STUDY OF GENETIC DIVERSITY IN WILD (*SUS SCROFA CRISTATUS*) AND DOMESTIC (*SUS SCROFA DOMESTICA*) PIGS TO FIND LEVEL OF HYBRIDIZATION BETWEEN THEM IN THE VICINITY OF RANTHAMBHORE NATIONAL PARK

Final Report

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

March, 2015

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March, 2015

Pandey, P., Nigam, P., Chauhan, N.P.S., and Goyal, S.P. (2015). Study of genetic diversity in wild (*Sus scrofa cristatus*) and domestic (*Sus scrofa domestica*) pigs to find level of hybridization between them in the vicinity of Ranthambhore National Park. Final Report. Wildlife Institute of India, Dehradun, India.

CONTENTS

Executive Summary

Wild pig *(Sus scrofa cristatus)* has a wide geographical range among all ungulates and terrestrial mammals found in the Indian subcontinent and forms an important prey-base for carnivores. Despite the variation with domestic pig in chromosome numbers, these animals can mate and produce fertile hybrids that have physical attributes similar to wild pig. A systematic study on wild pigs by the Wildlife Institute of India in Ranthambhore National Park revealed that wild pigs stray out of national park, raid agricultural crops and utilises the agro-ecosystem in peripheral villages for food resource and shelter and thus coming in contact of domestic pigs. As a result, there may be genetic hybridization between the wild and domestic pig populations. Hybridization between wild and domestic pigs may lead to introgression of alien alleles that can affect the genetic fitness and overall immune response. Thus in order to detect the hybridization and to quantify the impact on wild species, genetic assessment of wild and domestic pig is necessary with ultimate goal to find out extent of hybridization if any.

Therefore, a study on genetic assessment of wild and domestic pigs and to evaluate the use and efficacy of power fence in controlling crop damage caused by wild pigs as advised by the Training, Research and Academic Council (TRAC) was undertaken from 04.02.2012 to 03.08.2014 around Ranthambhore National Park. The objectives of the study were (a) To study genetic diversity in wild and domestic pigs in the vicinity of Ranthambhore National park, (b) To find the level of hybridization between wild and domestic pigs based on genetic variability, (c) To evaluate the use and efficacy of power fence in controlling crop damage caused by wild pigs, (d) to evaluate the use and efficacy of power fence in controlling crop damage caused by wild pigs and (e) Based on the findings of the project, examine the possibility of hybridization between wild and domestic pigs in other parts of the country for further study.

In view of this, report has been in two parts i.e. Section ï I describes the genetic assessment of wild and domestic pigs where as Section II is related to use and efficacy of power fence.

We systematically collected the biological samples of unrelated domestic pigs (n=65) from different villages surrounding the Ranthambhore National Park as a zone of interaction and also from different parts of Sawai Madhopur city as a control. For the genetic characterization and detection of event of introgression in wild pigs, we proposed collection of 30-40 blood samples in the project. The habituation of animals on baits could not been successful in spite of best efforts. This was due to the delayed receipt of permission to capture animals, in appropriate weather conditions making habituation procedure difficult and unsuccessful capture. In view of this, we collected other samples of wild pigs such as hair and faecal matter to meet the objectives of the project as these samples are equally amenable

for genetic analysis but require appropriate optimization of protocols. Thus we collected blood samples (n=6) of unrelated wild pigs representing Ranthambhore National Park. Apart from blood samples, we also collected wild pigs faecal (n=26) and hair samples (n=34). For genetic diversity and detection of event of introgression, we amplified partial fragment (662 base pairs) of control region from mitochondrial genome (Asch et al., 2011) and a panel of 10 highly polymorphic microsatellite markers in domestic (n=55) and wild pig samples (n=66).

We tested the applicability non-invasive faecal and hair samples for genetic assessment and evaluation of genetic introgression. Faecal DNA was of low molecular weight with PCR success rate restricted up to 200 base pair of mitochondrial DNA. We found PCI and Qiagen kit protocol for DNA extraction better for hair DNA extraction. As reported in the literature for PCR success (40 to 80%) in using such samples, our success rate in pig samples (hair and a faecal matter) was also between 60 and 90% whereas it was possible to obtain good quality data with all blood samples. Hence, collection of adequate invasive samples at large landscape would be difficult, therefore, we suggest use of non-invasive samples i.e. faecal matter and hair in future studies. Optimized protocols undertaken in this study for using non-invasive samples would have immense advantage for undertaking future cost effective studies on wild pigs.

First time we report presence of two haplotypes with one segregation site in 560 base pair of amplified sequence of control region in eastern most population of wild pigs. Two haplotypes (WP_Hap-1 and WP_Hap-2) were shared equally i.e. 50% each in the population. The overall haplotype diversity of wild pigs was found 0.6 ± 0.13 whereas the nucleotide diversity was 0.001. In total 13 haplotypes with 24 segregation sites were recorded in 590 base pair of amplified sequences of domestic pigs. Two haplotype (Hap-1 and Hap-9) were shared by 80% of domestic pigs examined so far. Seven haplotype were detected only once (DP_Hap-3, DP_Hap-5, DP_Hap-8, DP_Hap-10, DP_Hap-11, DP_Hap-12 and DP_Hap-13) indicating their different geographic origin. The overall haplotype diversity of domestic pigs was found 0.79 ± 0.04 whereas the nucleotide diversity was 0.01.

We selected eleven microsatellite markers for genotyping purposes. Of total samples (n=66), it has been possible to generate data complete data on multi locus genotyping only for 22 and were used for introgression purposes. Out of the eleven microsatellite markers tested on domestic and wild pig samples, three loci (S0090, S0026 and SW72) deviated from HWE whereas SW72 also showed presence of null alleles in domestic pigs. Six loci (SW122, SW24, S0090, S0225, S0226 and SW911) deviated from HWE whereas none of the loci showed sign of null alleles in wild pig samples analyzed. We found overall high genetic diversity in domestics pigs (Na=14.2, Ho=0.72 and He=0.86) as compared to the analyzed wild pig samples (Na=3.2, Ho=0.7 and He=0.7).

We did not find any common haplotype between individuals of wild and domestic pigs and thus the introgression at mitochondrial level can be ruled out in the analyzed samples. We analyzed microsatellite marker data of both domestic and wild pig samples. By using Bayesian MCMC approach implemented in Structure 2.3.1. indicated admixed nature (ca.55%) of the wild pig samples. Similar results were also observed during factorial component analysis (FCA) where the wild pig individuals showed more affinity towards domestic pigs. Thus we report first time the presence of hybrid wild pig individuals (wild at mitochondrial level and domestic at nuclear level) in RTR. Therefore, absence of mitochondrial genetic introgression and presence of nuclear genetic introgression suggests unidirectional hybridization.

Section II, describes the use and efficacy of power fence which was evaluated in controlling crop damage caused by wild pigs. Traditionally, wild pigs have been kept out of cultivations by scaring them away or restricting them with barriers. Scaring wild pigs with flash lights, fire, fire-crackers, crop protection guns, stone slings etc may effectively deter them sometimes. Most forms of effective barriers for wildlife such as trenches, rubble wall or conventional fences, are expensive to construct and maintain.

A power fence is purely a psychological barrier. Power fence is a relatively new control technique and not fatal for animals and only restricts their movements. Power fencing is most effective and safe to animals and to humans. If properly constructed and maintained, it can effectively keep most of the animals out. Power fencing system provides an economic and a practical solution to achieve maximum protection through effective control of animal trespass. In this study, we developed the pig-proof power fences by construction of fence line around the crop fields in Jaitpur village situated on the boundary of Ranthambhore National Park, and evaluated their efficacy in reducing crop damage. We compared the extent of wild pig crop-raiding in the fenced and adjacent unfenced areas.

The power unit had a solar panel, a 12 volt power battery, and an energizer to provide current to 1.5 km length of the two fences. We erected the electric fence as per the designs specified in June 2006 under different project when the fields were prepared for sowing crops. A fence design with posts at an interval of 8 meters and GI wires at the height 15, 37.5, 62.5, 100 and 135 cm were tested. Another design with posts at an interval of 8 meters and GI wires at the height 15, 37.5, 62.5, 100, 135, 165,195 and 225 cm was also constructed. The pig-proof fence was 4.5 feet in height with five strands; the first and third strands were connected to earthing, and the rest three i.e. 2^{nd}, 4^{th} and 5^{th} strands were live strands. The pig and nilgai-proof fence line was constructed along the forest boundary and crop fields, and it was 8.5 feet in height with 8 strands. The 1^{st}, 3^{rd} and 5^{th} strands were connected to earthing system, and the rest five strands i.e. 2^{nd}, 4^{th}, 6^{th}, 7^{th} and 8^{th} were live wires. The fences were monitored regularly by walking along the perimeter to evaluate their effectiveness and we discuss our

findings . Both the fences were maintained properly. We recorded high range of voltage (7.9-8.1 KV) at the energizer point. Average output voltage of the main fence i.e. nilgai-pig proof fence ranged from 5.5 to 6.5 KV. The voltage of small pig-proof fence was slightly on the lower side i.e. 5.2 to 6 KV. The voltage at 1000m fence length was higher than at shorter distances, which might be due to good earthing system. Overall the fences were found effective against pigs and nilgai.

We conclude that the present study support the ecological finding (of previous project of WII) about genetic introgression between wild and domestic pigs around Ranthambhore National Park. This study provides valuable information on the genetic structure of indigenous wild pig which would be useful for future conservation program. Wild pig in RTR is in a vulnerable state as a distinct genetic wild resource and we suggest for appropriate measures to be undertaken to minimize the contact zone between wild and domestic pigs in and around RTR by using appropriate physical barriers as designed and tested for its efficacy. This may enable to restore genetic diversity of wild pigs after few generation through back crossing with wild pigs. We also suggest there is a need to re-visit the study in RTR with using optimized protocols to document spatial distribution of hybrids using NGS (hair and fecal matter) for developing appropriate strategies of wild pig genetic resource adapted to hot climatic conditions (Estimated expenditure would be Rs.6.0 lakhs/year). In view of valuable wild pig genetic resource for human being, there is a need to assess extent of introgression from domestic to wild pig populations of different bioclimatic zones. We also suggest for use of both mtDNA and nuclear markers which avoids inheritance bias because they detect information on both the maternally and codominatly inherited regions.