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Short Communication: Genetic diversity of *Rana* (*Pelophylax*) *ridibunda* and *Bufo* (*Pseudepidalea*) *viridis* in different populations

TAYEBEH MOSLEHI, MAJID MAHDIEH, ALIREZA SHAYESTEHFAR, SEYED MEHDI TALEBI♀
Department of Biology, Faculty of Science, Arak University, 38156-88138, Arak, Markazi, Iran. Tel.: +98-86-4173317.♥email: seyedmehdi_talebi@yahoo.com

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Abstract. *Moslehi T, Mahdieh M, Shayestehfar A, Talebi SM. 2015. Genetic diversity of* Rana (Pelophylax) ridibunda *and* Bufo (Pseudepidalea) viridis *in different populations. Biodiversitas 16: 128-131.* In present study, genetic diversity of two genus of Anura order was investigated in Iran. For this reasons, four populations of *Rana* (*Pelophylax*) *ridibunda* (R1-R4) and three populations of *Bufo* (*Pseudepidalea*) *viridis* (B1-B3) were selected from different regions of Arak province. Random Amplified Polymorphic DNA (RAPD) marker was used for molecular investigation. Nine random primers were used that only four of them were suitable and revealing different bands pattern for further analysis. A total of 69 scorable bands (loci) were found. The obtained data were analyzed using MVSP software for clustering. The studied populations were separated from each other in the UPGMA tree as well as PCA and PCO plots. In the mentioned diagrams the populations of *B. (Pseudepidalea) viridis* were closely together, while the populations of other species were placed far from each other. The results of this study showed that high genetic variations were present among *R. ridibunda* populations, but this condition was in contrast to *B. viridis* populations, where these populations were near each other.

Key words*: Bufo*, genetic variations, population, *Rana*, RAPD.

INTRODUCTION

Amphibians have important roles in food chains and are important as indicators of ecosystem health (Welsh and Ollivier 1998). The amphibians of Iran consists of 13 species and five subspecies of frogs and toads belonging to five genera of four families, in addition eight species of salamanders belonging to four genera of two families (Rastegar-Pouyani et al. 2008). Despite of worthily studies of them in Iran by foreign (Schmidt 1952; Blandford 1876; Anderson 1957; Tuck 1974; Leviton et al. 1992) and native researchers (Hezaveh et al. 2009; Rastegar-Pouyani et al. 2011: Baloutch and Kami 1995), the amphibians still needs to study systematically.

Physiological, morphological and genetically variations were seen in populations of species that occurred in different habitat, these variations were created in response to contrasting environmental conditions (Talebi et al. 2014). Random amplified polymorphic DNA (RAPD) is a multilocus technique which allows obtaining information on the general polymorphism of a genome. Low expense, high efficiency in developing a large number of DNA markers in a short time and requirement for less sophisticated equipment, the simplicity and applicability, requirement of small amount of DNA without the requirement of cloning, sequencing or any other form of the molecular characterization of the genome has made the RAPD technique valuable (Bardakci 2001; Williams et al. 1990).

There was no previous study on genetic variation of amphibians in Arak Province, therefore in this study RAPD technique was used for investigation of genetic variation in some populations of two species namely, *Rana* (*Pelophylax*) *ridibunda* and *Bufo* (*Pseuopidalea*) *viridis* in Arak Province, because these species are dominant ones with more populations in this area. In the other hand, Arak is one of the most important industrial areas that can affect on migration of amphibians to other locations.

Materials and Methods

The materials for this study consisted of a total of 47 individuals selected from 7 different populations of *Rana* (*Pelophylax*) *ridibunda* and *Bufo* (*Pseuopidalea*) *viridis* in Arak province (Table 1). Individuals were sampled by using triangular ring frame 30-mesh dip nets and manual picking of substrates with field forceps, then samples dissected in the field and the liver was removed. Genomic DNA was extracted from 100% ethanol preserved liver using a genomic DNA purification protocol (Sambrook and Russel 2001). The isolated DNA was amplified using nine primers that were bought from Sinaclon Company (Table 2). PCR reactions were performed in a volume of 25 µL containing 2.5 µL PCR reaction buffer (10x), 1.5 µL $MgCl_2$ (25 mM), 2.5 µL dNTPs (10 mM), 2 µL primer (10µM), 14 ng of genomic DNA and 0.3 µL Taq polymerase (1 unit) (Kohler et al. 2000). Amplification was done with a programmable thermal cycler (Ependorf, AG, Hamburg, Germany) under following conditions: 94°C for 1.5 min, 45 cycles of 94°C for 30 sec, 42°C for 1 min and 72°C for

2 min, followed by one cycle of 72ºC for 10 min. (Kohler et al. 2000). The amplified fragments were separated on 2% agarose gels and stained with ethidium bromide and photographed under UV light. Fragment sizes were estimated by comparison with 1 kb DNA ladder. Bands were distinguished by Labworks software. The data were used to compute the genetic variation of species. MVSP software was used for statistical analyses. A dendrogram was constructed by the Unweighted Pair Group Method of Arithmetical Average (UPGMA).

Results

In this study, the extracted DNA from four populations of *R. ridibunda* (codes R1-R4) and three populations of *B. viridis* (codes B1-B3) were amplified by using nine random primers of RAPD technique. Between the nine tested primers, we selected four that yielded clear and repeatable band patterns. Other primers either failed to amplify any fragment or only amplified a few fragments, making them inappropriate for a study on variations. These four primers provided a distinct pattern of amplified fragments. The numbers of fragments were varied among the primers. In total 69 scorable bands (loci) obtained that were varying from 10 to 21 per primer. The primer ROTH-180-08 produced the highest number of bands in comparison with the other primers. This primer amplified 21 bands with molecular weight range from 550-10000 bp. Band at 1800 bp was unique band in B2 population from Delijan area. Primer ROTH-180-02 and ROTH-180-04 produced 19 bands with molecular weight ranged from 900-10000 bp and 700-8000 bp. In addition primer ROTH-180-05 created 10 polymorphic bands with molecular weight range from 700-7900 bp (Figure 1).

The studied populations of these species were separated from each other in the PCA and PCO plots (Figures 2 and 3) as well as UPGMA tree (Figure 4). As shown in the mentioned diagrams three populations of *B. viridis* were close together while the populations of *R. ridibunda* were placed separately. These showed that the genetic similarities were present among populations of *B. viridis*. But high genetics variations were found among *R. ridibunda* populations, so that the R3 population was placed in separate clade. Species studied were different in genetic characters and separated from each other in PCA and PCO plots as well as UPGMA tree (Figures 2 to 4).

Table 1. Sampled populations from different locations of Arak province .

Pop. code	Species/ populations	Location
R1	*Rana ridibunda*	Markazi Province, north eastern of Arak
R2	*Rana ridibunda*	Markazi Province, Delijan
R3	*Rana ridibunda*	Markazi Province, Shazand
R4	*Rana ridibunda*	Markazi Province, west of Arak
B1	*Bufo viridis*	Markazi Province, north eastern of Arak
B2	*Bufo viridis*	Markazi Province, Delijan
B3	*Bufo viridis*	Markazi Province, south eastern of Arak

Table 2. Used RAPD primers with their sequences

Primers	Sequences
ROTH-180-01	5'-GCACCCGACG-3'
ROTH-180-02	5'-CGCCCAAGC-3'
ROTH-180-03	5'-CCATGGCGCC-3'
ROTH-180-04	5'-CGCCGATCC-3'
ROTH-180-05	5'-ACCCCAGCCG-3'
ROTH-180-06	5'-GCACGCCGGGA-3'
ROTH-180-08	5'-CGCCCTCAGC-3'
ROTH-180-09	5'-GCACGGTGGG-3'
ROTH-180-10	5'-CGCCCTGGTC-3'

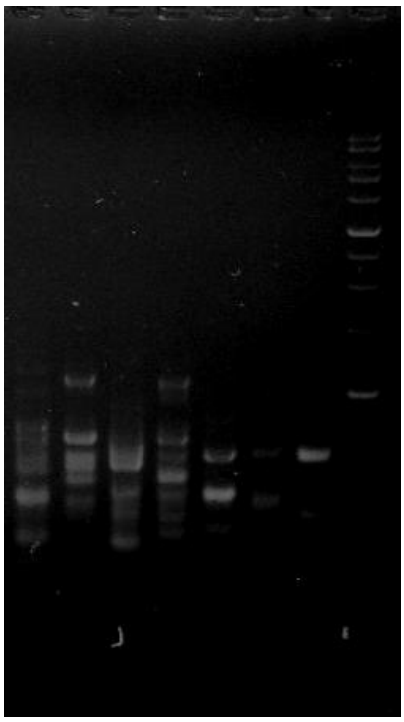

Figure 1. Band patterns on agarose gel (the right band related to ladder).

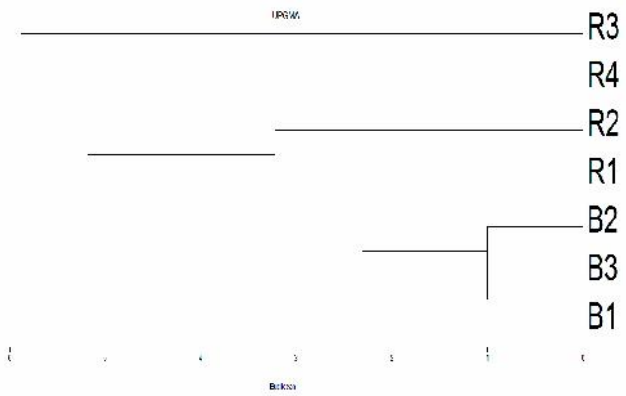

Figure 4. UPGMA tree based on the molecular data (details of symbols were given in Table 1).

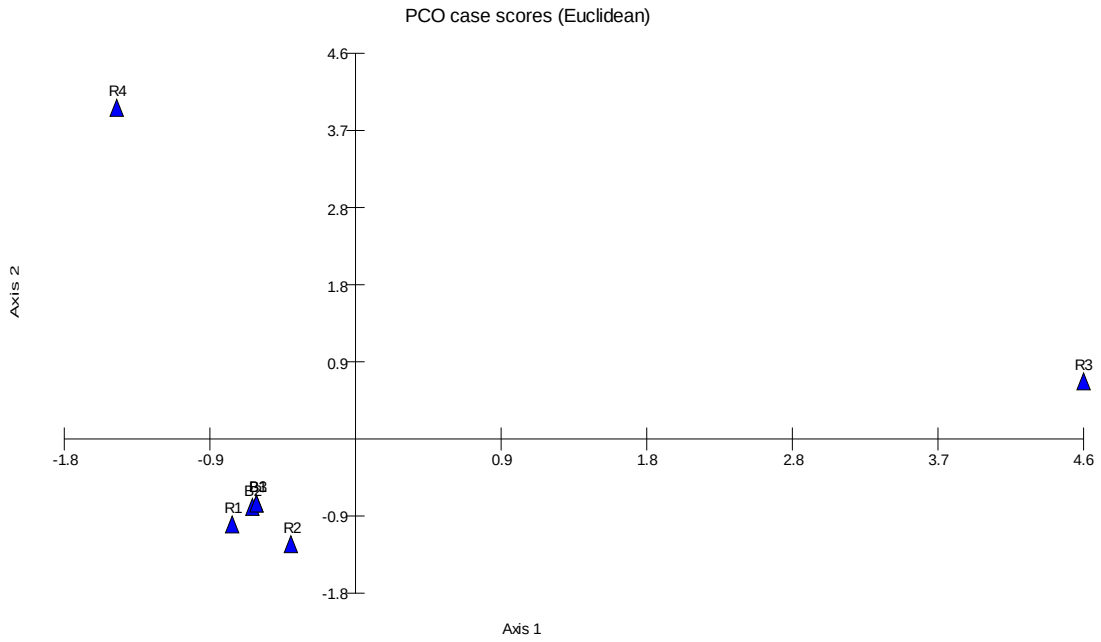

Figure 2. PCO plot of populations of *R. ridibunda* and *B. viridis* based on the RAPD data (details of symbols were given in Table 1)

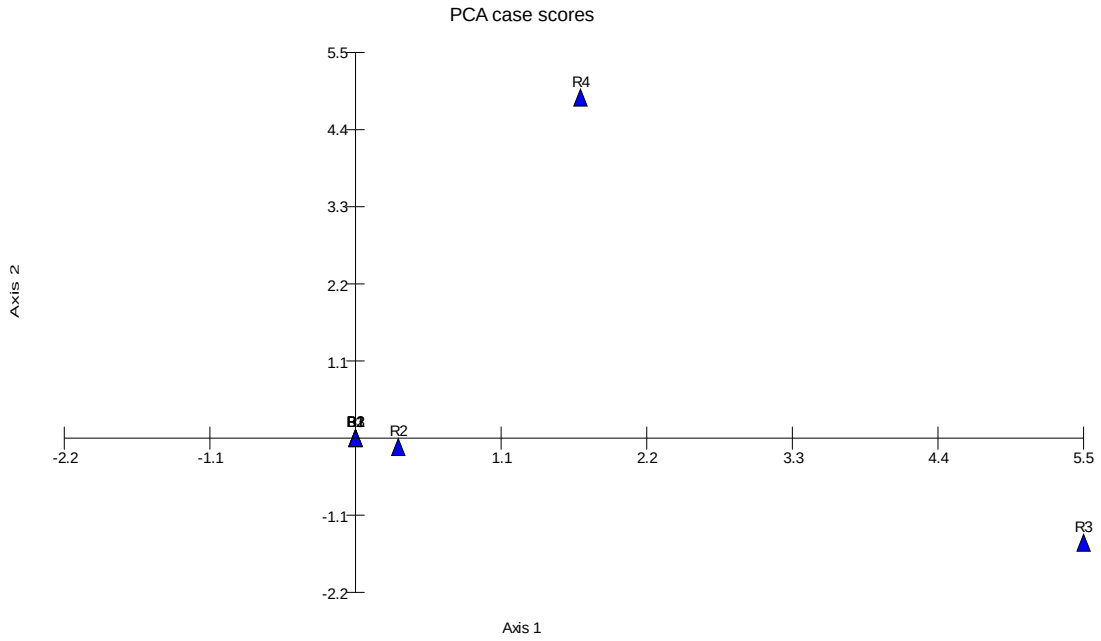

Figure 3. PCA plot of populations of *R. ridibunda* and *B. viridis* based on the RAPD data (details of symbols were given in Table 1)

Discussion

In this study the intra-specific genomic variations in four *R. ridibunda* and three *B. viridis* populations from different regions of Arak province were analyzed by using RAPD technique. The molecular technique RAPD analysis is currently used to differentiate between the genomes of the closely related species in order to determine the genetic distance and genetic diversity (Williams et al. 1990; Camargo et al. 2010). The studied populations of these species were separated from each other in the UPGMA tree as well as PCA and PCO plots. As shown in the mentioned diagrams populations of *B. viridis* were closely together while the populations of *R. ridibunda* were arranged separately.

A low genetic variability was discovered among the different *B. viridis* populations from various altitudes and located at short geographical distances and high similarity was seen between B1 and B3 populations. We believe that

the explanation for this result is the very short geographical distances (10 km) among the populations, together with the fact that all of the habitats were of the unpredictable type (rain pools), causing the toads to adapt to the wide ecological variations.

While, high genetic variations were found among *R. ridibunda* populations, so that the R3 population was placed in separate clade. R3 population from Shazand area was almost different genetically from others that may be due to presence of mountains between this area and Arak, different climate and lack of migration make it different from others populations. So it's a very important point to say that ecological and biological conditions are one of important reasons for genetic differentiation. The results of the present study are very similar to those of Degani and Kaplan (1999), who (s) studied the genetic variation of salamanders from different habitats, using RAPD. They discovered a very low genetic variation between two populations from semi-arid habitats (band sharing was 94%), and in contrast, a high genetic variation between populations from semi-arid and humid habitats (85-86% band sharing). Their results agree with the present study, in which ecological conditions affected genetic variation. Because one of the main ecological factors is altitude, which differed between populations and this factor influences other ecological factors such as wind, temperature as well as moisture. In study on *Triturus vittatus vittatus* by Pearlson and Degani (2007) also obtain similar result about relation between DNA variation and different site populations.

RAPD genetic analysis showed that there is molecular variation between different populations of these species in Arak province, because these samples clustered separately. These conditions hold true for many other species of anurans worldwide (Driscoll 1998; Baptista 2001; Marsh and Trenham 2001; Palo et al. 2004). Although local populations tend to be slightly different, this population differentiation is not strongly structured in geographic space, and the UPGMA tree indicated only a slight significant and relatively weak spatial structure at short geographic distances so it may be difficult to find a general explanation for genetic divergence among local populations across geographic space (Sokal et al. 1986). It is important to stress that even some of very close local populations might tend to be independent in respect to genetic variability. Thus, beyond micro evolutionary and ecological processes, human effects may help explaining patterns of genetic distances in this species (Telles et al. 2007).

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