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Short Communication

Study on genetic variability of *Cassidula aurisfelis* (snail) by random amplified polymorphic DNAs

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The genetic variability among individuals of *Cassidula aurisfelis* from Setiu Wetland, Terengganu Darul Iman was examined by using the random amplified polymorphic DNA (RAPD) technique. Ten oligonucleotide primers were screened and three primers were selected (OPA 02, OPA 04 and OPA 10) to amplify DNA from five samples of *C. aurisfelis* from Setiu Wetland, Terengganu. A total of 28 RAPD bands (RAPDs) with 17 polymorphic bands (60.71%) with size ranging from 300 – 1750 bp were scored from the population. Genetic distance for samples ranges from 0.135 to 0.269. Similarity index of samples ranges from 0.7179 to 0.8649 (mean 0.7810 ± 0.0497).

Key words: *Cassidula aurisfelis*, snail, genomic DNA, genetic variation, RAPD.

INTRODUCTION

Cassidula aurisfelis is known as Angulated Shoulder Ear snail, Angulate Vassidula or cat's Ear Cassidula in English (Smith, 1992). It belongs to the great division or phylum called the Mollusca and from class of gastropoda. The shell is one piece (univalve) and may be coiled and uncoiled. Body bilaterally symmetrical and unsegmented, usually with definite head. Feeding habits of snails are as varied as their shape and habitats, but all include the use of some adaptation of the radula.

Owing to recent innovation in molecular biological techniques, such as polymerase chain reaction (PCR) and DNA automated sequencing, nucleic acid data are becoming more and more important in biology (Hillis et al*.,* 1996). One of the modern marker techniques for studying genetic variability is Random Amplified Polymorphic DNAs (RAPD) (Williams et al*.,* 1990). The technique requires no prior knowledge of the genome and it needs only a small amount of DNA (Hadrys et al*.,* 1992). Using this technique polymorphism can be detected in closely related organism. In this investigation, relationship between samples of *C. aurisfelis* were evaluated using RAPD.

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MATERIALS AND METHODS

Sample collection

The samples of *C. aurisfelis* were collected from the area in Setiu Wetland, Setiu, Terengganu. 15 individuals were collected randomly around this area by hand packing. All the samples were collected during the low tide of water. The length, width, thickness and weight from each sample were measured.

DNA extraction and RAPD

DNA was extracted based on the phenol-chloroform method described by Brown et al. (1991) with some modifications. The quantity of DNA was measured by obtaining the absorbance reading at 260 nm and the purity of DNA was estimated by calculating the ratio of absorbance reading at 260 and 280 nm. 10 RAPD primers (Table 1) from Kit A (with 60 - 70% G-C) content were screened. Primers that have the basic of sharpness, clarity of the profile and the existence of polymorphism were chosen for further study (D'Amato and Corach, 1997).

The total reaction volume of 25 µl was used with the final concentration containing 1x reaction buffer, 50 ng genomic DNA, magnesium chloride 4.0 mM, *Taq* DNA polymerase (2 units), 0.4 mM dNTPs and 10 pM primer. The DNA was amplified by using a Master Cycles Gradient (Eppendorf). The amplification was programmed at 45 cycles for 30 s of denaturation at 94°C, 30 s of annealing temperature at 36°C, 1 min of primers extension at 72°C and final extension of 2 min at 72°C.

Table 1. Code, sequence, nucleotide length and G+C content of primers used in the Random Amplified Polymorphic DNA analysis.

Primer	Primer sequence (5' to 3')	Nucleotide length	G+C content (%)
OPA 01	CAGGCCCTTC	10-mers	70
OPA 02	TGCCGAGCTG	10-mers	70
OPA 03	AGTCAGCCAC	10-mers	60
OPA 04	AATCGGGCTG	10-mers	60
OPA 05	AGGGGTCTTG	10-mers	60
OPA 06	GGTCCCTGAC	10-mers	70
OPA 07	GAAACGGGTG	10-mers	60
OPA 08	GTGACGTAGG	10-mers	60
OPA 09	GGGTAACGCC	10-mers	70
OPA 10	GTGATCGCAG	10-mers	60

Table 2. Size of fragments, total number of fragments, number of polymorphic fragments and percentage of polymorphic of *Cassidula aurisfelis* generated from OPA 02, OPA 04 and OPA 10.

Primer	Size of fragments (bp)	Total number of fragments	Number of polymorphic fragments	Percentage of polymorphic (%)
OPA 02	400 - 1500	9	6	66.67
OPA 04	300 - 1750	10	5	50.00
OPA 10	300 - 1031	9	6	66.67
Total	-	28	17	60.71

PCR product was electrophoresed on 1.5% (w/v) agarose gel in 1x TBE buffer at 55 V for 1 to 2 h depending on the size of amplified fragment from each primer. The gel was stained in 1 µg/mL ethidium bromide for 20 to 30 min and photographed with Image Master VDS.

Data analysis

The RAPDistance Package Software Version 1.04 (Armstrong et al., 1994) and Numerical taxonomy and Multivariate Analysis System (NTSYS-pc) were used in this study. The molecular weights of band were estimated based on the standard bands from Gene Ruler DNA Ladder Marker. The presence of band was scored from the photograph. Only clear and reproducible bands were given consideration.

These bands were considered as polymorphic when they were absent in some sample in frequency greater than 1% (Jorde, 1995) and change in band intensity was not considered as polymorphism. Clear bands were scored as present (1) or absent (0) at particular position or distance migrated on the gel. The data matrix of 1's and 0's been prepared from the scorable bands and was entered into the data analysis package (Armstrong et al., 1994). The indexes of similarity were calculated across all possible pair wise comparisons of individual within and among population following the method of Nei and Li (1979). The formula was:

$$SI = 2NXY / (NX + NY)$$

NXY is the number of RAPD bands shared in common between individuals X and Y, NX and NY are the total number of bands scored in X and Y, respectively.

The index similarity was used to calculate the genetic distance values and to construct the dendrogarm. The dendrogarm provides a visual representation of the relationship of difference population of *C. aurisfelis*. The dendrograms were constructed using the Un-weighted Pair-Group Method of Arithmetic (UPGMA) employing Sequential, Agglomerative, Hierarchical, and Nested Clustering (SAHN) from NTSYS-pc program (Rohlf, 1994).

RESULTS AND DISCUSSION

Ten primers from the Operon 10 mers (Operon Kit A) (OPA 01 to OPA 10) with 60 – 70% GC content were used during the screening of the RAPD primers. The banding patterns were clear and reproducible bands selected. The primers selected are OPA 02, OPA 04 and OPA 10. These primers were selected to generate RAPD pattern of genomic DNA for all individuals of *C. aurisfelis*. The three primers (OPA 02, OPA 04 and OPA 10) were applied on five individuals of *C. aurisfelis*. The results showed different primers generated different fragment numbers and length of DNA amplification products as shown in Table 2. There were 28 fragments generated by the three primers. OPA 02 generated 9 fragments, OPA 04 generated 10 fragments (Figure 1) and OPA 10 generated 9 fragments. The size of bands ranged from 300 to 1750 bp.

In this study, the average similarity index for *C. aurisfelis* from Setiu wetland was 0.7179 to 0.8649. The similarity indices among individuals in each sample of *C. aurisfelis* are represented in Table 3. This high similarity in samples indicated low variability between individuals in

Table 3. Similarity Index of *Cassidula aurisfelis* individuals from Setiu Wetland.

Sample	1	2	3	4
1				
2	0.810811			
3	0.833333	0.800000		
4	0.864865	0.777778	0.800000	
5	0.750000	0.717949	0.736842	0.717949

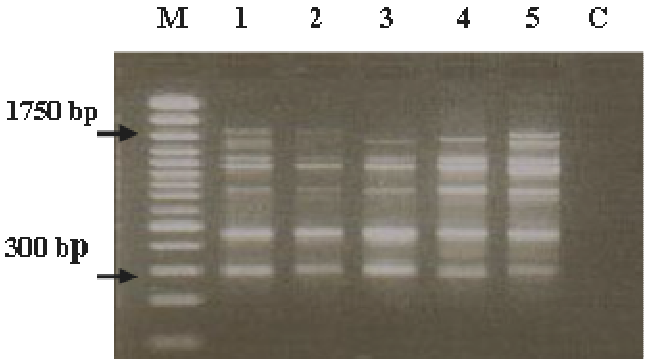

Figure 1. Banding patterns of RAPD fragments of *Cassidula aurisfelis* individuals using primer OPA 04. (Lane M is a marker 100 bp ladder plus. C is negative control. Individuals 1 to 5, left to right).

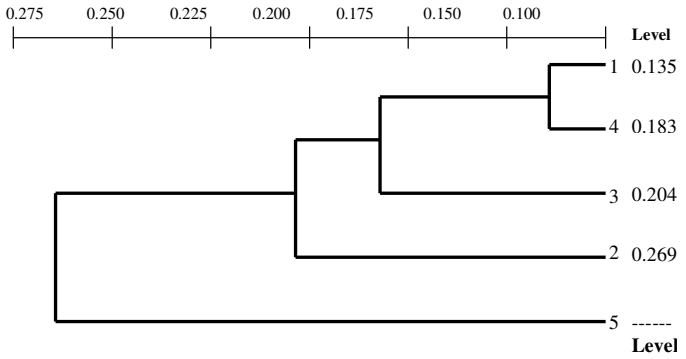

Figure 2. Dendrogram based on the genetic distance generated from Nei and Li's indices of *Cassidula aurisfelis* from Setiu Wetland. Data of RAPD generated by primer OPA 02, OPA 04 and OPA 10.

that area. The dendrogram produced is presented in Figure 2. Genetic distance levels of *C. aurisfelis* from Setiu Wetland ranged from 0.135 to 0.269. The UPGMA cluster analysis of *C. aurisfelis* based on the genetic distance generated from Nei and Li's. Small estimations of distance may indicate population substructure like subpopulations in which there is random mating, but between which there is a reduced amount of gene flow (Brent, 1996). However, small estimations of distance may also be present because the populations are completely isolated but have only been separated for a short period of time (Brent, 1996).

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