THE DETECTION OF DNA VARIATION IN MUTANT "STING-BROKEN HONEYBEES (*Apis mellifera*)" INDUCED BY GAMMA RADIATION

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ABSTRACT

Sting broken *Apis mellifera* was produced in Japan in order to prevent the allergy from bee stings and increase pollination. They are obtained by inducing gamma radiation to wild type *A. mellifera* at the larva stage. The difference between both types can not be detected at the developmental level. The size reduction in sting is visible after an abdomen dissection of sting broken workers. In order to avoid the death of sting broken bees, a method to detect the DNA variation between wild type *Apis mellifera* and sting broken honeybees was performed by using 6-MW primer (5'-CCGAC TCGAG NNNNN NATGT GG-3'), CTA_4DOP primer (5'-CTACT ACTAC TACCG ACTCG AG-3'), and various RAPD primers (5'-GCATC GACTT-3' as RAPD1; 5'-ACACT TCCCA-3' as RAPD2; 5'-GACGC TTGAC-3' as RAPD3; 5'-CGATT CCCGT-3' as RAPD4). The pattern of amplification was different between wild type *A. mellifera* and sting broken mutant. In addition, the high polymorphism of sting broken individuals was detected but in not wild type *A. mellifera* individuals. *Dra*I was used to cut genomic DNA of both. It seems that genomic DNA from wild type *A. mellifera* was cut better. Genomic DNA library of both types of bees were successfully made. The result indicates the random effect of gamma radiation to the genome of the sting broken type. Although the DNA variation from both can be detected, the behavior of foraging is not different. At present, there are still many experiments to perform on sting broken honeybees before distributing to bee farming.

1. INTRODUCTION

There are 2 species of honeybees that can survive in Japan. The first one is the native Japanese honeybee (*Apis cerana japonica*) while the second one is *A. mellifera* which was imported from the USA in 1877 [1]. Besides pollination, honeybees can also provide nutritional bee products such as honey, bee pollen, royal jelly, etc. In 1997, there was a high demand for bee products in Japan. Ninety percent of honey consumed in the country was imported, especially from China. To meet consumer demand there was a need for more honeybee hives. In 1997, there were about 24,000 colonies across Japan. The obvious danger to farmers working with honeybees is due to the ability of worker bees to sting, which can cause allergic symptoms. In order to provide for easier maintenance of honeybees to farmers, the breeding of sting broken honeybees was introduced. Two methods have been used to induce mutation in *A. mellifera*. The first method, was gamma (20 to 50 gray) irradiation of queens which had already completed copulation and had sperms in their spermatheca. The second method was gamma ray (30 gray) irradiation of honeybees at the developmental stages

where they metamorphose themselves from mature larvae into pupae. A higher rate of success was obtained by the second method [1]. Since it is difficult to differentiate the wild type from the mutant type at the developmental stage or through behavior observation we have tried to differentiate the two by trying to characterize variation at DNA level.

2. MATERIALS AND METHODS

Sample collection and genomic DNA isolation

Apis mellifera and sting-broken bees were provided by Amano, K. The honeybee hives were maintained at the Apiculture laboratory of National Institute of Livestock and Grassland Science, Ibaraki, Japan. The honeybees of both types were stored at –20°C immediately or in absolute ethanol and stored at 4°C. Genomic DNA was isolated from thoraxes of honeybees by the DNeasy tissue kit from Qiagen (catalog number 69504).

PCR amplification by 6-MW, CTA_4DOP, and some RAPD primers

The amplification of genomic DNA (200 ng) by 6-MW (5'-CCG ACT CGA GNN NNN NAT GTG G-3') and CTA_4DOP primers (5'-CTA CTA CTA CTA CCG ACT CGA G-3') was according to [2, 3, 4] respectively. PCR amplification by various primers for RAPD was performed under optimum conditions. RAPD primers were GCA TCG ACT T (as RAPD1), ACA CTT CCC A (as RAPD2), GAC GCT TGA C (as RAPD3), and CGA TTC CCG T (as RAPD4).

RAPD primers

RAPD 1, 2, and 3 primers were used. The PCR condition is at 94°C for 3 min. Then, it is followed by 35 cycles of 94°C for 1 min, 37°C for 1 min, and 72°C for 3 min. The last extension cycle is at 72°C for 10 min. According to **Figure 5**, there is no variation in selected *A. mellifera*.

Pattern of restriction digestions and genomic DNA library

Genomic DNA of *A. mellifera* was cut with various restriction enzymes under optimum conditions. Then, both DNA of *A. mellifera* and sting-broken bee were cut with *Dra*I. The cut DNA was selected and cloned into pBluescript II KS (+/-) phagemid vector. The clones were transformed into *E. coli* DH5α cells. White colonies were selected and the insert size was determined by restriction digestion with *Sal*I and *Bam*HI.

3. RESULTS AND DISCUSSION

Both genomic DNA of *A. mellifera* and sting-broken honeybees were isolated. Thoraxes were used because of the awareness of pollen DNA contamination. High molecular weight (M.W.) of genomic DNA (about 23 kb) were obtained from both samples. This indicated the good quality of DNA as shown in **Figure 1**.

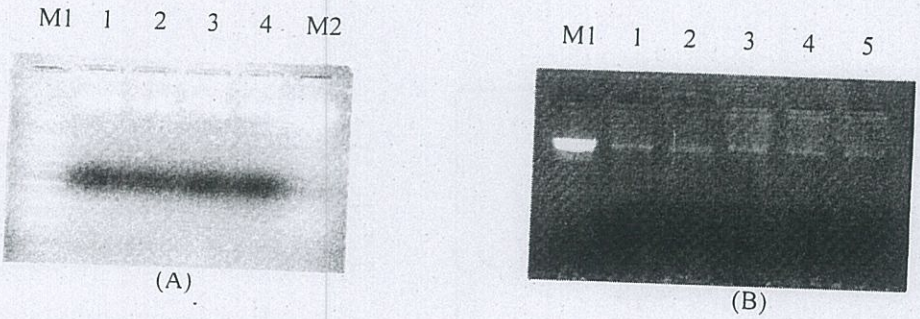


Figure 1. Genomic DNA of *A. mellifera* (A) and sting-broken bees (B). For **Figure 1(A)**, lane M1 shows 100 bp DNA ladder and lane M2 indicates lambda *Hin*dIII as markers. Lanes 1, 2, 3, and 4 indicate genomic DNA of *A. mellifera.* For **Figure 1(B)**, lane M1 contains lambda *Hin*dIII as a marker. Lanes 1, 2, 3, 4, and 5 contain genomic DNA of sting-broken bees.

Both genomic DNA were amplified by 6MW primer. In **Figure 2(B)** discretes bands were obtained while the products looked like smears in **Figure 2(A)**. The results indicate that the primer can bind more positions to the genome of *A. mellifera* **(Figure 2(A))** than the genome of sting-broken bee **Figure 2(B)**.

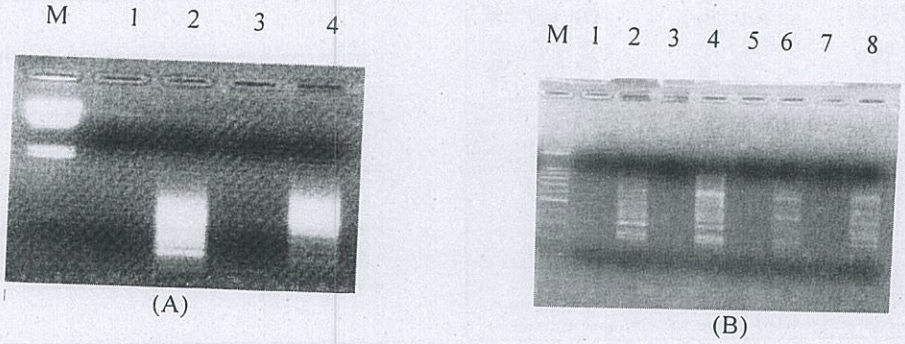


Figure 2. Pattern of PCR products from genomic DNA of *A. mellifera* (A) and sting-broken bee (B) after amplification by 6MW primer. For (A), lane M represents lambda *Hin*dIII marker. Lanes 1 and 3 contained negative reactions (without genomic DNA) while lanes 2 and 4 contain positive reactions (with genomic DNA). For (B), lane M represents 100 bp DNA ladder as marker. Lanes 1, 3, 5, and 7 indicate negative reactions containing no genomic DNA. In contrast, lanes 2, 4, 6, and 8 indicate positive reactions containing genomic DNA.

After amplification by 6MW primer, the PCR products were purified and re-amplified by CTA4DOP primer. From **Figure 3**, obvious bands were obtained from both samples. In **Figure 3 (A)**, four identical bands were visible in both *A. mellifera* individuals. In **Figure 3 (B)**, four individual sting-broken bees were used. Four sharp bands were visible in all individuals but there was an extra band (larger than 400 bp) from individual 3. Also, there was an extra band at about 300 bp from individual 4. The results indicate DNA variation in sting-broken bees.

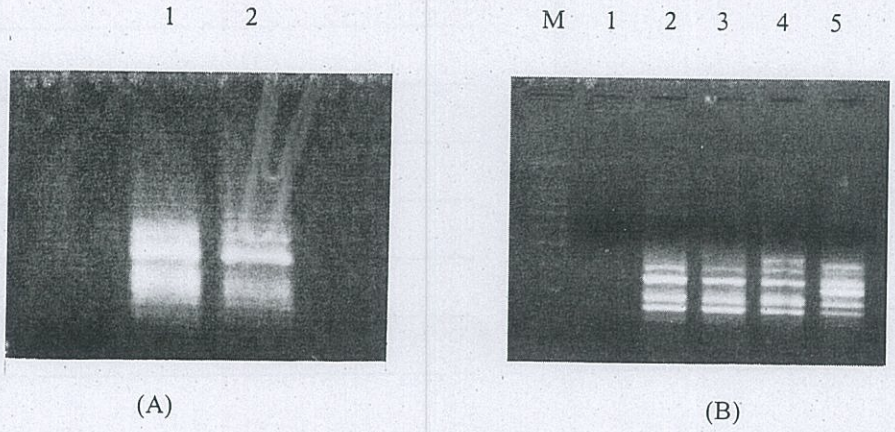


Figure 3. Pattern of PCR products after amplification by CTA4DOP; (A) represents *A. mellifera* and (B) sting-broken bee. For 3 (A), lanes 1 and 2 contain the PCR products of *A. mellifera* 1 and 2. For 3 (B), lane M represents 100 bp DNA ladder as marker. Lane 1 indicates a negative reaction containing no genomic DNA while lanes 2, 3, 4, and 5 indicate positive reactions containing PCR product mutants 1, 2, 3, and 4, respectively.

In order to separate each band of PCR products better, the electrophoresis was performed on polyacrylamide gel (PAGE) instead of agarose gel.

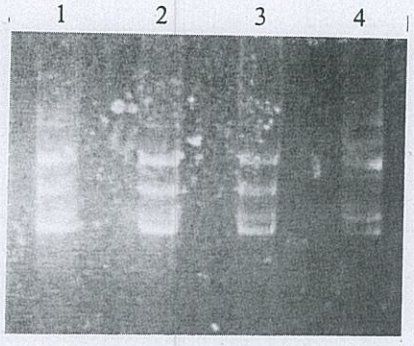


Figure 4. PAGE. Lanes 1, 2, 3, and 4 contain the samples from lanes 2, 3, 4, and 5 of **Figure 3(B)**, respectively. All samples were electrophoresed on 7.6% acrylamide gel and ethedium bromide stain.

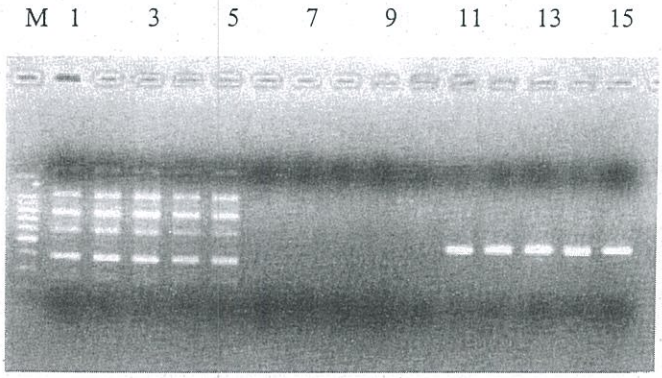


Figure 5. Amplification of genomic DNA of *A. mellifera* by RAPD 1-3 primers. Lane M contains 100 bp DNA ladder as a marker. The result shows that 6 bands at approximately 250, 400, 600, 800, larger than 1 kb, and larger than 1.5 kb were observed in lane 1-5 (RAPD 1). No products were visible at all in lane 7-11 (RAPD 2). Furthermore, only approximately 450 bp product is shown in lane 11-15 (RAPD 3).

Using same condition as in **Figure 5**, the genomic DNA of 5 individuals of sting-broken bees were amplified by RAPD 1, 2, and 3. The variations in sting-broken bees can be detected by RAPD 2 and 3 but not by RAPD 1 (**Figure 6**).

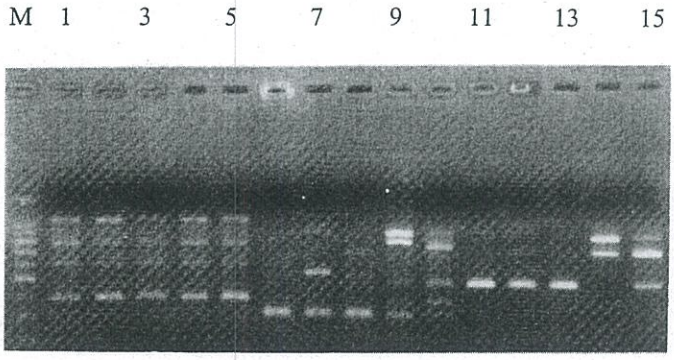


Figure 6. Amplification of genomic DNA of sting-broken bee by RAPD 1, 2, and 3 primers. Lane M contains 100 bp DNA ladder as a marker. The result shows that 4 bands at approximately 400, 600, 800, and larger than 1 kb were observed in lanes 1-5 (RAPD 1). Products of various sizes were visible in lanes 6-10 (RAPD 2) and lanes 11-15 (RAPD 3).

Using the same PCR conditions as for RAPD 1, 2, and 3, amplification by RAPD 4 was performed. There are satellite products from *A. mellifera*. Considering the major bands only, there are 2 products of about 500 bp and about 1.5 kb from *A. mellifera*. This indicates that there is no variation. In contrast, there is variation in sting-broken bee because there are extra bands in lanes 5-9.

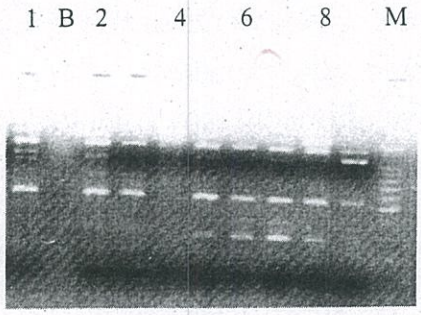


Figure 7. Amplification of genomic DNA of *A. mellifera* and sting-broken bee by RAPD 4. Lane M contains 100 bp DNA ladder as a marker while lane B is blank. Lanes 1-4 contain the PCR products of *A. mellifera* while lanes 5-9 contain the PCR products of sting-broken bee.

The pattern of restriction digestion can be used to detect the presence of genomic variation between *A. mellifera* and sting-broken bee. Since only one mutated nucleotide can change the pattern of digestion. Various restriction enzymes (*Hin*dIII, *Ava*I, *Dra*I, *Taq*I, *Hin*fI, *Hpa*II, *Sau*3AI, *Kpn*I, *Xho*I, *Eco*RI, *Eco*RV, *Bam*HI, and *Eco*RI) were used. Only *Dra*I, *Hin*fI, *Hpa*II, *Sau*3AI, and *Eco*RV can digest both genomic DNA (data not shown). *Dra*I was used for the further experiments. From **Figure 8**, it seems that *Dra*I can digest genomic DNA of *A. mellifera* better. This indicates that many restriction sites of *Dra*I have been changed in sting-broken bee DNA.

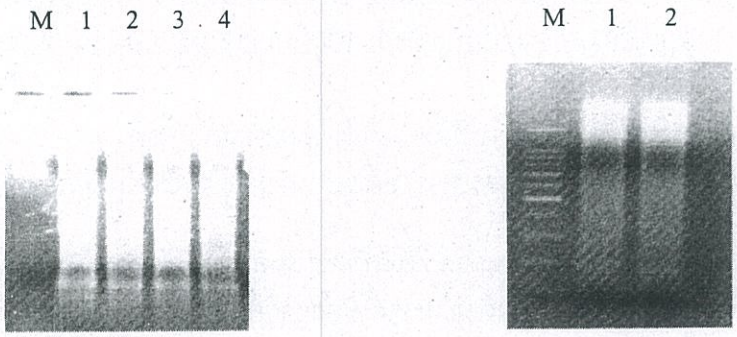
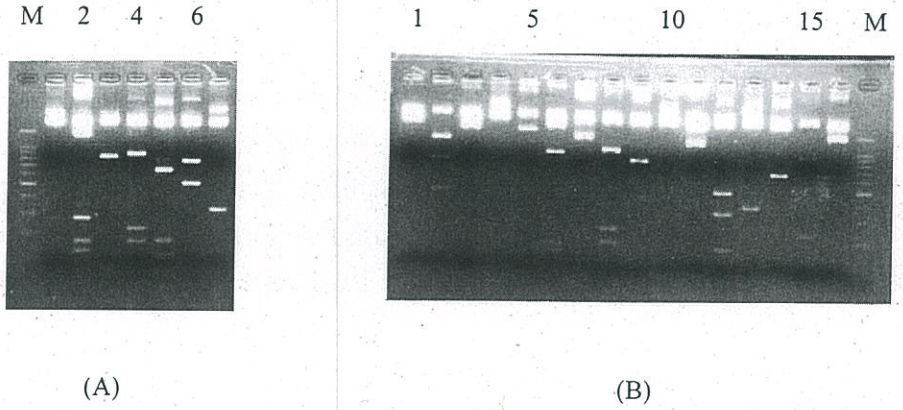


Figure 8. *Dra*I digestion of genomic DNA of *A. mellifera* (A) and sting-broken bee (B). For **Figure 8** (A), lane M contains lambda *Hin*dIII while lanes 1-4 contain digested genomic DNA. For **Figure 8(B)**, lane M contains 100 bp DNA ladder while lanes 1 and 2 contain digested genomic DNA.

The digested genomic DNA of both types were purified and cloned into pBlueScript SK (+/-) vector that was already cut by *Eco*RV. After transformation, white colonies were selected for plasmid preparation. The insert size was determined by the digestion of *Sal*I and *Bam*HI. Various insert sizes were obviously visible from both genomic DNA libraries (**Figure 9**). Both libraries will be used to determine the difference of some nucleotide sequences between *A. mellifera* and sting-broken bee in the future.



(A) (B)

Figure 9. Genomic DNA libraries of *A. mellifera* (A) and sting-broken bee (B). Lane M in both 9 (A) and (B) contains 100 bp DNA ladder.

4. CONCLUSION

There are DNA variations in sting-broken bee because of the different patterns from the amplification by 6-MW primer (5'-CCG ACT CGA GNN NNN NAT GTG G-3'), CTA4DOP primer (5'-CTA CTA CTA CTA CCG ACT CGA G-3'), RAPD1 (GCA TCG ACT T), RAPD2 primer (ACA CTT CCC A), RAPD3 primer (GAC GCT TGA C, and RAPD4 primer (CGA TTC CCG T). Furthermore, the ability of *Dra*I to cut genomic DNA of sting-broken bee is less than that of *A. mellifera*.

5. ACKNOWLEDGMENTS

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