genome. Elsik et al. (2014) also determined the presence of *copia*, *R2* and *I* retrotransposons together with *mariner* and *piggyBac* transposons in the genome of the honeybee. Similarly, Wang et al. (2017) reported that queen bees and drones contain *mariner*, *piggyBac*, *R2*, *copia*, *Bel-Pao* and *I* transposons but worker bees only contain *mariner*, *piggyBac* and *R2* transposons.

There are many studies related to the effect of retrotransposon movements on species identification (Leśniowska-Nowak et al. 2021), organ differentiation (Ramos et al. 2011; Zhu et al. 2012) and abiotic/biotic stress (Ghonaim et al. 2021) conditions by using retrotransposon-based molecular markers. Most of these studies were performed in plant genomes because transposons constitute most of plant genomes.

Conclusions

To our best knowledge, there is no investigation related to plant-specific retrotransposons in the honeybee. The findings could be preliminary results to understand the difference between *Nikita* and *Sukkula* retrotransposons' movements related to retrotransposon effects on an important species for humanity's future. Deep sequencing of honeybee genome will provide better understand the relationships among different organisms in different species.

Declarations

Conflict of Interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Tables

Due to technical limitations, Table 1 is only available as a download in the Supplemental Files section.

Figures

Figure 1

IRAP-PCR results of Nikita retrotransposon (M, marker; Q, queen; W, worker; L, larva; NC, Negative control).

Figure 2

IRAP-PCR results of Sukkula retrotransposon (M, marker; Q, queen; W, worker; L, larva; NC, Negative control).

Figure 3

Phylogenetic tree analysis of transposons belonging to insect and plant species.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.docx](#)