

Genetic variation of Caucasian pit viper (*Gloydius halys*) populations in Iran using mtDNA marker

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Abstract

The concept of genetic variation is a vital factor to investigate the effect of evolutionary process on genus *Gloydius*' populations. Having a vast distribution in many parts of Asia, genus *Gloydius* (Serpentes: Crotalinae) is from the venomous group of snakes, and consists of thirteen species. Caucasian Pitviper (*Gloydius halys*) is scattered from Northwest to Northeast Iran in wide variety of terrestrial and mountainous, forestal and bush-lands habitats. This study aims to analyze biodiversity, genetic variation and genetic structure among the Caucasian Pitviper populations in Alborz habitats. For this purpose, 18 individuals representing two populations (northern and northeastern Alborz) were captured, and, some ventral scale specimens were collected. Polymorphism of a mitochondrial gene *Cytb*, was investigated in these samples. For this purpose, using PCR, a segment of this gene with 1067 base pair length was amplified and the amplified sequence was then subjected to sequencing. The genealogy analysis was conducted to determine the best evolutionary model using Bayesian and maximum likelihood approaches. Our results revealed that the Alborz populations were separated by 0.99 and 95.34 posterior probability and bootstrap values, respectively. Besides, 13 unique haplotypes out of 18 sequenced individuals (7 from northern and 6 from northeastern population), were analyzed and consequently a considerable distinction was detected between north and northeastern population haplotypes by 17 mutational steps. Also, a significantly

high genetic variation ($F_{st} > 0.25$, $p < 0.01$) was found between populations in the north and northeast Alborz through the Analysis of Molecular Variance (AMOVA). On the other hand, using neutrality tests, the incidence of spontaneous expansion was determined in north and northeast populations. As a conclusion, we believe that the genetic distance between these two populations is due to their local adaptability. Hence, they can be considered as two distinct conservational units in terms of biodiversity Conservational Plans. High levels of genetic diversity (mean $H-O = 0.76$) were detected and no evidence of a recent bottleneck in any sampling site could be discovered. We found that *G. halys* exhibits a low, although significant, level of genetic differentiation on a fine geographic scale (overall $F_{ST} = 0.013$). The degree of differentiation is comparably low with regard to values reported for other vipers and more similar to those in Colubridae. In addition, using local based F_{ST} estimates, we detected a significant difference in the extent of genetic drift between the centrally located and marginal local populations as predicted by the stepping-stone model of migration. Thus, from a short-term perspective, primary attention should be given to the peripheral local populations, due to limited immigration and stronger influence of genetic drift. Since all habitat patches are situated along the river and have a linear order it is important to preserve each habitat site to maintain gene flow across the meta population.