EVALUATION OF MHC GENE HETEROZYGOSITY IN ISOLATED TIGER POPULATION

Dr. S. K. Gupta

Technical Report No. 2016/010

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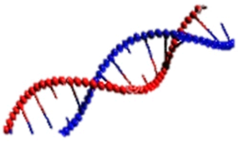

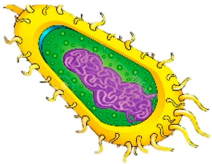

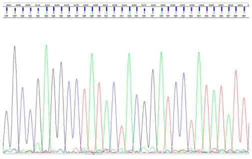

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Evaluation of MHC Gene Heterozygosity in Isolated Tiger Population

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1.	**Title of the project**	Evaluation of MHC Gene Heterozygosity in Isolated Tiger Population
2.	**Duration of the project**	4 years
3.	**Name of the investigator**	Dr. S.K. Gupta (Scientist-D), skg@wii.gov.in
4.	**Names of the research fellow**	Mr. Ajit Kumar (Senior Research Fellow)
5.	**Final budget**	₹28.87 lakhs
6.	**Name of the funding agency**	Wildlife Institute of India, Dehradun
7.	**Date of submission of final report**	May 30, 2016

8. **List of M.Sc. dissertations awarded**

Bhim Singh (2015), M.Sc. (Biotechnology) from Graphic Era University, Dehradun

9. **Publications**

Gupta SK and Kumar A (2014) Molecular identification of man-eating carnivores from scat samples. Conservation Genetics Resources, 6:271–274.

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EXECUTIVE SUMMARY

Bengal tiger (*Panthera tigris tigris*) populations in India have been exposed to various anthropogenic influences that potentially affect their genetic structure. A small and isolated population faces an uncertain future from the impact of a variety of potential threats, including climate change, human encroachment (habitat degradation) and infectious diseases. Opportunities to gather genetic data from natural populations have been enhanced by the development of non-invasive genetic techniques in the past two decades. These techniques involve the extraction of DNA from materials such as scat, shed hair and remains of dead animals.

Firstly, this project aims to standardize the protocols for successful amplification of the MHC class *I* gene from tiger scat samples. This has not been done so far. Secondly, the project aims to establish infrastructure for molecular cloning at the Wildlife Institute of India. A total of 138 samples were collected from various tiger reserves of India—the Ranthambore, Bandhavgarh, Bandipur and Wayanad tiger reserves for MHC gene standardization. The results indicated that 30–50 ng/µl DNA extracted from scats with an OD260/OD280 ratio between 1.75 and 1.90 can significantly increase the PCR success rate. A panel of nine microsatellite loci and about 250 bp of exons 2 and 3 of the MHC class *I* gene were assessed to estimate the genetic variability in these populations. The results obtained from the microsatellite loci revealed that the genetic diversity in these tiger populations are moderate. The genetic diversity of the Ranthambore (RTR) tiger population was found to be slightly lower than that of the Bandhavgarh (BTR) and Bandipur–Wayanad (BdTR) populations in terms of number of alleles as well as heterozygosity level. This might be due to the loss or weakness of connectivity of Ranthambore with the other tiger reserves. Structure analysis clearly assigned the RTR, BTR and BWTR populations to three distinct clusters.

The amplified PCR product of the MHC gene was cloned prior to sequencing to obtain unambiguous nucleotide sequences. Removal of putatively false and spurious nucleotides from the sequences provided a clear estimation of the MHC gene diversity among the tiger populations. After MHC gene was cloned, a maximum of eight alleles were identified in single individuals, suggesting the presence of at least four MHC class *I* loci. Interestingly, a high level of polymorphism (hot spot region) was observed between 179 and 240 bp of exon 2, which had a high polymorphic amino acid residue content, which is responsible for the specificity of recognition of antigens. The sequence of the exon 2 regions is more polymorphic compared with exon 3. The corresponding amino acid sequence consisted of 89 residues in the α-1 domain and 82 residues in the α-2 domain. A high level of MHC variation was found in all the tiger populations, with 30 alleles in exon 2 and 24 alleles in exon 3 from 30 and 18 individuals, respectively. No significant difference in MHC diversity was detected between the isolated population of RTR and connected tiger populations of BTR and BdTR . The MHC gene of the RTR and BTR populations exhibited almost the same level of nucleotide diversity. However, relatively high nucleotide diversity was observed in Bandipur (BdTR). Twenty polymorphic sites were observed in both the MHC α-1 (exon 2) domain and the α-2 (exon 3) domain. Such high polymorphism in the MHC gene is linked with the resistance of individuals as well as populations to disease. The high rate of non-synonymous substitutions provided clear evidence of positive selection and shaping of the genetic variation in the tiger populations. Phylogenetic analysis of the MHC gene sequences of the domestic cat, tiger, Asiatic lion and cheetah revealed trans-species polymorphism (TSP). The current investigation of MHC polymorphism indicated that the tigers of RTR, BTR and BdTR have comparable immunological fitness. This study will help long-term conservation and tiger reintroduction plans by identifying immunologically fit populations from which individuals may be selected. The results do not suggest that the small and isolated population of RTR is genetically compromised at the MHC gene locus.

Citation:

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