Conservation of Red Junglefowl (Gallus gallus) in India

Technical Report · January 2012

CITATION
1

READS
6,602

5 authors, including:

Sathyakumar Sambandam
Wildlife Institute of India
236 PUBLICATIONS **1,587** CITATIONS
SEE PROFILE

Merwyn Fernandes
Manipal Academy of Higher Education
60 PUBLICATIONS **460** CITATIONS
SEE PROFILE

Mukesh Thakur
Zoological Survey of India
134 PUBLICATIONS **671** CITATIONS
SEE PROFILE

Rahul Kaul
Wildlife Trust of India
63 PUBLICATIONS **375** CITATIONS
SEE PROFILE

Conservation of Red Junglefowl (*Gallus gallus*) in India

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

i.	Title of the project	: Conservation of Red Junglefowl (Gallus gallus) in India
ii.	Duration of the project	: September2006 – August,31 2011
iii.	Name of the investigator(s)	: S. Sathyakumar, Scientist – F Principal Investigator Rahul Kaul, Wildlife Trust of India, Noida & Rajiv S. Kalsi, MLN College, Yamuna Nagar (Co-Investigators)
iv.	Name of the research fellow(s)	: Mr. Merwyn Fernandes, SRF Mr. Mukesh, SRF
v.	Total cost of the project	: Rs. 48.99 lakhs
vi.	Name of the funding agency	: Grant-in-Aid
vii.	Name of the collaborator(s)	: Central Avian Research Institute, Bareilly, Uttar Pradesh (Dr. Deepak Sharma) National Bureau of Animal Genetic Resource, Karnal, Haryana (Dr. Ramesh S. Vijh)
viii.	Date of submission of final report	: March 2012
ix.	List of MSc. Dissertations awarded	:

Dohling , L.M. 2009. Status, Distribution and Relative abundance of Galliformes in Nongkhyllem Wildlife Sanctuary, Meghalaya. M.Sc. dissertation. FRI University, Dehradun. 46 pp.

Kesiezie, K. 2009. Status, Distribution and Relative Abundance of Galliformes in Khonoma Nature Conservation and Tragopan Sanctuary, Nagaland. M.Sc. dissertation. FRI University, Dehradun.

x. List of Ph.D. thesis awarded/ submitted /under submission. :

Mukesh, 2012. Molecular genetic diversity of Red Junglefowl (*Gallus gallus*) in Northern India and its admixture with domestic chicken. Ph.D. Thesis, Kurukshetra University, Kurkushetra, Haryana.

Fernandes, M. (in prep.). Spatial and temporal pattern in occupancy and abundance of Red Junglefowl (*Gallus gallus*) in Western Shivalik Landscape, India. Ph.D Thesis, Saurashtra University, Rajkot, Gujarat.

xi. Copy of publications appended :

2009 Merwyn Fernandes, Mukesh, S. Sathyakumar, Rahul Kaul, Rajiv Kalsi and Deepak Sharma. 2009. Conservation of Red Junglefowl in India. **International Journal for Galliformes Conservation** 1: 94-101.

2010. Mukesh, Merwyn Fernandes, R.P. Mandhan and S. Sathyakumar. 2010. Comparison of different parameters of invasive and non invasive sampling methods for microsatellite genotyping: a case study from Red Junglefowl and its application for other Galliformes. **Journal of Research in Biology** 1: 38-43.

2011. Mukesh, Kalsi, R.S., Mandhan, R.P. and Sathyakumar, S., 2011 Genetic Diversity Studies of Red Junglefowl Across its distribution Range in Northern India. **Asian Journal of Biotechnology**. ISSN 1996-0700 / DOI: 10.3923/ajbkr.2011. Published online, January 2011

Dohling,L.M. and S. Sathyakumar 2011. Relative abundance of galliformes in Nonkhyllem Wildlife Sanctuary, Meghalaya. NeBio 2: 4-8.

Mukesh, Javed, R., Gaur, U., Jianlin, H., and Sathyakumar, S. 2011. Cross-species applicability of chicken microsatellite markers for investigation of genetic diversity in Indian duck (*Anas* platyrhynchos) populations. African Journal of Biotechnology, 10 (76), 17623-17631. Available online: http://www.academicjournals.org/AJB/PDF/pdf2011/30Nov/Mukesh%20et%20al.pdf

Mukesh and Sathyakumar, S. 2011. Eighteen polymorphic microsatellites for domestic pigeon Columba livia var. domestica developed by cross species amplification of chicken markers. **Journal of Genetics**, 90, 86–89.http://www.ias.ac.in/jgenet/OnlineResources/90/e86.pdf

Mukesh, Rai, I.D., Mandhan, R.P. and Sathyakumar, S., 2011. A Panel of polymorphic microsatellite markers in Himalayan Monal *Lophophorus impejanus* developed by cross-species amplification and their applicability in other Galliformes. **European Journal of Wildlife Research.** DOI 10.1007/s10344-011-0494-1. Springer Verlag. Published online on 18 January 2011.

Gene Sequences submitted:

1. Thakur, M., Singh, S.K., Shukla, M., Goyal, S.P. and Sathyakumar, S. JQ796700 (2012). Nucleotide sequence submitted in NCBI data base. Direct submission. *Francolinus pondicerianus* 12S ribosomal RNA gene. GenBank: JQ796700.
2. Thakur, M., Singh, S.K., Shukla, M., Goyal, S.P. and Sathyakumar, S. JQ796701 (2012). Nucleotide sequence submitted in NCBI data base. Direct submission. *Ithaginis cruentus* 12S ribosomal RNA gene. GenBank: JQ796701
3. Thakur, M., Singh, S.K., Shukla, M., Goyal, S.P. and Sathyakumar, S. JQ796702 (2012). Nucleotide sequence submitted in NCBI data base. Direct submission. *Lophura nycthemera* 12S ribosomal RNA gene. GenBank: JQ796702
4. Thakur, M., Singh, S.K., Shukla, M., Goyal, S.P. and Sathyakumar, S. JQ796703 (2012). Nucleotide sequence submitted in NCBI data base. Direct submission. *Pavo cristatus* 12S ribosomal RNA gene. GenBank: JQ796703
5. Thakur, M., Singh, S.K., Shukla, M., Goyal, S.P. and Sathyakumar, S. JQ796704 (2012). Nucleotide sequence submitted in NCBI data base. Direct submission. *Francolinus pondicerianus* cytochrome b (CYTB) gene. GenBank: JQ796704
6. Thakur, M., Singh, S.K., Shukla, M., Goyal, S.P. and Sathyakumar, S. JQ796705 (2012). Nucleotide sequence submitted in NCBI data base. Direct submission. *Lophophorus impejanus* cytochrome b (CYTB) gene. GenBank: JQ796705
7. Thakur, M., Singh, S.K., Shukla, M., Goyal, S.P. and Sathyakumar, S. JQ796706 (2012). Nucleotide sequence submitted in NCBI data base. Direct submission. *Pavo cristatus* cytochrome b (CYTB) gene. GenBank: JQ796706
8. Thakur, M., Singh, S.K., Shukla, M., Goyal, S.P. and Sathyakumar, S. JQ796707 (2012). Nucleotide sequence submitted in NCBI data base. Direct submission. *Pavo muticus imperator* cytochrome b-like (CYTB) gene. GenBank: JQ796707

Seminars

International:

1. Fernandes Merwyn., Mukesh, Sathyakumar, S., Kaul, Kalsi R.S. and Sharma D. **"**Conservation of Red Junglefowl (*Gallus gallus*) in India- A Research Initiative"- presented in ***4th International Galliformes Symposium*** organized by World Pheasants Association (WPA) held at Chengdu, Sichuan, China, **14- 21 October, 2007**.

2. Mukesh, M. Fernandes, S. Sathyakumar, R. Kaul, R.S.Kalsi, R. K. Vijh and R.P.Mandhan. "Discrimination of Red Junglefowl (Gallus gallus) and Domestic chicken (Gallus domesticus) using morphological traits and STR markers and its application in wildlife forensic"- presented in International Symposium on Emerging Trends in Forensic Science organized by Department of Forensic Science, Punjabi University, Patiala, **22-24 February, 2010.**
3. Mukesh, Merwyn Fernandes, S. Sathyakumar, R.S.Kalsi, Rahul Kaul and R.P. Mandhan."Evaluation of genetic diversity and admixture analysis of Red junglefowl with domestic chicken in India: Preliminary findings"- presented in 5th International Galliformes Symposium organized by World Pheasants Association (WPA) held at Chiang Mai, Thailand, **7-14 November 2010.**
4. Mukesh, S. Sathyakumar, R.S.Kalsi, R. K. Vijh, Merwyn Fernandes and R.P.Mandhan. "Assessment of genetic diversity of Red Junglefowl (Gallus gallus) population in Himachal Pradesh, India"- poster presented in 5th International Galliformes Symposium organized by World Pheasants Association (WPA) held at Chiang Mai, Thailand, **7-14 November 2010**.
5. Merwyn Fernandes, Mukesh, S. Sathyakumar, K. Ramesh. "Ecogeographical determinants of range limit and distribution pattern of Red Junglefowl and Grey Junglefowl in India"- presented in 5th International Galliformes Symposium organized by World Pheasants Association (WPA) held at Chiang Mai, Thailand, **7-14 November 2010.**
6. Mukesh, M. Fernandes, Rajiv S.Kalsi, Rahul Kaul, and S. Sathyakumar. Genetic characterization of Red junglefowl (Gallus gallus) in India. Abstract accepted in 100th Annual Meeting of PSA (Poultry Science Association) to be held in St. Louis, Missouri, USA during **July16-19, 2011.**
7. Mukesh, Merwyn Fernandes, S.Sathyakumar, R.Kaul and R.S. Kalsi. Purity assessment of captive Red Junglefowl Gallus gallus populations using morphological traits and microsatellite markers. International conference on Indian Ornithology (ICIO) **2011**. Organised by SACON held at Coimbatore, Thailand, **19-23 November, 2011.**

Book Chapter:

Fernandes, M, Mukesh, Sathyakumar, S., Kaul, R., Kalsi, R.S., and Sharma, D. 2007. Conservation of Red Junglefowl (*Gallus gallus*) in India: Towards mapping abundance and genetic diversity (*in press*). (IN) Sathyakumar, S & Sivakumar, K. (Eds.) **Galliformes of India**. **ENVIS Bulletin**. Wildlife and Protected Areas: Vol.10 (1). Wildlife Institute of India, Dehradun. Pp. 61-68.

Conservation of Red Junglefowl *Gallus gallus* in India

Merwyn Fernandes and **Mukesh**
Senior Research Fellow
Wildlife Institute of India, Dehradun, Uttarakhand

S. Sathyakumar
Principal Investigator
Wildlife Institute of India, Dehradun, Uttarakhand

Rahul Kaul
Co - Investigator
Wildlife Trust of India, Noida, Uttar Pradesh

Rajiv S.Kalsi
Co - Investigator
M.L.N. College, Yamuna Nagar, Haryana

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

March 2012

Collaborating agencies:
Central Avian Research Institute, Bareilly, Uttar Pradesh (Dr. Deepak Sharma)
National Bureau of Animal Genetic Resources, Karnal, Haryana (Dr. Ramesh K. Vijh)

Published by:
Wildlife Institute of India
P.O. Box # 18.
Chandrabani
Dehradun 248001
Uttarakhand, India
Tel: +91-135-2640111-115
Fax: +91-135-2640117
Website: http://www.wii.gov.in

Citation:
Sathyakumar, S., Fernandes, Merwyn., Mukesh., Kaul, R. and Kalsi, R.S., 2012. Conservation of Red Junglefowl *Gallus gallus* in India. Final Project Report. Wildlife Institute of India, Dehradun. 98 Pp.

Layout and design:
Virendra Sharma
Wildlife Institute of India

Photo Credits:
Dhritiman Mukherjee (Front cover)
Atul Dhamankar (Back cover)

Printing:
M.S. Printers and creators**,**
93, Subhash Road
Dehradun 248001
Email: mspc2007@gmail.com

CONTENTS

ACKNOWLEDGEMENTS

We would like to express our gratitude and appreciation to the under mentioned individuals and institutions for their expert guidance, advice and assistance for the timely and successful completion of this project.

- The Chairman and members of the Training, Research, Academic Council (TRAC), Wildlife Institute of India (WII) for granting approval to this project.

- At WII, we thank Shri P.R. Sinha, Director, Dr. V. B. Mathur, Dean and Dr. K. Sankar, Research Coordinator for providing encouragement and support. Dr S.P. Goyal, Faculty-in-Charge, Wildlife Forensic Cell and Shri Qamar Qureshi Faculty- in-Charge, IT and RS and GIS cell for providing the necessary facilities and cooperation.

- The State Government and Chief Wildlife Warden - Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Haryana, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Odisha, Punjab, Sikkim, Tripura, Uttarakhand, Uttar Pradesh and West Bengal for granting permission. The Park Directors, Wildlife/Forest Managers, Zoo Directors, Range Officers and Field Staff in the above mentioned RJF range States for providing the necessary help and cooperation during the study.

- At WII, Shri B. C. Choudhury, Dr. Y. V. Jhala, Shri Pratap Singh, Dr. Dhananjay Mohan, Shri N K Vasu, Dr. Bivash Pandav, Dr. K. Sivakumar, Dr. K. Ramesh and Research Associates, SRFs, JRFs, and Researchers of the 'All India Tiger Monitoring Program' and M.Sc. students for providing information on the species and assistance during the field surveys.

- Shri S.K. Mukherjee, former Director, WII, Director Wildlife Trust of India, Dr Ramesh Kumar, MLN College, Dr. R.P. Singh, Director Central Avian Research Institute, Dr B.K. Joshi, Director National Bureau of Animal Genetic Resources, Dr. R.D. Jakati, Director, Indira Gandhi National Forest Academy, Dr. Asad R. Rahmani, Director, Bombay Natural History Society for their support in various ways

- Dr. M.C. Sathyanarayan, Dr. Sushil Dutta, Mrs. Sonali Ghosh, Dr. Kashmira Kakati, Dr. Christy Willams, Shri Manoj Nair, Dr. R. Jayapal, Dr. Rashid Raza, Dr. Advait Edgaonkar, Dr. Dilip Chetri (Primate Conservation Centre), Dr. Bibhuti P. Lahkar, Mr. Udayan Borthakur, Dr. Feroz Ahmed and other resource people at Aaranyak, Mr. Partyush, Mr. Rishi Kumar Sharma (NCF), Mr. Joseph Vattakaven (WWF) for their help during field work.

- Dr. Peter J. Garson, Dr. Philip McGowan, Dr. Han Jianlin, Dr Stephen Weigend, Mr. John Corder for discussions and photographs

❖ Mr. Ved Prakash Ola, Mrs. Nilanjana Roy, Ms Swati Saini and the staff of IT and RS and GIS cell and Forensic Cell, Library and other sections at WII.

❖ Dr. Madhu Sudan Tantia, Dr. Uma Gaur, Dr. Ruheena Javed, Ms. Jyoti and Ms. Priyanka are thanked for their help during genetic analysis at National Bureau of Animal Genetic Resources Karnal, Haryana.

❖ Project Managers, Researchers and Staff at Wildlife Trust of India and staff at MLN College, Yamuna Nagar.

❖ This study would be incomplete without the strenuous efforts put in by the Bird trappers, Field Assistants and local villagers in the various study sites pan India.

Authors

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Conservation of Red Junglefowl *Gallus gallus* in India

Executive Summary

The Red Junglefowl (RJF) is believed to be the wild ancestor of all domestic chicken in the world. there still exist a strong ethno-cultural bond where the wild males are used to invigorate the domestic stock in order to enhance the first generation individuals that are used in the context of cultural and religious relevance. Concerns were raised on the genetic endangerment of RJF due to introgression of domestic genes into the wild population. There needs to address these concerns and maintain uncontaminated RJF population in wild and captivity. keeping this in view, the Wildlife Institute of India, carried out a research project from 2006 to 2011 in two phases that dealt with status, distribution, genetic diversity, interactions between wild RJF and domestic chicken and introgression of domestic genes into the wild and captive stocks.

The RJF listed in the "Least Concern" category of IUCN with an extent of occurrence of about 5,100,000 km^2. One of the subspecies *G g murghi* has its distribution within India. In order to address the issues of status and distribution we resorted to using presence-only models. These models overcome the cost and time constraints when dealing with a large ranging species. Species site locations were all collated by using primary field data, network of field biologist, literature records, museum specimens and archived databases. A total of 500 geo-rectified data points were used along with predictable variables such as bioclimatic factors, digital elevation model and forest cover. These variables were used to run maximum entropy models using the product function, the test data has an AUC score of 0.979, the jackknife test for variable importance was annual precipitation and precipitation of the driest quarter that contributed 46% to the model. The total predicted probability suitable area in India is approx 354,978 km2. There are three distinct landscapes within India namely north (12%), central (52%) and northeastern (36%).The central landscape is isolated and does not connect either to the north or northeastern landscape. The north and northeastern landscape is connected to each other through the forest patches in Bhutan and Nepal. The

PA network accounts for nearly 13% of the area with the National Parks (34) representing 4.32% and the Wildlife Sanctuaries (135) representing 8.52%, while nearly 90% of the area lies outside the purview of the PA network system. The species is still reported from 205 districts out of the 270 districts in range 21 states.

Genetic diversity, population differentiation and phylogenetic analysis of RJF populations were assessed in 19 RJF range states of India. In total, 385 samples (306 RJF & 79 domestic chickens) were collected and genotyped with 26 microsatellite markers. Altogether, 628 alleles were observed across five RJF and one domestic chicken population. Observed and effective number of alleles ranged from 9 to 49 and 2.96 to 12.40 with mean (± s.e.) number of alleles 24.15 (± 8.31) and 6.50 (± 2.71), respectively. Effective number of alleles was less than the observed number of alleles for all the loci. The overall observed heterozygosity ranged from 0.23 and 0.79, with mean value of 0.52 ± 0.13, while expected heterozygosity ranged 0.62 to 0.92 with mean value of 0.82 ± 0.08. PIC value ranged from 0.56 to 0.91 with mean value 0.80 (±0.09) and therefore all microsatellite markers were informative in the present study. Mean observed number of alleles & mean observed heterozygosity was highest in Northern RJF population, *i.e.* N_a 21.12 ±7.14 & Ho 0.61 ±0.17 and lowest in central RJF population, *i.e.* N_a 1.92 ±0.89 & Ho 0.35 ±0.42, respectively. Total number of private alleles ranged from 1 to 179 in South-Eastern and Northern RJF population, respectively while no private was found in Central RJF population. The analysis of molecular variance (AMOVA) revealed a total of 6% variation was attributed to among populations while 94% variance was within population. The minimum population differentiation or maximum gene flow was between Northern and Eastern RJF population (Nm 10.846) while maximum population differentiation or minimum gene flow was between Central and Eastern RJF population (Nm 0.911). The overall, Nm values were quite high, suggesting the high gene flow among RJF populations. Nei's genetic distance indicated that the Central Indian RJF population is least similar or most distant (D_A= 0.942) with domestic chicken, while the northeastern RJF population is most identical or least genetically distant (D_A = 0.255) with domestic chicken. The UPGMA dendrogram was generated based on Nei's genetic distance. The RJF populations in India formed three clusters: (i) central and southeastern, (ii) northern

and eastern, and (iii) northeastern and domestic chicken. The multi-factorial correspondence analysis also revealed the similar pattern of clustering the RJF populations.

In order to study interactions, observation were recorded from 13 sites with mixed groups all observations were in the pre-dawn hours. A total of 51 encounters were recorded. The interest was to elucidate whether an interaction between the wild and domestics fowls was mutualistic or agnostic during the breeding and nonbreeding season. From the 10 observation recorded during the breeding season there were no interaction between the wild and feral population suggesting that there might be a spatial segregation between these two populations. While interactions during the nonbreeding season suggest that that males are intolerable to each other when in close proximity, while the females are tolerated and move about freely within the groups.

Genetic characterisation and maintaining studbooks is the key step towards formulating management action plan for conservation breeding or release program for any captive species. We collected 220 RJF samples (blood/feathers) from 14 captive centers and investigated population genetic structure and admixture analysis of RJF with domestic chicken using 23 highly polymorphic microsatellite markers. Bayesian clustering analysis revealed three distinct groups that indicated the genetic integrity among the birds of 14 centers. We presumed genetic integrity would have been resulted due to exchange of birds between zoos or the founders would have been introduced from the same wild population. The global performance of STRUCTURE assigning individuals was 169/220=76.81% while 8.63% individuals remained unassigned to any of three clusters. Each RJF stock was independently investigated for admixture analysis with a pooled domestic chicken population and ten birds were found to be hybrids out of 220 birds collected from 14 captive centers.

based on the study, we recommend the following

- As this study could not survey all areas within RJF's distribution range, we suggest that there is a need to increase efforts to understand whether the species is prevalent within forested tracts outside the PA network, especially Bihar, Haryana,

Punjab, Sikkim and Uttar Pradesh where the present distribution is highly fragmented with growing pressures on the existing PA of these States.

- Similarly, in the States of Andhra Pradesh, Jammu & Kashmir and Maharashtra, extensive field surveys should be carried out to ascertain the presence/absence and exact distribution limits of RJF as these States encompass the limits or edges of the distribution range of this species.
- Special focus surveys/studies are required at range overlaps between *G.g. murghi* and *G.g. spadiceus* (northeastern States) and also between RJF and Grey Junglefowl (central India).
- Based on our samples collected from zoos/captive centres (Table 5.1), admixed birdwere identified (Table 5.4). These admixed individuals (hybrids between RJF and domestic chicken) that are kept in zoos/captive centres should be removed from these captive stocks to avoid any further hybridisation. They should not be exchanged with any other zoos/captive centres and should not be released back into the wild. The list of individual birds in the zoos/captive centres that have been identified as 'not admixed' have been provided to these centres.
- For RJF individuals in zoos/captive centres that were not sampled during the study or born or added after the sampling, similar genetic analysis should be carried out. Such individuals should not be used /exchanged for any breeding programme.
- As there are chances of silent breeding between RJF and domestic chicken, hence the use of domestic hens as foster parents should be avoided.

1.0 INTRODUCTION

"The argument that a species is in no danger, because it is very common is a complete fallacy…"

Charles S. Elton

1.1. INTRODUCTION

Resilience and dominance of the human race has allowed it to colonise and modify most of the habitable land areas. It is this mobility and the induced effects that have synergistically brought about habitat destruction, fragmentation and species introduction and have been reflected as the three key drivers of extinction (Rhymer and Simberloff 1996). In order to comprehend the effects of human civilisation, one of the most critical aspects is the transition of a hunter-gatherer to herder-farmer form of life (Zelder 2006a). This transition and exercise of dominion over other species is being singled out as the most consequential event in the history of human kind (Diamond 2002). It is this strong selective mutualistic relationship that has been imbibed and its evolution through a partnership that has shaped the biology of the species and the cultural context of humans (Helback 1959). The degree of flexibility (genetic plasticity) of the species to adapt physiologically and behaviourally to new selective pressures induced by human kind, plays a central role in the process called domestication (Zelder 2006b).

A large number of species have been domesticated into different breeds or varieties, primarily for food and/or aesthetics. Darwin (1868) had suggested that there are changes in the morphological and physiological appearance in relation to the wild counterparts. The close proximity of feral/domestic breed and the effective gene flow to and from the wild counterparts has raised concerns in regards to the purity, possibly loss of the progenitor (Hanotte and Jianlin 2005) and the effect of hybridised individuals in the system (Randi 2008). But, natural hybridisation *per se* has been seen as an important driver in the evolution of species (Stebbins 1950, Grant and Grant 1992, Dowling and Secor 1997, Gardner 1997) while anthropogenic hybridisation a result of forced intervention is of concern. It is imperative that we conserve or identify the non-hybridised individuals, however, this would largely be impossible due to hybrid swamps and to tease out whether the 5% or less proportion of hybridisation is an effect of admixture or natural (Allendorf et al. 2001). The last two concerns have been highlighted in this species (Brisbin 1995, Brisbin et al. 2002 and Brisbin and Peterson 2007) and it is imperative that there needs to be an investigation within India as the species has not been evaluated.

The word 'pheasants' are referred to those members of subfamily Phasianinae Horsfield (1821), that are characterised by greater sexual dichromatism, dimorphism, and largely possess chicken-like morphological and behavioural traits (Delacour 1977). They are large, ground dwelling birds with brightly coloured plumage and represent the family

Phasianidae of the order Galliformes. Johnsgard (1986) hypothesised that the pheasants that exist today evolved from generalised partridge-like ancestors and that early radiation of the partridge and pheasant lineages perhaps occurred in Southeast Asia. There are 67 species (including subspecies) belonging to 20 genera of pheasants in the world (Madge and McGowan 2002), except for the Congo peafowl *Afropavo congensis*, the rest are Asian in origin (Delacour 1977). Currently, 34 species of family Phasianidae are listed in the IUCN criteria developed by IUCN/Species Survival Commission/Pheasant Specialist Group (McGowan and Garson 1995, Fuller and Garson 2000). Junglefowls are members of the family phasianidae, medium sized tropical birds that are highly dimorphic where the males have a fleshy erect comb on the crown hence the name '*Gallus*'.

1.1.1. The Junglefowl Genus: *Gallus*

The genus *Gallus* is represented by four species namely Green Junglefowl *Gallus varius*, Grey Junglefowl *Gallus sonneratii*, Ceylon Junglefowl *Gallus lafayettii* and Red Junglefowl *Gallus gallus* confined to the Indomalayan sub-region of which the latter three occur within the Indian subcontinent, whereas the Green Junglefowl is restricted to Java and the neighbouring island eastwards (Fig 1.1a). Out of these four species, three species *viz.* green, grey and ceylon are endemic and have restricted distribution, whereas the RJF is widely distributed, having distinct clinal intergradation between five recognised subspecies. These subspecies differ chiefly in colour and shape of the neck hackles in male and size and colour of the facial lappet. The five subspecies that are recognised are Cochin-Chinese Red Junglefowl, Burmese Red Junglefowl, Javan Red Junglefowl, Indian Red Junglefowl and Tonkinese Red Junglefowl. The distribution of these five subspecies is given below in Fig 1.1b.

1. Cochin-Chinese Red Junglefowl *G.g gallus* (Linnaeus, 1758) occurs in Cambodia, C and S Vietnam, C and S Laos and E Thailand. The neck hackles of cock are long and golden orange to bright red and ear lappets large and white.
2. Burmese Red Junglefowl *G.g spadiceus* (Bonnatere, 1791) occurs in SW Yunnan (China) and northeastern parts of Arunachal Pradesh, Myanmar, Thailand, peninsular Malaysia and N Sumatra. In comparison to *G.g. gallus*, the neck hackles are golden yellow, comb and facial lappet red and all are short.
3. Javan Red Junglefowl *G.g. bankiva* (Temminck, 1813) occurs in S. Sumatra, Java and Bali. The neck hackles are rounded, golden-yellow and even shorter.
4. Indian Red Junglefowl *G.g. murghi* (Robinson and Kloss, 1920) occurs in India, Nepal, Bhutan and Bangladesh. The neck hackles are yellow, longer with a black shaft streaks, ear lappets pinkish and small, hen is paler than other race.

5. Tonkinese Red Junglefowl *G.g. jabouillei* (Delacour and Kinnear, 1928) occurs in N. Vietnam, SE Yunnan and Hainan. These are darker and redder than *G.g. gallus*. Their hackles are short, less pointed and golden yellow. Facial lappet and comb are short and all red.

1.1.2. Red Junglefowl *Gallus Gallus*

The Red Junglefowl (RJF) males are more conspicuous than the female, by presence of characteristic red comb, wattle, golden yellow hackles with a prominent black streak, blackish coloured breast, two long metallic green sickle shaped tail feathers with a white patch present at the rump. The female is slender, thin and smaller in size (41-46 cm) than compared to the male (65-78 cm). The female has elongated golden-buff, black centred, feathers across the nape and mantle with rudimentary or no comb. The rest of the upperparts are rufous brown, finely vermiculated with black and has rufous brown under parts streaked with buff. The males and females have reddish to pale white ear lappets colour, blackish brown, slender, smooth and thin legs with a spur present in the males. The first year male is superficially similar to adult male, but much duller and hackles less developed. The chicks are precocial when born, and distinguished from the domestic chicks by presence of blackish-brown eye stripe (Ali and Ripley 1983). They prefer well watered areas around nullahs, streams and in moist mixed forests below 3,000m and have been recorded in mangrove forests and scrub jungles with patches of cultivation (Madge and McGowan 2002).

They are generally found in small groups or parties, with one cock to several hens. They are very shy, secretive birds that scurry into cover on slightest disturbance, and are known to fly considerable distances when flushed. Usually, found during dawn and dusk in the open forest tracts, trails, fire lines and fields abutting the forest where they come to feed (Ali and Ripley 1983). During the day, they are found in the thickets of bushes and at noon often resting in the shade of shrubberies. They roost in trees and bamboo clumps. Nests are built on the forest floor, within thickets of the shrubs. The clutch size varies from 4-7 eggs per clutch and the incubation period is 20-21 days (Ali and Ripley 1983, Madge and McGowan 2002 and Rasmussen and Anderton 2005).

In terms of threats perception to the species, the International Union for Conservation of Nature (IUCN) has listed it as “Least Concern”, while the Indian Wildlife (Protection) Act, 1972 has listed it in Schedule IV, Convention of International Trade in Endangered Species of Wild Fauna and Flora (CITES) has not listed the species (Fuller and Garson 2000).

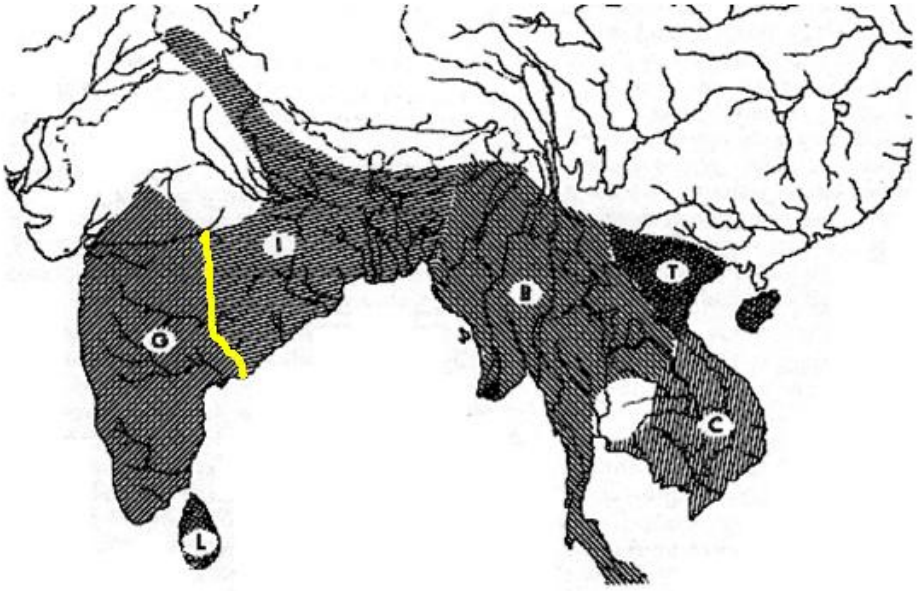


Figure 1.1a: Global Distribution of Junglefowl (*Gallus* spp) and its subspecies.

Source: Johnsgard (1986)

G = Grey Junglefowl, L= Ceylon Junglefowl, B= Burmese Red Junglefowl, T=Tonkinese Junglefowl, C= Cochin-Chinese Junglefowl.

Source: Tring Museum. UK.

Figure 1.1b. The five subspecies of Red Junglefowl

1. *G.g. jabouillei*, 2. *G.g. bankiva*, 3. *G.g. gallus*, 4. *G.g. spadiceus* and 5. *G.g. murghi*

1.1.3. RJF – The Wild Ancestor of Domestic Chicken

Archaeological discoveries in the Indus Valley and in Hebei Province, China, suggest that chickens were probably domesticated from the Red Junglefowl *Gallus gallus* (henceforth RJF), as early as 5400 BC (West and Zhou, 1989). Darwin (1868) was the first to indicate that chicken were derived from RJF and since then, there has been considerable controversy regarding its ancestry. There have been two schools of thought, one the monophyletic origin (Fumihito et al. 1996) and the other multiple origin (Liu et al. 2006), but both schools suggested that RJF was the progenitor for domestic fowl (Hillel et al. 2003, Moiseyeva 1998). Molecular evidence for hybridisation between species in the genus *Gallus* raised the possibility that other junglefowl species were also progenitors for the domestic fowl (Nishibori et al. 2005) which complicates this issue. The RJF, resulting from centuries of domestication, breeding and hybridisation with feral/domestic chicken, is now at the risk of being lost (Peterson and Brisbin 1999) this has been highlighted in the IUCN Action Plan (IUCN 2000, pages 7 & 46) for Pheasants (IUCN 2000, www.iucn.org). The pure wild RJF may, however, represent a pool of wild genes for future breeding and research purposes.

One of the primary premises in present day conservation programs is to maximize the conservation of the genetic diversity available for potential future use. The chicken is by far the most common domesticated animal and the most important production animal in the world. The total chicken population is about 30 billion, with breeds specialised for either egg production or rapid growth and meat production (Jensen 2005). Chicken are widely used as models of human diseases, including muscular dystrophy, immunological diseases and thyroid insufficiency and it will be possible to find and investigate candidate genes affecting such traits, in the benefit of human medicine (Jensen 2005). The putative wild ancestors of our major livestock species are now either extinct (e.g. the auroch the wild ancestor of cattle or the ancestral species of the Old World camelids) or low in numbers and threatened by extinction due to various reason (Hanotte and Jianlin 2005). Hence it is imperative to know the distribution of the species that are ancestors of domestic varieties which may have significant relevance to the poultry industry and to understand their genetic diversity, genetic distance between their populations and the domestic chicken as it is considered to be the precursor of all domestic poultry. Hence, it forms a very important genetic resource which can be used for enhancement of domestic varieties/breeds. It also has relevance to International patenting of strains under Intellectual Property rights and the information produced from this report can be used by the government and research organisations.

Keeping this in view a research project “Conservation of Red Junglefowl *Gallus gallus* in India” was initiated by the Wildlife Institute of India in 2006 with the following objectives in two phases.

1.2. OBJECTIVES

- Assessing the status and distribution of RJF in India.
- Identification of pure RJF populations by molecular genetic studies.
- Study social interactions between wild RJF and domestic RJF.
- Propose conservation action plan for the identified RJF populations.

Of these, with the exception of the first; that was completed in phase I, the remaining objectives were addressed in phase II.

1.3. Literature Review

Archaeological discoveries suggest that chickens were probably domesticated from the RJF, as early as 5400 BC (West and Zhou 1989) and Darwin (1868) was the first to indicate that chicken were derived from RJF. Hence there were apprehensions of the possible hybridsation between village chicken causing introgression in the wild (Brisbin 1995, Peterson and Brisbin 1999) and with the assistance of molecular techniques this phenomenon was said to have dire consequences on the wild RJF (Brisbin et al. 2002, Brisbin and Peterson 2007).

Due to their brilliantly coloured plumage and highly dimorphic nature, this species has contributed in understanding complex issues such as sexual selection. In captivity, mate selection within males (Zuk et al. 1990a, Ligon 1990, Parker and Ligon 2002) and females (Johnson et al. 1993, Zuk et al. 1995a,b), ornamentation (Zuk 1990c, Zuk 1993) and effects of parasite load on selection and endocrinology (Zuk 1990b, d, Zuk et al. 1995a) and whether these choices are repeated were studied by Johnsen and Zuk (1996) and vocalisation was studied by Chappell et al. (1995) all these studies involved captive RJF. Considering there might be some difference in behaviour through time, Håkansson and Jensen (2005) studied behavioural and morphological variations in captive and wild RJF. There were studies to understand plumage variation within species and between hybrids (Morejohn 1953, 1955, 1968 a, b).

In India, much has been written about the distribution and ecology of RJF by British Naturalists and Ornithologists (Hume and Marshall 1879, Beebe 1922, Baker 1920) highlighting their habit of coming out on the roadsides to feed grit early in the morning. They have also been clear in their assessment about the association of this species with Sal forest (*Shorea robusta*). However, even before this period, RJF finds mention in the *Tuzuk-e-Jehangiri* written between 1605 and 1623 by the Mughal emperor Jehangir (Beveridge 1909).

Bump and Bohl (1961) were perhaps the first who conducted ecological studies on this species. These studies were followed by Collias and Collias (1967) who studied this

species in North central India. Kalsi (1992) studied the habitat use of this species in Kalesar Forest Reserve of Haryana and suggested that the species preferred mixed forest with cultivation more than other habitats, a view endorsed later by Javed and Rahmani (2000). Thus, this species appears to be predominantly an inhabitant of edge habitat, seldom found very deep inside a forest. A few observations on the morphological traits of the captive stock of RJF have been carried out (Kaul et al. 2004).

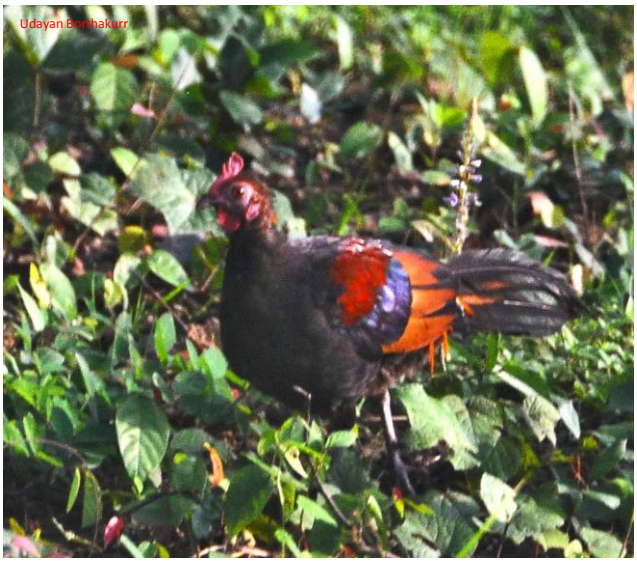
Fumihito *et al.* (1996) found RJF to be the real matriarchic origin of all the types of domestic poultry. The DNA finger printing analysis of various breeds and species was carried out to evaluate the genetic relationship between domestic and jungle fowl (Yamashita *et al.,* 1994 and Okumura *et al*., 2006). Vanhala *et al.* (1998) evaluated the genetic variability and divergence of eight chicken lines using microsatellite markers. While analysing the genomes of domestic and RJF with 14 microsatellite markers belonging to 11 linkage groups, Romanov and Weigend (2001) observed that RJF formed a separate branch in the phylogenetic tree and demonstrated a specific allele distribution when compared to domestic fowl. Niu *et al.* (2002) sequenced the first 539 bases of D-loop of *mt* DNA from five Chinese native chicken breeds and white leghorn and compared with RJF. Their domestic breed, *Gallus gallus domesticus* was found to be the closest to the RJF. Brisbin *et al.* (2002) have characterised the genetic status of populations of RJF and have emphasized on assessment of RJF populations in Southern Asia.

Irina *et al.* (2003) reassessed the published data to determine the origin and relatedness among the four postulated chicken breeds and with basic ancestral species of Jungle fowls, *Gallus gallus*. Hillel *et al.* (2003) analysed 52 diverse chicken populations using 22 dinucleotide microsatellite markers in an European project on chicken biodiversity, revealing that the RJF and unselected breeds and broilers had more and wide heterogeneity as compared to the layers and experimental lines. Ya-Bo *et al.* (2006); Cuc *et al.* (2006) and Chen *et al.* (2008) provided evidence of the applicability of microsatellite markers to determine the genetic relatedness among different Chinese chicken populations and evaluation of genetic variations.

In India, some reports have also presented on genetic diversity and relationship studies of different breeds of domestic chicken (Sharma, 1998; Sharma *et al*., 2001; Sharma and Singh 2001; Pandey *et al*., 2002 and Ahlawat *et al*., 2004). However, some work on comparative genomic studies between RJF and domestic chicken, in reference to detect random differences between the both genomes has been carried out by Sharma (2006). Kanginakudru *et al*. (2008) studied RJF population of Haryana and supported multiple domestication of modern day chicken. At landscape level, no work has been carried out till now regarding evaluation of molecular genetic diversity and assessment of genetic purity of RJF population in India at a landscape level.

Plate 1: Red Junglefowl (a) Male (b) Female

Plate 2: Red Junglefowl (a) eclipse plumage (b) Pink earlobe (c) White earlobe



2.0 STATUS AND DISTRIBUTION OF RED JUNGLEFOWL IN INDIA

"What biologists want to know about is the process of decline in range and number…"

Michael E Soulé

2.1 INTRODUCTION

In context of declining forests, comprehending the scale of rapid growing changes in landuse patterns, human demography and accompanied cultural changes and their synergistic effects (Geist and Lambin 2002) may have caused many more species to reach their critical threshold levels (Brooks et al., 2006). How species have coped with these changes that lead to their extirpation is of concern, but such understandings have been ignored at the scale of the species range. An understanding of range reduction and accompanied decline in numbers is vital for the preservation of biodiversity and these concerns are of fundamental interest to conservation biology (Simberloff 1986).

Depending on whether these processes are localised or widespread, there are two different hypotheses for range extinctions, the demographic hypothesis and contagion hypothesis. The contagion hypothesis makes the implicit assumptions that the extinction factor must extend across the entire range of the species and is uniformly distributed because any portion that is not impacted by the extinction factor would expect the species to persist. In addition, the intensity of the factor must be uniformly distributed, or else peripheral populations might persist where the intensity of the factor is reduced. The other important aspects of the factor should be synchronous and sudden onset. If the onset is staggered then, those populations that are last impacted regardless of their location may persist longer regardless of their historic range size. The widespread colonisation and domination of the human race has formulated this contagion through a suite of disturbances (Channell and Lomolino 2000). Due to this the International Union for Conservation of Nature (IUCN) had set up a framework that prioritises species for conservation. But determining the critical threshold-abundance values for specific species can be impractical especially for large geographical ranging species. Hence, alternative measures were suggested (Clement et al., 2011) where six of the nine major institutional templates of global biodiversity conservation prioritisation, conceptually fit into the framework of "irreplaceability" and this is central to the theory of spatial conservation planning (Margules and Pressey 2000). Hence the persistence of such sites will be crucial in maintaining the 10% target that was adopted at the Convention of Biological Diversity (CBD).

Even if biodiversity is reduced conceptually to the species level, quantifying and managing it would normally be extremely time consuming and expensive. Hence, preferentially, endangered and threatened species are the focus since when confronted

with anthropogenic perturbation they are generally among the first to go locally extinct (Woodroffe and Ginsberg 1998). Relatively, common species perform better with most pressures and hence can be (Noss 1996; Lambeck 1997; Coppolillo et al., 2004) and have been used in a working landscape (Githiru et al., 2007). Although, commonness being a rare phenomenon has received very little attention through the years, there is increased focus in understanding the role and function that they play in the community (Gaston 2009). In part, this has been driven simply by a recognition of key gaps in understanding, but it has also followed from deepening concerns over declines of many common species and the consequences these declines might have (Gaston 2011). In order to understand reasons for such declines it is imperative to understand factors that govern the distribution of the species.

Hutchinson's concept of "n-dimensional hypervolume" considered basic niche concept with species existence (Hutchinson 1957). These n-dimensions can be environmental conditions that define a range at which species can persist. These climatic-envelope models provide an essential conceptual tool for understanding range limits (Gaston 2009) though independent of its implications for inter and intra-specific interactions and community organisation (Soberon, 2007). Hence an useful autecological construct to test ecological and evolutionary theories that underpin the fields of biogeography and biological conservation.

"Species niche model," "ecological niche model" or even "niche-theory model" are synonyms that have been used to describe Species Distribution Modelling (SDM). These models have variously been described as estimating the fundamental (potential) niche, realised (actual) niche, the multivariate species niche (Rotenberry et al., 2006). The distributional maps resulting from the application of climatic niche models are often referred to as predictions of (species) geographical range (Graham et al., 2004). Some authors even distinguish between ecological niche models, which they define as models of potential distribution, and species distribution models of actual distributions (Peterson et al., 2008). SDM's are widely acknowledged technique to accurately determine the ranges of the target species (Anderson et al., 2002, Raxworthy et al., 2003), and have been widely used for various applications such as aspects of resource management, conservation planning, biodiversity assessment, reserve designs, habitat management, ecological restoration, invasive species, risk assessments, population, community and ecosystem modelling to name a few (Guisan and Thuiller 2005). The number of methods available for modelling patterns of species distribution are numerous (Guisan and Zimmermann 2000, Scott et al. 2002) and evaluating the relative performance of different methods remains an ongoing challenge (e.g. Loiselle et al., 2003, Thuiller 2003, Ottaviani et al., 2004, Vaughan and Ormerod 2005, Elith et al., 2006, Pearson et al., 2006) in ecology and conservation

biology. But the main concern is at what scale are these ecological patterns being studied? Presently, this is being guided by the scale of the data that is being made available (Levin 1992).

The RJF is listed in the "Least Concern" category of IUCN (www.iucn.org, accessed on Jan 1, 2012) with an extent of occurrence (breeding/resident) approximating to 5,100,000 km^2 (www.birdlife.org accessed on Jan 1, 2012) with the subspecies *G g murghi* having a distribution range of 652,000 km^2 (Fernandes et al., 2009). There are a few studies that delve into the status, ecology (Collias and Collias 1967) and distribution (Fernandes et al., 2009) of the species from its entire range. Most of the information gathered on the species is either in the form of opportunistic sightings and anecdotal records that are listed in the avian guides for the oriental region (Hume and Marshall 1879, Baker 1920, Beebe 1922, Ali and Ripley 1983, Madge and McGowan 2002, Rasmussen and Anderton 2005). While several techniques that have been developed, aid research work in the field, it would take years to get the required information for such a large ranging species. In an attempt to overcome time, surveys were aimed at collecting information on the RJF status, distribution through a network of researchers, conservationists and other informed personnel with the associated goals with this data collection effort.

1. Development of database with site information for the species.
2. Generation of current distribution of the RJF in India.

There were two broad bases for which data was collected and interpreted, one at the national level and other at a local scale namely Protected Area (PA) [National park (NP) and Wildlife Sanctuary (WLS)] and Reserve Forest (RF).

2.2. METHODOLOGY

Ethics Statement

All research (surveys and sample collection) was conducted with relevant permissions granted from incumbent administrators. Published literature and archived database were used as information for the species.

2.2.1. Species and Occurrence Data

Data on the distribution of the species was obtained from primary and secondary sources. The primary source of information came through surveys that were conducted in the different RJF range states. The secondary source of information was mainly through literature review, questionnaires, and personal communications, and from a network of NGO's and local villagers. Apart from this archived database Global Biodiversity Information

Facility (www.gbif.net accessed on September 3, 2010) and museum records (Smithsonian Natural History and Bombay Natural History Society) were sought for supplementary information regarding past records for the species. Records in the GBIF were cross-checked for duplication.

2.2.2. Environmental layers

The environmental layers were made up of Bioclimatic, topographical and landcover variables. We extracted bioclimatic data from the WORLDCLIM data set at 30 arc seconds Version 1.4 available at http://www.worldclim.org/bioclim.htm (Hijmans et al., 2005). This dataset, ranging over a 50 year period (1950 to 2000) and collected over several globally located weather stations, uses annual trends, extremes and seasonality of temperature and precipitation to derive biologically meaningful variables. A total of 19 bioclimatic variables (annual mean temperature, mean monthly temperature range, isothermality (2/7*100), temperature seasonality (standard deviation of monthly temperature*100), maximum temperature of the warmest month, minimum temperature of the coldest month, temperature annual range (5–6), mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of wettest month, precipitation of driest month, precipitation seasonality (CV), precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, precipitation of coldest quarter) were used for the initial analyses. Topographical variable derived from the Digital Elevation Model (DEM) was extracted from Advanced Spacehorne Thermal Emission and Reflection Radiometer (ASTER) data (www. http://www.gdem.aster.ersdac.or.jp/) and global cover type data was extracted from http://ies.jrc.ec.europa.eu/global-land-cover-2009 (Bartholomé and Belward, 2005; henceforth referred as GLC 2009). All environmental variables were rescaled to 1 km spatial resolution. All bioclimatic variables were tested for multicollinearity by examining cross-correlation (Pearson correlation coefficient, r). Only one variable from a set of highly collinear variable ($r \geq 0.7$) was included in the model based on its potential biological relevance to the distribution of the species and the ease of interpretation.

2.3. Modelling Procedure

Modelling was undertaken in a two step procedure namely: Rule based models and Presence-only models. Presence information collected was mapped using GIS tools (ArcMap 9.2) to prepare the distribution for the species. The secondary source of information was verified during the surveys. Areas outside the historical distributional range such as an altitude of more than 3000m and wetlands were left outside the purview of the distributional map. Presence-only modelling was used (Tsoar et al., 2007, Elith and

Leathwick 2009) as it is preferred to other robust statistical modelling methods (Rota et al., 2011).

2.3.1. Rule based Modelling

The elevation range and forest cover where the RJF were reported were used to delineate existing spatial layers to construct "rule-based model". The rule-based model works on the basis of Boolean logic, which relies on well-established available knowledge and prescribes the area to be either suitable (1) or unsuitable (0) without any other middle level (Lenton et al. 2000). The existing information available in the literature (Ali and Ripley 1983 and Johnsgard 1986) combined with personal field records was utilised to identify suitable broad characteristics. Unique numbers were assigned to each category in all the variables and initials maps of potential areas and otherwise for each variable were prepared. Overlay analysis was then, performed in Arc/info software to obtain final map depicting potential area of distribution for RJF.

2.3.2. Presence-only Modelling

Wide ranging vagile organisms may potentially use or occupy the location but not at the time of the survey (issues of detection) and hence in some situations we cannot estimate a sample of unused sites, including georeferenced natural history collections records. There are three general approaches to modelling habitat suitability when data on species presence are available. One approach is to calculate some measure of similarity to the presence location by using environmental variables. There are many modelling algorithms that have been formulated in the last decade that use such an approach. To judge the sensitivity of our rule-based method to reductions, we computed the relative amount of climate space that would have been detected had our samples been a random subset of the original localities using Maxent.

2.3.2.a. Maxent

Maxent is a machine learning process and a statistical method (Phillips et al., 2006, Elith et al., 2010), used to develop SDMs with presence-only species occurrence data (Dudik et al., 2007). Maxent models the species distribution directly by estimating the density of environmental covariates conditional on species presence. The raw output is an exponential function that assigns a probability to each site, where the value is dependent on the number of background and occurrence sites (Phillips et al., 2006). The output is a logistic output that estimates the probability of presence (Phillips and Dudik 2008).

We used Maxent version 3.3.3.e and ran the model with product features for convergence threshold, maximum iterations (500) and background points (10,000) and reduced regularisation value (0.5) in order to reduce overfitting with a combination of feature classes. Jackknife variable importance features was used to assess the relative importance of environmental predictors. This generates an estimate of probability of

presence of the species which varies from 0 to 1, where 0 being lowest and 1 being the highest probability(http://www.cs.princeton.edu/~schapire/maxent/).

2.4. Model Validation

Testing or validation forms an important part to assess the predictive performance of a model. In this case, we could not get an independent data set for testing the model performance. Therefore we used the 50-fold partitioning cross-validation method to create 'training' and 'testing' data this ensures quasi-independent data for model testing (Fielding and Bell 1997, Elith et al., 2006). Independent model validation was carried out for the testing and training dataset using sensitivity, specificity analysis for which mean and standard deviations was used.

2.5. RESULTS AND DISCUSSION

2.5.1 Distribution

From the field surveys, questionnaires survey, literature reviews, informal interviews and databases, the present study reports RJF from 21 States in India which is in accordance to Hume and Marshall (1879), Ali and Ripley (1983) and Madge and McGowan (2002). It is reported to be 'present' in 205 districts out of the 270 districts of India and in 34 NP and 135 WLS (Appendix 1). Based on the rule based model, the RJF distribution was prepared for India, where the potential available area of occurrence is estimated to be *ca.* 807,780 km^2 (Fernandes et al., 2009) while in the predictive modelling, the estimated potential area available *ca*. 354,978 km^2 for India. The total area including areas outside the political boundary of India was *ca* 462,729.15 km^2 and this included Pakistan, Nepal, Bhutan and Bangladesh. Such large discrepancies within the rule-based model are primarily due to the fact that the rule-based model did not exclude areas such as urbanised and other developed areas, waterbodies and agricultural land, which was delineated by the landuse class cover layer (GLC 2009) that was used in the predictive model. The PA network accounts for nearly 13% of the area (45,592.97 km^2), where the NPs represent 4.32% (15,335.84 km^2) and the WLSs represent 8.52% (30,257.13 km^2). Approximately, 90% of the distributional area lies outside the purview of the present PA system and the northeastern landscape has only 2.88% of the area within the purview of the PA network.

2.5.2. Predictive Modelling

The product function model in Maxent predicted potential suitable habitat for RJF with an AUC value of 0.979 for the test data. The maxent model internal jackknife test of variable importance showed that annual precipitation and precipitation in the driest quarters presented the higher gain for the model than compared to other variables, due to their overall contribution (46 percent).

From the predictive modelling (Fig 2.1) it appears that the once continuous distribution of the species as reported by (Ali and Ripley 1983, Madge and McGowan 2002, Rasmussen and Anderton 2005) is presently divided into two major landscapes, namely the northern (north and northeastern) and central. The distribution in central (186,507.99 km^2) landscape of India is disconnected from that of the north (44,103.69 km^2) and northeastern (124,366.32 km^2) States. The northern landscape is continuous and is represented by Bhutan, Nepal and India (Fig 2.2). The Indian part of the distribution for the north is represented through the administrative States of Jammu and Kashmir, Himachal Pradesh, Punjab, Haryana, Uttarakhand, Uttar Pradesh, Bihar, and the northern districts of West Bengal, the northeastern landscape is represented through the administrative States of Sikkim, Arunachal Pradesh, Assam, Nagaland, Manipur, Mizoram, Tripura and Meghalaya and the central landscape is represented by the administrative States of Madhya Pradesh, Chhattisgarh, Andhra Pradesh, Odisha, Jharkhand and the southern districts for Bihar and West Bengal.

Within the central landscape for the State of Madhya Pradesh, presently RJF is not reported beyond the west bank of the Pench River (R. Jayapal pers comm. 2009) though reported records for the species are from Satpura Hills (Ali and Ripley 1983) while the southern distribution of RJF is demarcated to be near Rajahmundry and River Godavari being the barrier, but Nagula et al. (1997) reported the presence of the species at Eturnagaram WLS in the Warangal District, while surveys in 2007 did not record the species. Additionally, the species was recorded on the west bank of the River Godavari at Pollavaram WLS, in the West Godavari District. In the State of Maharashtra, the species has been recorded from from Nawegaon NP and Nagzira WLS (CWLW- Maharashtra 2009).

Being a forest surrogate species the range of distribution is restricted in certain states due to geographical barriers, environmental elevation gradients and unavailability of forest cover. The later being the main factor that has affected the geographic range for RJF and hence the distribution is discontinuous. As suggested by McKinney (2007), that a wide ranging species with a large geographic range sizes are more resistant to global extinctions, and persist for longer periods of time than those with smaller ranges, this attribute may have assisted in the species to be reported within all earlier reported states. Creation of PAs as a mandate of the state and the ability of a generalist to inhabit wide ranging habitats from mangrove forest to mixed-Pine dominated forest may also have facilitated this species to persist. Though RJF, is a widely distributed species the absence in certain PAs such as Barnawapara WS (Chhattisgarh) seems to be a result of continuous management practices that were undertaken. Addressing and accounting for such human induced interventions in the form of habitat restoration, is difficult (Rouget et al. 2003, Leemans and Serneels 2004). Similarly the non-detectability of the species in small size PAs of Assam (Bherjan, Borajan

and Padumoni) seem to suggest that human-induced disturbance may have affected the species, though the present study reported the presence of the species from neighbouring tea-estates at Digboi and also from the Hollangapar Gibbon WLS. Another underlining effect may be the prevalence of hunting that is still practiced and common by the indigenous ethnic groups (Aiyadurai 2007). All this may potentially result in declines in abundance of individuals in parts of the range of the species without necessarily leading to local extinction. The intensity and effects of such activities might have an impact on the local abundance of the species, but due to absence of such baseline data, consideration of such plausible effects on the abundance is speculative. Though, these changes may occur and may pass unnoticed due to apathy for common species, especially in areas outside the purview of the PA network there are strong indications that these changed patterns of habitat fragmentation and connection are known to have at least as large an impact on the range of the species (Jetz et al. 2007). These effects have been addressed while taking into account the level of changes that occur at the molecular level in the species for northern India (Mukesh et al. 2011). This is the first instance that the distribution of a common avian species has been mapped within India. The need and importance for such an objective can be inferred from other common species decline such as Vulture (Gyps spp), when reported records had fallen to abysmal low level (Prakash et al. 2003).

The other important facet that was needed to be addressed is the issue of hybridization that has been reported within the species (Brisbin 1995). This brings into question the issue of species identification especially when morphological characters used to identify the species are deceptive (Brisbin and Peterson 2007). These anomalies may have led authors to report the species from areas outside their distributional range. There are other instances, when the species might have been introduced to a particular area as reported by Ali and Ripley (1983) these areas were not considered to be the part of present geographic distribution range for the species.

2.6. LIMITATIONS

Although the SDM have helped to map and contributed to our understanding of the RJF distribution by revealing places where to search for undetected new populations and helped find areas, there are some inherent limitations with the methods that have been used. These limitations are mostly with the resolution of the available data. Most of the data used, provide only coarse scale descriptions of the habitat, small-scale features are difficult to capture hence, finer scale interpretations were not feasible. This also limits our understanding of in building interspecific and intraspecific interactions that are known to have an effect on the distribution of a species.

Table 2.1. Area representation for the various landcover class that is available for the Red Junglefowl

Land class	Global Area (km^2)	India Area (km^2)		
		North	Central	Northeastern
Rainfed croplands	89001.45	10402.29	57709.08	5284.26
Mosaic cropland (50-70%) / vegetation (grass/shrub/forest) (20-50%)	87717.87	8710.92	58569.48	7050.6
Mosaic vegetation (grass/shrub/forest) (50-70%) / cropland (20-50%)	72909.18	9811.53	18022.95	18634.05
Closed to open (>15%) broadleaved evergreen or semi-deciduous forest (>5m)	44994.87	1118.43	4119.48	30676.32
Closed (>40%) broadleaved deciduous forest (>5m)	36769.68	3515.49	24969.78	2864.97
Open (15-40%) broadleaved deciduous forest/woodland (>5m)	672.3	0	0	368.1
Closed (>40%) needleleaved evergreen forest (>5m)	15093.27	2296.8	422.37	8266.5
Closed to open (>15%) mixed broadleaved and needleleaved forest (>5m)	16784.1	2234.97	2742.39	5991.75
Mosaic forest or shrub (50-70%) / grassland (20-50%)	460.26	142.2	2.07	31.95
Mosaic grassland (50-70%) / forest or shrub (20-50%)	712.8	228.6	3.51	94.23
Closed to open (>15%) (broadleaved or needleleaved, evergreen or deciduous) shrub (<5m)	88211.88	5021.82	18298.35	41998.59
Closed to open (>15%) herbaceous vegetation (grass, savannas or lichens/mosses)	5080.5	188.37	838.26	2641.14
Sparse (<15%) vegetation	23.67	0	23.67	0
Closed (>40%) broadleaved forest /shrub permanently flooded - Saline or brackish water	2130.66	0.36	106.65	0
Artificial surfaces and associated areas (Urban areas >50%)	2166.66	431.91	679.95	463.86

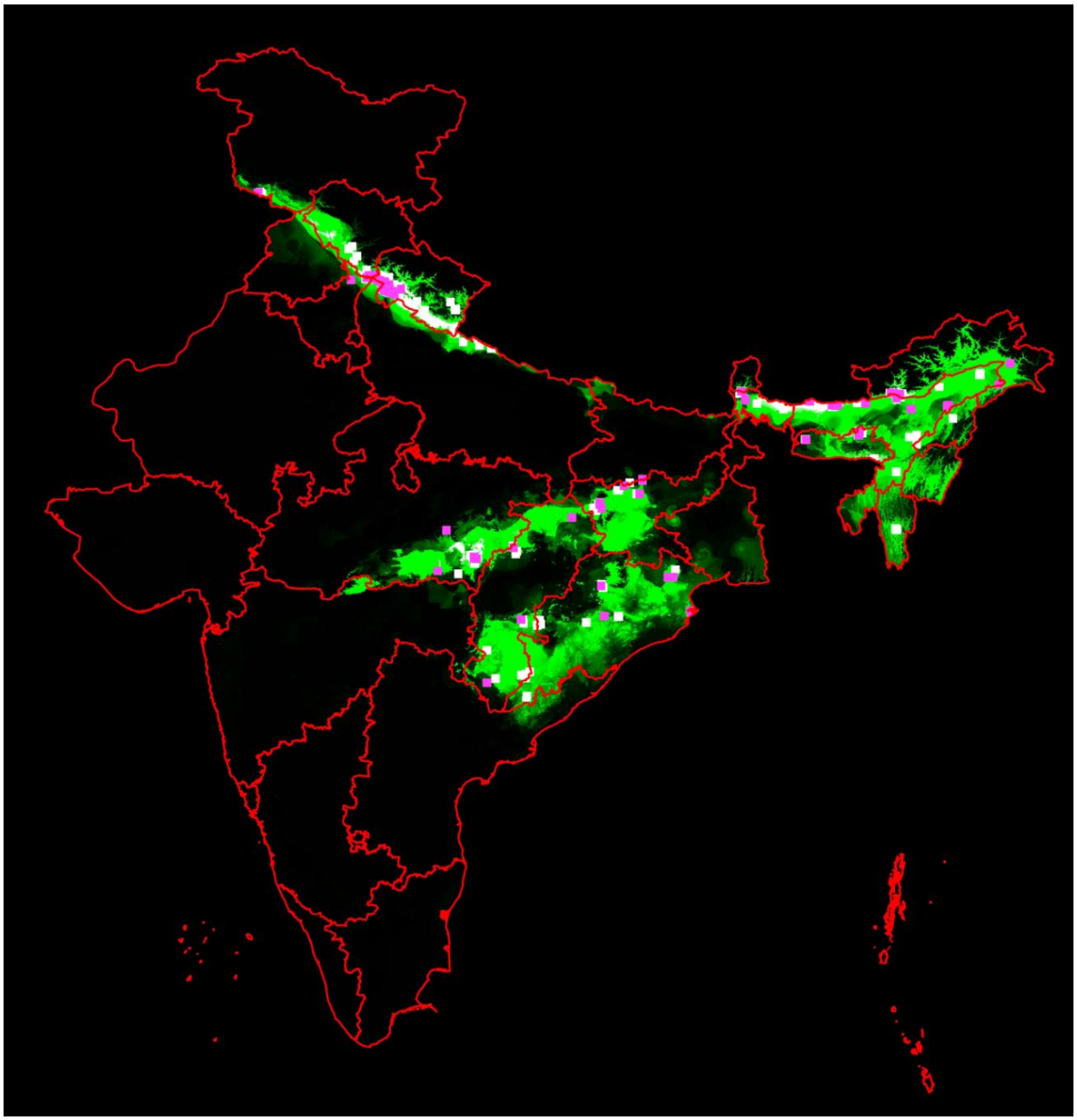


Figure 2.1. Predictive distribution for Red Junglefowl in India.

The green areas indicate the predicted distribution of Red Junglefowl. Presence records that were used for training dataset are indicated as white squares, while the test dataset are indicated as pink squares.

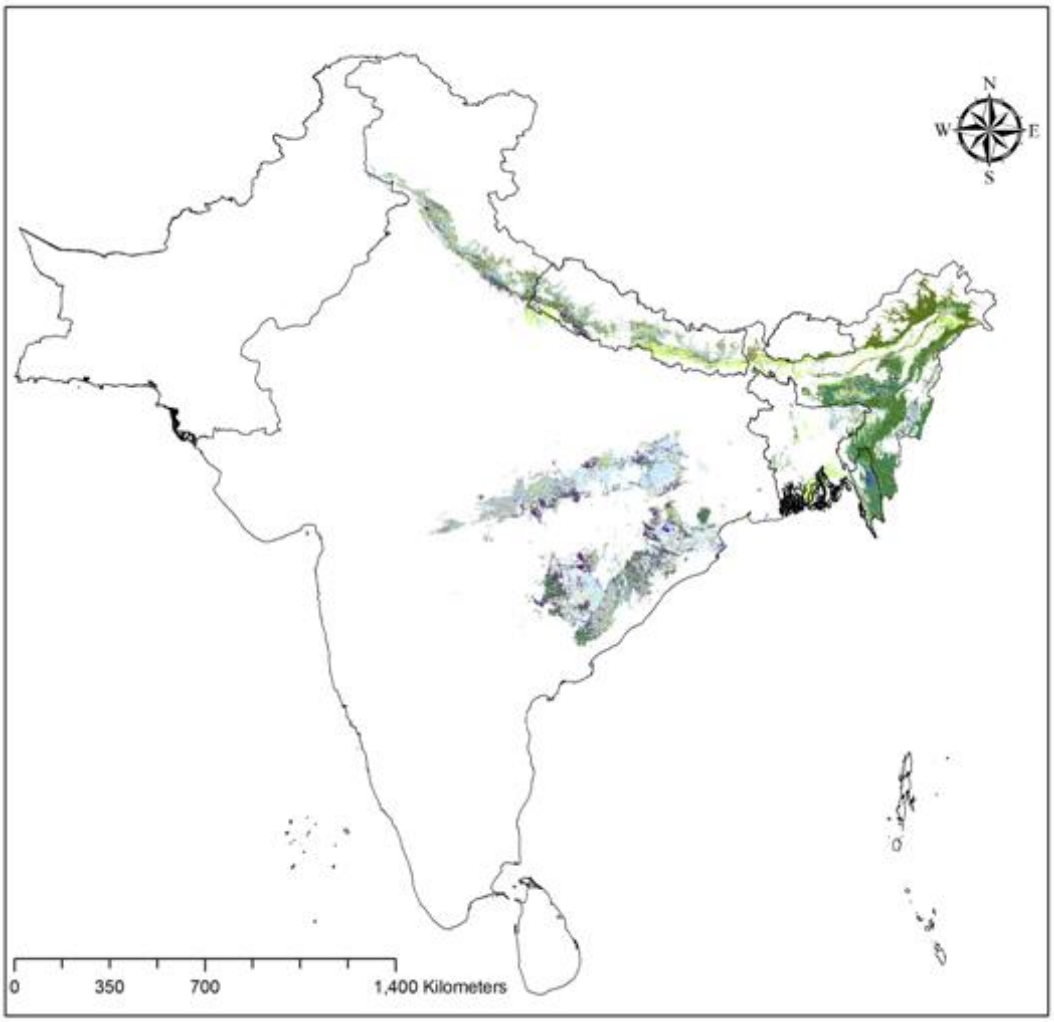


Figure 2.2. Predicted distribution of Red Junglefowl along with land use classes (GLC-2009).

Legend

- Rainfed croplands
- Mosaic cropland (50-70%) / vegetation (grass/shrub/forest) (20-50%)
- Mosaic vegetation (grass/shrub/forest) (50-70%) / cropland (20-50%)
- Closed to open (>15%) broadleaved evergreen / semi-deciduous forest (>5m)
- Closed (>40%) broadleaved deciduous forest (>5m)
- Open (15-40%) broadleaved deciduous forest / woodland (>5m)
- Closed (>40%) needleleaved evergreen forest (>5m)
- Closed to open (>15%) mixed broadleaved and needleleaved forest (>5m)
- Mosaic forest / shrub(50-70%) / grassland (20-50%)
- Mosaic grassland (50-70%) / forest/ shrub (20-50%)
- Closed to open (>15%) (broadleaved/needleleaved, evergreen/ deciduous) shrub(<5m)
- Closed to open (>15%) herbaceous vegetation (grass, savannas/lichens/mosses)
- Sparse (<15%) vegetation
- Closed (>40%) broadleaved forest /shrub permanently flooded Saline/brackish water
- Artificial surfaces and associated areas (Urban areas >50%)
- Bare areas
- Water bodies

Plate 3: Habitats of Red Junglefowl in India

Top left :Mixed Sal Forest
Mid left: Riverine Forest
Low left: Grassland and Woodlands

Top right: Moist Evergreen Forest
Mid right: Bamboo mixed Forest
Low right: Mangrove Forest

Plate 4: DNA extraction and agarose gel electrophoresis in the lab.

3.0 GENETIC DIVERSITY, POPULATION DIFFERENTIATION AND PHYLOGETIC ANALYSIS OF RED JUNGLEFOWL IN INDIA

"*It requires a very unusual mind to undertake the analysis of the obvious*."

Alfred North Whitehead

3.1 INTRODUCTION

RJF is the single most important species to mankind due to the economic and cultural significance to human civilization. It has been shown that RJF is the main or only wild ancestor of all domestic breeds (West and Zhou, 1989; Fumihito et al*.,* 1994 and 1996) while Eriksson *et al.* (2008) provided evidence for a hybrid origin of the domestic chicken, most likely from Grey Junglefowl (*Gallus sonneratii*). Andersson et al*.* (1994) have stated that 'populations of domestic animals and their wild ancestors provide a valuable source of genetic diversity that may be exploited to develop animal models for quantitative traits of biological and medicinal interest'. Conservation of genetically pure wild forms or their representatives have great potential to make significant contribution to the study of some economically important genetic traits of domestic forms (Brisbin, 1995). Unlike other domestic species, where the ancestor from which the present day animals evolved do not exist, the domestic chicken offers an unique system to compare the effect of natural selection and therefore it has immense value. Although RJF is considered abundant both in captivity and in wild, today this bird is facing the threat of extinction in terms of genetic purity leading to an inference that there may not be any pure RJF populations in the wild (Peterson and Brisbin, 1998). Brisbin and Peterson (2007) crossed genetically pure male RJF with domestic female chicken to create contaminated lines of known purity and the results they got were very surprising as the phenotypic characters generally used as indicators of purity (mainly male eclipse plumage, leg color, reduced or absent female comb) all appeared and persisted to at least some extent in domestically contaminated progeny and more so in successive more pure generations of the experiment. Therefore, recent studies suggest that such phenotypic characters may have little, if any, utility in characterising RJF stock as to their genetic purity. To approach these questions of genetic purity, however apart from phenotypic markers, additional tools drawn from molecular genetics have yet to be applied and these molecular tools may be helpful in detecting the occurrence of such genetic contamination.

Recent advances in molecular technology have provided new opportunities to assess genetic variability at DNA level. DNA polymorphisms, such as amplified fragment length

polymorphism (AFLP), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), single nucleotide polymorphism (SNP), minisatellites and microsatellites are extensions of markers for detection of classical sequence polymorphisms.

AFLPs and RAPDs typically are dominant markers, resulting in complicated allele distribution patterns, in which it is difficult to distinguish heterozygotes from homozygotes of the dominant allele, thus causing serious loss of information (Piepho and Koch, 2000 and Ma et al. 2004). It has been reported that RAPDs were difficult to reproduce (Schierwater and Ender, 1993) and they provide relatively little information for evolutionary rates and gene genealogies. They are random markers, and provide limited power for making inferences about molecular clock and kinship relationships.

SNPs and RFLPs are two basic tools for detecting genomic variation in both mitochondrial and nuclear genomes. Both can be analyzed by their haplogroups (arrangement of groups according to haplotypes). SNPs are easier to characterize by their known nucleotide substitution pattern. SNPs have recently been recognized as the most widespread type of sequence variation (Brumfield et al., 2003) in either the nuclear or mt DNA genome, comparable with microsatellites that are used mostly in the nuclear genome. Both microsatellites and SNPs are valuable genetic markers for revealing evolutionary history at different depths. The fact that SNPs occur throughout the genome makes them ideal for analyses of species differences, historical genealogies and all levels of evolution (Lewis, 2002 and Brumfield et al., 2003). SNPs make the comparison of genomic diversities and histories of different species more straightforward than possible with microsatellites due to their lower variation and fewer alleles compared with microsatellites. The main complication in SNP analysis is a bias towards analyzing only the most variable loci. This bias is usually introduced by the limited number of individuals initially used to screen for polymorphisms and it is correctable. The use of SNPs as markers in population analyses and phylogenetic studies is a new approach but it is expected to become a widely used marker following the innovation of high throughput, accurate and cheap methods for SNP identification, inference, and statistical analysis. (Syvänen, 2001 and Brumfield et al., 2003).

Microsatellites are DNA motifs of tandemly repeated units of one to six base pairs. They are highly polymorphic and widely distributed throughout eukaryotic genomes and show co-dominant inheritance (Groen et al. 1994; Kavaca et al. 1999). Microsatellites display relatively higher mutation rates compared to the eukaryotic substitutes, averaging around 10-9 mutation per nucleotide per generation (Ellegren, 2000). The simplest mechanism for microsatellite mutation is replication slippage with unequal crossing-over being an alternative model. Two main mutation models have been associated: the infinite allele model (IAM) and the stepwise mutation model (SMM). The IAM states that all

mutation events will create a new allele and that new allele may have more than one repeat difference with the 'parental allele'. The SMM predicts that mutation will result in an allele which is one repeat larger or smaller. Many microsatellites have recently become available in chicken and have been mapped in reference populations (Crooijmans et al. 1993; Cheng et al. 1994; Crooijmans et al. 1996 and Groenen et al. 2000). These markers provide a powerful tool for quantitative trait loci (QTL) research, and have also been successfully used to study the genetic relationship between and within chicken populations (Ponsuksili et al. 1999; Zhou 1999, Romanov et al. 2001; Rosenberg et al., 2001 and Vanhala et al, 1998). Reliable information on allele frequencies was obtained from chicken blood or DNA pools using minisatellite markers (Dunnington et al. 1990 and Dunnington et al, 1994; Hillel et al. 1989 and Hillel et al. 1990) as well as microsatellites (Crooijmans et al. 1996 and Khatib et al. 1994). Some research on origin, phylogeny, genetic diversity and domestication of RJF (Yamashita et al. 1994, Fumihito et al. 1996, Vanhala et al. 1998, Romanov and Weigend 2001, Niu et al. 2002, Irinia et al. 2003, Hillel et al. 2003, Olowfeso et al. 2005, Yu Ya-Ba et al. 2006, Sharma 2006 and Mukesh et al., 2011) has been carried out.

The pattern of genetic contamination of wild RJF, in an east-west direction along Southeast Asia as proposed by Peterson and Brisbin (1998) may also be contentious. Irrespective of the apparent sampling inadequacy, the threat of hybridisation of RJF with domestic chicken in India is real and needs to be addressed urgently. The extent of hybridization between wild RJF and domestic fowl is stressed to be of international importance in the IUCN Action Plan (pages 7 & 46) for Pheasants (2000). Further, there has been no study conducted so far to understand the insight of the RJF population structures in India which might help in prioritizing the RJF populations for their conservation programmes. An investigation therefore was necessary to provide more information on the status of RJF in India and to suggest the ways in which to safeguard wild population. For this purpose, a number of markers were identified, evaluated and applied. Phenotypic trials were conducted only for baseline data.

3.2 Literature Review

Darwin (1868) was the probably first to indicate that chicken were derived from RJF since then there has been considerable controversy regarding its ancestry. There have been two schools of thought one that defends monophyletic origin (Fumihito et al., 1996) and the other on multiple origins (Liu et al., 2006 and Kanginakudru et al., 2008). Eriksson et al. (2008) provided evidence of presence of yellow skin color in domestic chicken which has been come from Grey Junglefowl (Gallus sonneratii) and suggested a hybrid origin of the domestic chicken. Molecular evidences for hybridisation between species in the genus Gallus raised the possibility that the other junglefowl species could also be the progenitors

of domestic fowl (Nishibori et al., 2005) which makes this issue more complicated. The distribution from east to west most probably was along the silk route during the 6th and 8th Centuries BC, via Turkistan, Iran and then to the Mediterranean region and central Europe. The historical records indicate that the initial domestication of the fowl was primarily for cultural and religious purposes. Fowls were kept for feathers, white and black magic and fighting, which must have influenced the selection of the birds, especially in terms of color and morphology (Crawford, 1990).

3.3 Methods for measuring genetic diversity

Genetic variation between populations can be the result of a number of factors including natural and artificial selection, mutation, migration, genetic drift and non-random mating (Hedrick, 1975). While breeding domesticated animals, man has strongly forced the accumulation of genetic differences between breeds and populations by isolating and selecting them for favourable traits. Therefore, to set up efficient conservation and utilization measures reliable information about genetic differences between individuals, populations and breeds are required. Quantitative assessment of genetic diversity within and among populations is an important tool for decision making in genetic conservation and utilization plans. The most widely used method to quantify these genetic diversities is by utilizing phenotypic characters, biochemical traits and molecular markers (Van Zeveren et al., 1990; Gueye, 1998; Weigend and Romanov, 2001 and Msoffe et al., 2001 & 2004).

3.3.1 Phenotypic and biochemical markers

Morphological and biochemical (protein) polymorphisms are among the first to determine the relationship between breeds (Moiseyeva et al., 1994 and Romanov, 1999). Phenotypic markers are cheap and easy to apply but they are subjected to environmental influences due to the nature of the qualitative and quantitative traits to be considered. Nikiforov et al. (1998) compared the Russian, Mediterranean and Asian chicken breeds with the RJF using morphological traits and clustered them into five different groups. Similarly, protein polymorphisms/ biochemical markers have been applied to estimate the genetic variation within and among chicken populations (Mina et al., 1991 and Romanov, 1994). The diversity of the local chickens reported so far is mostly on phenotypes including adult body weight, egg weight, reproduction performance and immune responses to various diseases (Gueye, 1998 and Msoffe et al., 2004). Limited reports have addressed the genetic diversity of the indigenous chickens (Van Marle-Köster and Nel, 2000 and Tadelle, 2003) with the primary aim to understand the extent of genetic variation within and among populations.

3.3.2 Molecular markers

a). Amplified Fragment Length Polymorphism (AFLP)

Amplified Fragment Length Polymorphism (AFLP) is a DNA fingerprinting technique that is based on the detection of DNA fragments, subjected to restriction enzymes, followed by selective PCR amplification. The AFLP technique can be done at a reasonable cost and has extensively been used, particularly in the genome mapping of plants. The technique has also been used for genetic analysis in chicken (Lee et al., 2000 and De Marchi et al., 2003).

b). Random Amplified Polymorphic DNA (RAPD)

Random amplified polymorphic DNA (RAPD) is also a technique that can be applied in evaluation of genetic diversity in Chicken (Mohd-Azmi et al., 2000; Sharma et al., 2001 and Romanov and Weigend, 2001). The RAPD technique is based on the use of a number of short arbitrary primers in one PCR reaction. These random primers generate several amplification products, which differ in size and may be characterized by simple agarose electrophoresis.

c). Restricted Fragment Length Polymorphism (RFLP)

RFLP generally refers to the differences in banding patterns obtained from DNA fragments, after digestion with restriction enzymes. Restriction enzymes (RE) bind to specific sequences and cut the DNA at a specific cleavage site. The DNA fragments of different lengths are then subjected to electrophoresis and fragments migrate according to their weights, the smaller fragments faster and the larger fragments slower. This application of RFLP is the more conventionally used, for example, for detection of diseases. Various restriction enzymes have already been shown to be useful in obtaining RFLP patterns in chicken (Xu et al., 2005). It is often required, that many enzymes need to be tested in the initial phase to identify the polymorphism, but even then it is still an easy and relatively cheap marker to use (Dodgson et al., 1997). Potential disadvantages of the RFLP technique are the dimorphic nature, since a RFLP only indicates the presence or absence of a cleavage site, and therefore does not provide a great deal of genotypic information. Large amounts of DNA are also required for RFLP analyses and the technique is relatively time consuming.

d). Single Nucleotide Polymorphisms (SNPs)

SNP refers to the substitution of one nucleotide by another. It could also be an addition or deletion of one or more nucleotides, causing the polymorphism (Xu et al., 2005). SNPs are bi-allelic markers, indicating a specific mutation (polymorphism) in two alleles only. In order to obtain information from a SNP marker, which is similar in complexity to that obtained from a microsatellite marker, at least five SNP markers are required (Beuzen et al., 2000). SNPs are also found in coding regions directly associated with the protein function. The inheritance pattern is more stable, making them more suitable markers for selection

over time (Beuzen et al., 2000). Most RFLP and AFLP markers are also the result of a SNP in a restriction enzyme recognition site, which confirms the importance of SNP markers.

e). Minisatellites

Minisatellites were first described as hypervariable tandem repeats, when found in the human genome. They were found to be longer repeats than microsatellites, consisting of up to 200bp. This led to the use of the term variable number of tandem repeat (VNTRs) loci in reference to repetitive units that include mini and microsatellites. Minisatellites are also well distributed throughout the eukaryotic genome (Bruford and Wayne, 1993). Minisatellite markers have certain limitations, as they do not uniformly mark the genome, the marker fragment is difficult to clone and they are dominant markers if the repeat is used as a probe, which reduces the potential information for genotyping (Dodgson et al., 1997).

f). Microsatellites

Microsatellites consist of tandem repeats between one and six bp, repeated up to 60 times and referred to as simple sequence loci. These domains were first demonstrated by Hamada and colleagues, during the eighties (Tautz, 1989). Repeat units may consist of (A)n, (TG)n, (CA)n or (AAT)n repeat. For example in most vertebrates the (CA)n repeat is the most common motif (Beuzen et al., 2000). Microsatellites are highly polymorphic due to the variation in the number of repeats. It is not uncommon to find up to 10 alleles per locus and heterozygosity values of 60% in a relatively small number of samples. Microsatellites are well distributed in animal genomes and are multiallelic, codominant and can be detected by PCR technology (Tautz, 1989). Tautz (1989) indicated that they could be involved in gene regulation or act as signals for recombination, as a certain amount of crossing over takes place within (GT)n repeat sequences. However, tandem repeat loci may also have a function in the packaging and condensing of the DNA in eukaryotic chromosomes. It was for example found that (GT)n repeat sequences are much more frequent in euchromatin than heterochromatin and therefore could be an important determinant in distinguishing between hetero and euchromatin. Microsatellites are found in both coding and non coding regions of eukaryotic genomes and are generated by a mechanism referred to as slippage (Tautz et al., 1986). Slippage occurs when normal pairing of repeats is altered during replication. A backward slippage causes an insertion of a repeat in the new strand and a forward slippage a deletion.

Two main mutation models have been associated with mutation at microsatellite loci: the infinite allele model (IAM) and the stepwise mutation model (SMM). The IAM states that all mutation events will create a new allele and that new allele may have more than one repeat difference with the ‘parental allele’. The SMM predicts that mutation will result in an allele which is one repeat larger or smaller. Here, difference in size conveys additional information about ancestral relationship between alleles. Under the IAM, alleles in different

individual can be either "same" or "different". Under the SMM, alleles can also either be the same or different but also alleles with large size differences will be more distantly related than alleles with small size difference. Wright's F-statistic underestimates population variation and is based on the IAM; Slatkin's R-statistic overestimates population variation and is based on the SMM. The average mutation rate of microsatellites ranges from 10-2 to 10-6 per locus per generation, among different species and different types of microsatellites. Microsatellites can be isolated by various methods. The development of microsatellite markers requires the construction of a genomic library. Briefly, the construction of a genomic library involves cloning the DNA of a specific species (e.g. chicken DNA) as follows: firstly, the genomic DNA is digested using restriction enzymes, which yield small DNA fragments. The DNA fragments are then cloned into vectors such as phages or plasmids, which allow proliferation in bacterial cells. The next step involves hybridization where thousands of clones are screened with synthetic polynucleotides such as (TG)13, (CAC)5, and (GAT)4 which are radioactively labeled with 32P-ATP. Positive clones are then isolated and sequenced (Crooijmans et al., 1993). The sequence information is used to synthesize PCR primers, which are then tested on a panel of unrelated animals. This step is essential to ensure that primers work optimally and there is no cross reactions (Crooijmans et al., 1993 and Crooijmans et al., 1997). Various microsatellites have been mapped for chicken (Cheng et al., 1994; Crooijmans et al., 1996 and Groenen et al., 2000) and used for studying the genetic relationship between and within chicken populations (Hillel et al., 2003, Osman et al., 2005a & b and Kaya et al., 2008). As a consequence these elements have become most valuable markers in studies on genetic variability, parentage verifications and genome mapping projects. A comparison of the molecular markers is given in Table 3.1.

g). Mitochondrial DNA (mt DNA)

Mammalian mitochondrial DNA is a small (15-20 kb) circular molecule, comprising of about 37 genes coding for 22 tRNAs, 2 rRNAs and 13 mRNAs. Within the coding region cytochrome b is the most widely used gene for phylogenetic work. This gene evolves slowly in terms of non-synonymous substitutions. In the non-coding region the major control region for mt DNA expression is the displacement loop (D-loop), which has also been used in evolution studies. The D-loop is also widely used in evolution studies and it has a rate of nucleotide substitution five to ten times higher than that of nuclear DNA. The mt DNA polymorphisms have been widely used to investigate the structure of populations, interspecies variability, evolutionary relationships between populations or species and for the identification of maternal lineages. The mt DNA has some importance characteristics that include maternal inheritance, high mutation rate, high copy number and lack of recombination (unlike autosomal or X chromosome specific loci). The D-loop is mainly used

for intraspecies variation studies (Niu et al., 2002) while the cytochrome b is for interspecies variation (Shen et al., 2002).

h). Y chromosome

The Y chromosome is the only portion of the mammalian genome that is exclusively paternally transmitted and therefore defines patrilineages. The Y chromosome consists of pseudoautosomal regions at the tips of both the long and short arm; the sex-determining gene (SRY) on the short arm and heterochromatin on the long arm. With exception to the pseudoautosomal region, the Y-chromosome acts as a single non recombining unit which is male specific and effectively haploid. Y-specific single nucleotide polymorphisms have slow mutation rate (10-9 per mutation site per year) and this contrasts the high rates observed in microsatellites (3 x 10-3 per mutation locus per generation). Studies of Y chromosome polymorphism are still uncommon due to the lack of polymorphic markers in most ruminant species. In cattle Y-specific microsatellite markers have been reported and they include INRA124, INRA189 and BM861. In horse six markers have recently been isolated (ECAYM2, ECAYP9, ECAYH12, ECAYE1, ECAYJ10, ECAYA16). Recently in sheep, a novel A/G single nucleotide polymorphism (SNP) located in the male-specific region of the Y chromosome (MSY) has been identified using comparative sequencing. A polymorphic microsatellite SRYM18 with at least 4 alleles has also been described (Meadows et al., 2006).

3.3.3 Development of Microsatellite Markers In Chicken

The chicken is the first bird, as well as the first agricultural animal, to have its genome sequenced and analyzed. As the first livestock species to be fully sequenced, the chicken genome sequence is a landmark in both avian biology and agriculture (Burt, 2005) and therefore provides a vast number of microsatellite markers for diversity studies. A number of microsatellite markers based on the degree of polymorphism and genome coverage have been recommended for the Measurement of Domestic Animals Diversity (MoDAD) (FAO, 2004a), for application in diversity studies that is available on the FAO website (www.dad.fao.org/en/refer/library/guidelin/marker.pdf). Various microsatellites have been developed for diversity assessment in chicken (Crooijmans et al., 1993; Cheng et al., 1994; Crooijmans et al., 1996; AVIANDIV, Weigend et al., 1998 and Groenen et al., 2000).

Table 3.1 Comparison of commonly used genetic markers

Feature	RFLP	SSR	RAPD	AFLP	Isozymes
Origin	Anonymous/ Genic	Anonymous	Anonymous	Anonymous	Genic
Maximum theoretical number of possible loci in analysis	Limited by the restriction site polymorphism (tens of thousands)	Limited by the size of genome and number of simple repeats in a genome (tens of thousands)	Limited by the size of genome, and by nucleotide polymorphism (tens of thousands)	Limited by the restriction site polymorphism (tens of thousands)	Limited by the number of enzyme genes and histochemical enzyme assays available (30-50)
Dominance	Codominant	Codominant	Dominant	Dominant	Codominant
Null alleles	Rare	Occasional to common	Not applicable	Not applicable	Rare
Transferability	Across genera	Within genus or species	Within species	Within species	Across 6-FAMilies and genera
Reproducibility	High to very high	Medium to high	Low to medium	Medium to high	Very high
required per sample	2-10 mg DNA	10-20 ng DNA	2-10 ng DNA	0.2-1 µg DNA	Several mg of tissue
Ease of development	Difficult	Difficult	Easy	Moderate	Moderate
Ease of assay	Difficult	Easy to moderate	Easy to moderate	Moderate to difficult	Easy to moderate
Automation / multiplexing	Difficult	Possible	Possible	Possible	Difficult
Genome and QTL mapping potential	Good	Good	Very good	Very good	Limited
Comparative mapping potential	Good	Limited	Very limited	Very limited	Excellent
Candidate gene mapping potential	Limited	Useless	Useless	Useless	Limited
Potential for Adaptive genetic variation	Limited	Limited	Limited	Limited	Good
Development	Moderate	Expensive	Inexpensive	Moderate	Inexpensive
Assay	Moderate	Moderate	Inexpensive	Moderate	Inexpensive
Equipment	Moderate	Moderate to expensive	Moderate	Moderate to expensive	Inexpensive

3.4 MATERIALS AND METHODS

3.4.1 Study Area

The historical distribution range of RJF in India was divided into five different zones viz., Northern (Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Haryana, Punjab and Uttar Pradesh), Central (Madhya Pradesh and Chhattisgarh), Eastern (Bihar, Jharkhand, West Bengal and Sikkim), Southeast (Odisha and Andhra Pradesh) and Northeast (Assam, Arunachal Pradesh, Nagaland, Mizoram, Manipur, Tripura and Meghalaya).

3.4.2 Live trapping and sample collection

A modified version of foot hold snare was designed (Petrides, 1946). First a noose was made using a fishline and then tied up with an anchor or a bamboo stick. Approximately, 20-25 nooses of such kind were tied up on a string of 10-12 m long (Ramesh et al., 2008). For live trapping, the snares were placed along the open forest trails or fire lines or agriculture fields at the forest fringes where RJF foraged. The snares design and their setting up in the field are shown in Figure 3.1 (A-F). At few places, a local country chicken was also used as decoy to attract the RJF towards the snares. These snares were monitored regularly and in the event of a capture, the bird was carefully handled and 1.0 ml of blood sample was drawn through brachial vein-puncture method using a 1 ml of disposable syringe. Collected blood samples were stored in two ways (A) in DNA zol®BD and (B) on FTA cards (Mackey et al., 1996 and 1997). The details of samples and their sampling locations are shown in Table 3.2.

The Sample collection protocol using DNA zol®BD was as follows:

1. Approximately 0.5 ml of blood was taken and mixed with 1.0 ml of DNA zol®BD.
2. The mixture was shaken vigorously by hand for 15-20 sec to lystae all the nucleated red blood corpuscles.
3. Then 0.4ml of Isopropnal was added to the DNA zol®BD – blood lysate.
4. It was shaken vigorously and stored at room temperature till isolation of DNA.
5. The identity/ number, sex, location and date of collection were recorded. An adequate precaution was taken during storage and transportation of the samples.
6. The Sample collection protocol using FTA cards was as follows:
7. 1 or 2 drops of blood was placed on the card in a concentric circular motion within the printed circle area and dried it before folding for storage at room temperature.
8. One FTA card was used for one individual samples and the identity/ number, sex, location and date was recorded. FTA card was safely stored and care was taken for it not to get soiled or exposed to humidity during transportation.

3.4.3 Genomic DNA extraction

DNA isolation is of crucial importance, since pure and high molecular weight DNA is absolute requirement for the success and reliability of downstream steps involved in fragment analysis. Any impurities or contamination in the DNA can inhibit or alter enzymatic reactions, electrophoresis and DNA quantification. Genomic DNA was isolated using the protocol in the accordance of type of sample and their storage method.

3.4.3.1 Isolation of genomic DNA from whole blood using DNA zol®BD

The isolation of genomic DNA stored in DNA zol®BD was carried out as follows:

A). Lysis: 1 ml of DNA zol®BD was mixed with 0.5 ml of whole blood by vortexing.

B). DNA precipitation : 0.4 ml of isopropanol was added to the DNA zol®BD-blood lysate. It was vortexed or shaken vigorously and stored it for 5 min at room temperature. The g-DNA sedimented by centrifugation at 6,000 × g for 6 min.

C). DNA wash: Following centrifugation, the supernatant was removed and 0.5 ml of DNA zol®BD was added to DNA pellet. It was revortexed or shaken till the DNA pellet was completely dispersed. The resulting mixture was centrifuged at 6,000 × g for 5 min. The supernatant was then removed and DNA pellet was washed by mixing with 1 ml of 75% ethanol and centrifuged at 6,000 × g for 5 min.

D). DNA solubilisation: The DNA pellet was washed with ethanol and flow through was removed by decanting. The tubes were stored vertically and the remaining ethanol was removed with a micropipette. Without drying the DNA pellet, 200 µl of 8 mM NaOH was added and solubilised DNA by incubation at room temperature for 3-5 min followed by repetitive pipetting or vortexing. The alkaline DNA solution was neutralized with 0.1 M HEPES. Alternatively to 8 mM NaOH, DNA could be solubilised in water. However, it takes more effort and time to fully solubilise the DNA pellet in water. Typical yield of DNA was 20-40 µg/ml. An adequate amount of 8 mM NaOH or water was added to achieve a DNA concentration of about 0.1 µg/µl. At higher concentrations, the solution is extremely viscous due to the presence of high molecular weight DNA. Alkaline solution was neutralized by CO2 from the air. 8 mM NaOH was prepared once a month from a 2-4 M NaOH stock solution of less than 6 months old.

3.4.3.2 Isolation of genomic DNA stored on FTA cards

FTA cards are designed for room temperature collection, shipment, archiving, and purification of nucleic acids from a wide variety of biological samples for PCR analysis. FTA cards are impregnated with a patented chemical formula that lyses cell membranes and denatures proteins upon contact. Nucleic acids are immobilized and protected from UV damage and microbial and fungal attack.

The isolation of genomic DNA stored on FTA cards was carried out as follows:

A. FTA card was placed on a clean, dry and flat surface and a sample disc of size 1.2 mm was taken using Harris Micro Punch.

B. The sample disc was then placed in a PCR amplification tube and 200 µL of FTA Purification Reagent was added.

C. The tube was moderately shaken by hand and incubated for 5 minutes at room temperature.

D. All spent FTA purification reagent was removed and discarded using a pipette.

E. The steps A-D were repeated twice, for a total of three washes with FTA Purification Reagent.

F. 200 µL of TE-1X buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) was added to PCR Tube.

G. Incubated for 5 minutes at room temperature and all spent TE-1X buffer was removed with a pipette.

H. The steps F-G were repeated once for a total of two washes with TE-1X buffer.

I. The discs were allowed to dry at room temperature for about one hour, or heat assisted the drying of disc at 56°C for 10 minutes. The FTA discs were now ready for PCR.

J. Alternatively, for elution of DNA, threes discs of 1.2 mm size were placed in a PCR tube and added 50 µl of DNase free water.

K. The tubes were incubated at 90°C for 10 min to release much of the DNA.

3.4.4 Quantity and quality assessment of isolated DNA

3.4.4.1 Quantity assessment of isolated DNA

DNA quantification can be done using spectrophotometric measurement of UV absorption at wavelengths 230, 260 and 280 nm. Measures of DNA purity can be determined by the A260:A280 and A260:A230 ratios. These ratios provide indications of protein, and polyphenol and carbohydrate contamination, respectively. The DNA should show a clear absorbance peak at 260 nm. The A260 value provides a measure of concentration (roughly 1.0 reading at A260 is equivalent to 50 mg/ ml). A pure DNA solution has an A260:A280 ratio of 1.8 ± 0.1. The concentration of unknown double stranded DNA samples was estimated using the following formula:

$$\text{DNA Concentration } (\mu g/ml) = \frac{\text{OD 260 X dil. Factor X 50 } \mu g/ml}{1000}$$

Table 3.2 Details of Red Junglefowl samples collected from different States

Zone	RJF range state	Locality of the samples collected		Total samples
		Protected area (# samples)	Captive center (# samples)	
North	Jammu & Kashmir	Nandini WS (1), Surinsar-Mansar WS (1)		2
	Himachal Pradesh	Simbalbara WS (2)	Chail (8), Renuka (12), Kufri (19), Shimla (15), Gopalpur (23)	79
	Uttarakhand	Rajaji NP (2), Corbett NP (1), WII (4), Terai East FD (1)	Nainital zoo (5)	13
	Haryana		Morni (109)	109
	Uttar Pradesh	Kishanpur WS (2), Dudhwa NP (1), Katerniaghat WS (1), Pilibheet (16)	Kanpur (1), Lucknow (6)	27
	Delhi		Delhi zoo (24)	24
Central	Chattisgarh	Udanti WS (2)		2
Eastern	Bihar	Gautambuddha WS (1), Valmiki NP (3), Kaimur WS (1), Bhimbandh WS (3)	Patna (2)	10
	Jharkhand		Tata (10)	10
	West Bengal		Padmaja Naidu ZP(4)	4
	Sikkim	Kitam (1)		1
South-East	Andhra Pradesh		Vizag zoo (4)	4
	Odisha	Kutub WS (1), Kotgarh (1), Bhittarkanika WS (1), Kyandadmala (1)		4
North-East	Meghalaya	Dadengiri (1), Nongkhyllem (1)		2
	Mizoram	Khuanglung (1)		1
	Assam	Manas WS (2), Nameri (1), Hailakandi (1),		2
	Nagaland	Khelma (1)		1
	Tripura		Sepahijala (7)	7
	Manipur	Sanapati (1), Bishnupur (3)		4

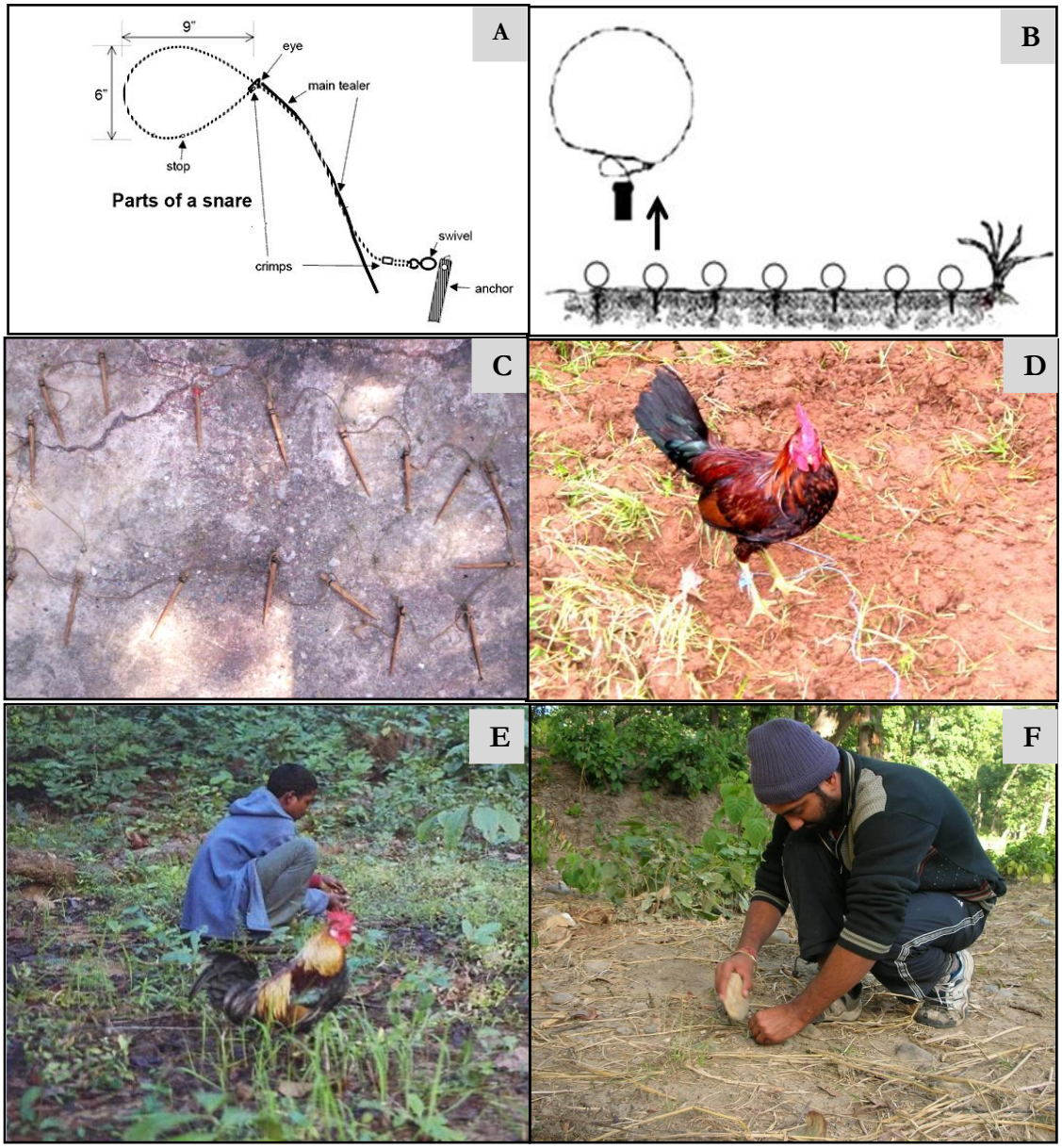


Figure 3.1 Trap design (A-C) and their setting up in the field for live trapping of wild Red Junglefowl (D-F)

3.4.4.2 Quality assessment of isolated DNA

Requirements: Agarose, TBE buffer, Ethidium bromide, DNA samples, DNA ladder and DNA loading dye.

Protocol

A). Casting the gel

- The gel casting tray was prepared for casting the agarose gel by making it grease free using 70 % ethanol.
- 0.8 % agarose in TBE buffer. (0.8g in 100 ml TBE buffer) was prepared and boiled in microwave oven to dissolve.
- 4µl/100ml of ethidium bromide (EtBr-20mg/ml) was added after cooling down the temperature of gel about 50^{o}C.
- The comb was placed in gel casting tray and the prepared gel was poured without formation of air bubbles into it and allowed for polymerization. The gel was 3-5 mm thick.
- After the gel had solidified, the comb was removed carefully by wriggling back and forth gently and then lifting up carefully, so as not to rip the bottom of the well.

B). Preparation and loading of samples

- While the gel was cooling, 2µl of loading dye was mixed with 2 µl of each DNA sample to be loaded. Molecular size standard was also prepared by mixing 2 µl of 1 kb ladder with 2 µl of loading dye.
- The casted gel along with the tray was inserted horizontally into the electrophoresis chamber and the top of the gel was flooded with fresh running buffer (1X TBE) to cover the gel to depth of about 1 mm.
- The solution (DNA sample with dye) was sucked into the pipette and the tip was placed at the top of the well and gently the solution was expelled into the well.

C). Gel electrophoresis and visualization of DNA

- The lid and power leads were placed to the apparatus and the gel was electrophorised at 100 volts for 30-60 mins or till the tracking dye reaches up to three-fourth of the way across the gel.
- The DNA bands were visualized by placing the tray onto High Performance UV Transilluminator and photographed using gel documentation system (Figure 3.2).

3.4.5 Selection of Microsatellite markers

A set of 30 highly polymorphic microsatellite markers developed for chicken and earlier used in 'European Chicken Biodiversity Project' (AVIANDIV, Weigend et al., 1998) were selected for the present study (Table 3.3). The selection criteria were primarily based on high degree of polymorphism of the markers. The primers were synthesized and forward primer of each marker was labeled with fluorescent dyes (6-FAM, VIC, PET, NED) at the 5' end.

Table 3.3 Selected microsatellite markers used in the present study

Marker	Chrom	Map Position [cM (Mb)]	Primer Sequence *(5' -> 3')* (Forward & Reverse)	Allele range	Genbank Accession
ADL0268	1	288 (82.96)	CTCCACCCCTCTCAGAACTA CAACTTCCCATCTACCTACT	102-116	G01688
MCW0206	2	104 (30.49)	ACATCTAGAATTGACTGTTCAC CTTGACAGTGATGCATTAAATG	221-249	AF030579
LEI0166	3	300 (103.36)	CTCCTGCCCTTAGCTACGCA TATCCCCTGGCTGGGAGTTT	354-370	X85531
MCW0020	1	460 (156.62)	TCTTCTTTGACATGAATTGGCA GCAAGGAAGATTTTGTACAAAATC	179-185	L40055
MCW0037	3	317 (106.71)	ACCGGTGCCATCAATTACCTATTA GAAAGCTCACATGACACTGCGAAA	154-160	L43676
LEI0192	6	31 (2.41)	TGCCAGAGCTTCAGTCTGT GTCATTACTGTTATGTTTATTGC	244-370	Z83797
ADL0112	10	120 (20.83)	GGCTTAAGCTGACCCATTAT ATCTCAAATGTAATGCGTGC	120-134	G01725
MCW0295	4	75 (16.09)	ATCACTACAGAACACCCTCTC TATGTATGCACGCAGATATCC	88-106	G32052
MCW0067	8	59 (8.89)	GCACTACTGTGTGCTGCAGTTT GAGATGTAGTTGCCACATTCCGAC	176-186	G31945
MCW0104	13	74 (16.60)	TAGCACAACTCAAGCTGTGAG AGACTTGCACAGCTGTGTACC	190-234	L43640
MCW0111	1	118 (39.97)	GCTCCATGTGAAGTGGTTTA ATGTCCACTTGTCAATGATG	96-120	L48909
MCW0034	2	233 (69.66)	TGCACGCACTTACATACTTAGAGA TGTCCTTCCAATTACATTCATGGG	212-246	L43674
MCW0222	3	85 (19.35)	GCAGTTACATTGAAATGATTCC TTCTCAAAACACCTAGAAGAC	220-226	G31996
LEI0094	4	153 (50.65)	GATCTCACCAGTATGAGCTGC TCTCACACTGTAACACAGTGC	247-287	X83246
MCW0216	13	47 (11.88)	GGGTTTTACAGGATGGGACG AGTTTCACTCCCAGGGCTCG	139-149	AF030586
MCW0081	5	151 (45.68)	GTTGCTGAGAGCCTGGTGCAG CCTGTATGTGGAATTACTTCTC	112-135	L43636
MCW0330	17	41 (7.01)	TGGACCTCATCAGTCTGACAG AATGTTCTCATAGAGTTCCTGC	256-300	G32085
LEI0234	2	50 (10.72)	ATGCATCAGATTGGTATTCAA CGTGGCTGTGAACAAATATG	216-364	Z94837
MCW0103	3	201 (67.76)	AACTGCGTTGAGAGTGAATGC TTTCCTAACTGGATGCTTCTG	266-270	G31956
MCW0098	4	217 (78.89)	GGCTGCTTTGTGCTCTTCTCG CGATGGTCGTAATTCTCACGT	261-265	L40074
MCW0284	4	167 (53.91)	CAGAGCTGGATTGGTGTCAAG GCCTTAGGAAAAACTCCTAAGG	235-243	G32043
MCW0069	E60C0	47	GCACTCGAGAAAACTTCCTGCG	158-176	L43684

	4W23	(1.21)	ATTGCTTCAGCAAGCATGGGAGGA		
MCW0016	3	247 (~90)	ATGGCGCAGAAGGCAAAGCGATAT TGGCTTCTGAAGCAGTTGCTATGG	162-206	L40041
MCW0078	5	93 (26.44)	CCACACGGAGAGGAGAAGGTCT TAGCATATGAGTGTACTGAGCTTC	135-147	L43686
MCW0014	6	50 (6.38)	TATTGGCTCTAGGAACTGTC GAAATGAAGGTAAGACTAGC	164-182	L40040
MCW0183	7	86 (23.42)	ATCCCAGTGTCGAGTATCCGA TGAGATTTACTGGAGCCTGCC	296-326	G31974
MCW0123	14	45 (13.61)	CCACTAGAAAAGAACATCCTC GGCTGATGTAAGAAGGGATGA	76-100	L43645
MCW0165	23	1 (0.59)	CAGACATGCATGCCCAGATGA GATCCAGTCCTGCAGGCTGC	114-118	L43663
MCW0248	W29	19 (0.58)	GTTGTTCAAAAGAAGATGCATG TTGCATTAACTGGGCACTTTC	205-225	G32016
ADL0278	8	94 (29.24)	CCAGCAGTCTACCTTCCTAT TGTCATCCAAGAACAGTGTG	114-126	G01698

3.4.6 Polymerase chain reaction (PCR)

The microsatellites provide an abundant class of closely spaced highly informative markers that can be amplified by PCR. In addition, they can be genotyped simply by their size differences. The PCR process is a relatively straightforward laboratory technique, requiring only a very small amount of the source DNA.

3.4.7 PCR cycle

The PCR cycle is a 3-step process:

(1) Denaturation. The first step of the PCR process is to create single-stranded DNA templates for replication. The double-stranded DNA molecules are denatured to form single DNA strands by an increase in temperature (to around 94°C for 2 min);

(2) Primer annealing. The second step is to specify a region on the DNA to be replicated. Specific primer pairs to the flanking regions of gene of interest were added to the reaction so that DNA region between the flanking primers will be amplified. Usually primers anneal at a temperature around 55°C for 30-40 sec.

(3) Primer extension. The third step is to amplify the specified DNA region. With the primers annealed to the binding sites to direct amplification, the Taq DNA polymerase synthesizes new complementary strands, which can then be denatured, annealed with primers, and amplified again. The concentration of the target sequence is doubled with each PCR cycle. After n PCR cycles, and there are 2n replicated DNA molecules in the resulting PCR mixture. Typically, the PCR cycle is repeated for as many as 30-40 cycles, taking several hours to complete and resulting in the formation of millions of copies of desired target DNA fragment.

Polymerase chain reactions (PCR) were performed on an Applied Biosystems thermal cycler (2700 and 2720) in a 10µl reaction mixture containing 1× PCR Buffer (50 mM KCl, 10 mM tris–HCl), 1.5 mM MgCl2, 200 µM of each dNTP, 1.25 µg BSA, 4 p-mole of each primer, 0.5 unit of Taq DNA polymerase (MBI, Fermentas) and approximately 25 ng genomic DNA. The amplification conditions were initial denaturation at 94°C for 2-min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 45 s and extension at 72°C for 2 min with a final extension at 72°C for 15 min. Annealing temperature for each microsatellite loci was first tested as reported in the published literature and then modified accordingly to get the optimum amplification. Approximately, 5 µl of PCR products were mixed with 1 µl of loading dye and then loaded onto a 2% agarose gel along with the size standard and visualised over trans-illuminator to detect amplification (Figure 3.3).

3.4.8 Post PCR multiplexing of microsatellite loci for fragment analysis

Primers producing visible and expected bands were labeled with fluorescent dyes (6-FAM, VIC, PET, NED) at the 5' end. PCR products of different sizes and dyes were pooled for one capillary injection for maximizing the throughput. It was important to pool PCR products together at the correct ratios, in order to get similar fluorescent intensities across all loci in the pooling.

In a pool the following ratio was used.

- PCR products labeled with VIC dye – 2.0 µl
- PCR products labeled with PET dye– 1.5 µl
- PCR products labeled with NED dye– 1.0 µl
- PCR products labeled with 6-FAM dye– 1.0 µl

The PCR products were pooled in such a way that the markers labeled with the same dye colour should not overlap the size ranges. A computer program Multiplex manger version 1.0 was used for pooling of PCR products and generating the multiplex panel. An output of Multiplex manager was shown in Figure 3.4. Thus, five multiplex panels of the thirty microsatellites (six markers in each panel) were generated as shown in the Table 3.4.

3.4.9 Size standard

Gene Scan 500 (-250) LIZ TM developed and supplied by Applied Biosystem, USA was used as internal lane size standard for fragment analysis. LIZ size standard yield size fragments between 50-500 bp providing 16 single standard labeled fragments of 35, 50, 75, 100, 139, 150, 160, 200, 300, 340, 350, 400, 450, 490 and 500 bases.

Each of the DNA fragments labeled with a proprietary fluorophore, which results in a single peak when run under denaturing condition. Internal lane size standard was run with every sample for accurate sizing. The genotyping reaction components were as follows:

- Pooled PCR Product- 1.0 µl
- Hi-Di Formamide-8.85 µl
- Gene Scan 500 (-250) LIZ ^{TM} – 0.15 µl

3.4.10 Denaturation

The genotyping reactions were mixed well and transferred to 96-well plate and denatured at 950C for 5 min. Fragment analysis was carried out in 3130 automated DNA sequencer (Applied Biosystem, USA).

3.4.11 Fragment analysis by automated genetic analyzer

Automated Genetic Analyzer- ABI 3130 Avant, (Applied Biosystem, USA) was provided with four capillaries with different array sizes (22cm, 36cm, 50cm and 80cm). Genotyping was performed using 36cm array size. The large surface area of a capillary allowed heat generated during electrophoresis to be dissipated efficiently, allowing high voltage electrophoresis. The result was rapid, high-resolution separation of DNA fragments. Polymer POP 7 (Performance Optimized Polymer) was used for sizing and separating the DNA fragments. Plate records were prepared and size standard was added to Automated DNA Sequencer prior to set up of the run and 96 well plate was linked and finally the run was started.

3.4.12 Data extraction

The electrophoresed data was extracted from Automated DNA Sequencer and allele sizing was done in GeneMapper software version 3.7 (Applied Biosystem, USA) as shown in Figure 3.5. The sizing of the alleles was extrapolated from the regression curve (Figure 3.6) drawn using Gene Scan LIZ 500 (-250) internal size standard. The data was exported as text file and imported in MS Excel for further refinement. The data for all the microsatellite studied was compiled in one excel sheet and then subjected for further statistical analysis.

3.4.13 Statistical analysis of microsatellite data

The data generated for 30 loci was first checked for any associated error and then subjected to further analysis. The heterozygosity measures were calculated using the appropriate software.

3.4.13.1 Genotyping errors

Following errors can commonly occur during amplification of microsatellite loci by polymerase chain reaction (PCR):

- Null alleles – one or more alleles fail to amplify during PCR.
- Stuttering – slight changes occur in the allele sizes during PCR.
- Large allele dropout – large alleles do not amplify as efficiently as small alleles.

These errors may affect the interpretation of microsatellite allele data at later stages. Therefore, these associated errors were assessed using the computer program MICROCHECKER version 2.2 (Van Oosterhout *et al*., 2004). The program uses a Monte Carlo simulation (bootstrap) method to generate expected homozygote and heterozygote allele size difference frequencies. The Hardy-Weinberg theory of equilibrium is used to calculate expected allele frequencies and the frequency of any null alleles detected.

3.4.13.2 Genetic diversity statistics

Different parameters of genetic diversity estimates were calculated using following softwares.

- Program CONVERT version 1.31 (Glaubitz, 2004) and CREATE version 1.33 (Coombs *et al.,* 2008) were used for inter conversion of different file format required for different genetic analysis software.
- Diversity estimates *i.e.* observed (Na) and effective number of alleles (Ne), observed (Ho) and expected heterozygosity (He) were calculated using POPGENE version 1.32 software (Yeh *et al*., 1999).
- Null allele frequencies and polymorphic information content, a measures of marker's informativeness were predicted using CERVUS version 3.0 software (Kalinowski *et al.,* 2007)
- Graphical representation of genetic diversity estimates *i.e.* allelic pattern across population, graphs for allele frequencies of microsatellite loci and AMOVA was drawn using GENEALEX version 6.4 software (Peakall and Smouse, 2006).
- Nei's genetic distances and UPGMA dendrogram was generated using POPGENE version 1.32 software (Yeh *et al*., 1999) and TFPGA version 1.3 software (Miller, 1997).
- Significance tests for deviation from Hardy Weinberg Equilibrium equation was tested using GENEPOP version 4.0 (Raymond and Rousset, 1995) and TFPGA version 1.3 software (Miller, 1997).
- Multifactorial correspondence analysis was done using GENETIX version 4.02 (Belkhir *et al.,* 2004).
- Analysis of molecular variance (AMOVA), population differentiation (F_{ST}) and gene flow (N_m) were estimated using GENEALEX version 6.4 software (Peakall and Smouse, 2006).

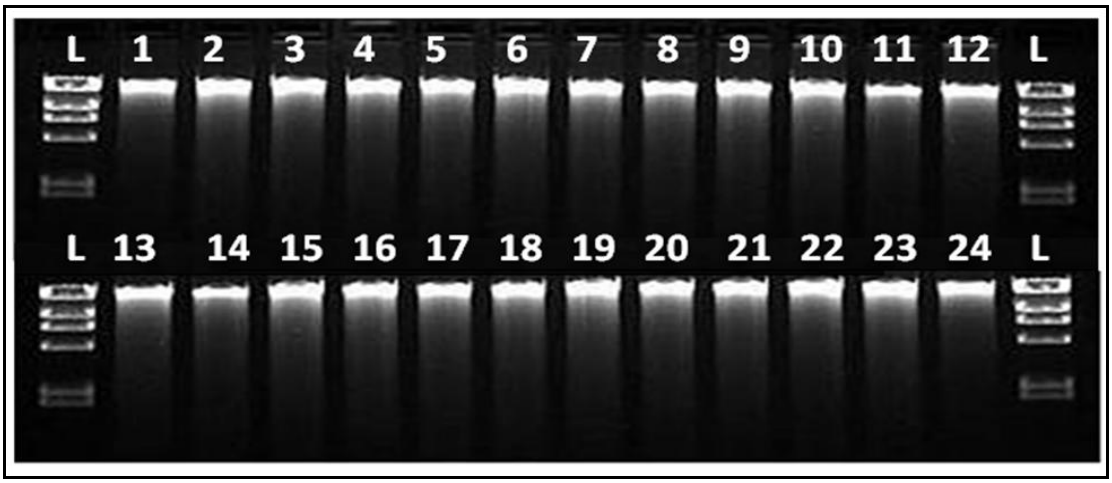


Figure 3.2 Agarose gel electrophoresis (0.8% agarose) of extracted DNA (L- 1Kb DNA Ladder, Lane 1 to 24- RJF DNA samples

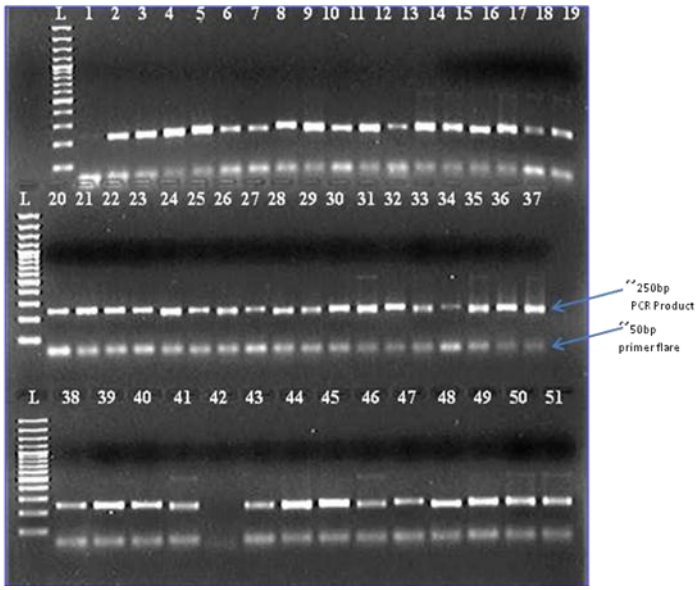


Figure 3.3 PCR amplification of locus MCW0206 with red junglefowl DNA samples (L- 100bp DNA Ladder, Lane 1 to 51- PCR Products)

Table 3.4 Five multiplex panels of thirty microsatellite markers

Multiplex Panel	Microsatellite loci	Dye color	Size Ranges (bp)
Mplex-1	ADL0268	NED	102-116
	MCW0067	6-FAM	176-186
	LEI0234	VIC	216-364
	MCW0165	PET	114-118
	MCW0206	NED	221-249
	MCW0295	6-FAM	88-106
Mplex -2	ADL0112	NED	120-134
	MCW0034	6-FAM	212-246
	MCW0103	VIC	266-270
	MCW0078	PET	135-147
	MCW0248	PET	205-225
	MCW0111	6-FAM	96-120
Mplex- 3	MCW0037	NED	154-160
	MCW0216	6-FAM	139-149
	MCW0330	VIC	256-300
	MCW0014	PET	164-182
	LEI0166	NED	354-370
	MCW0104	6-FAM	190-234
Mplex- 4	MCW0020	NED	179-185
	LEI0094	6-FAM	247-287
	MCW0081	VIC	112-135
	MCW0016	PET	162-206
	MCW0123	PET	76-100
	MCW0098	VIC	261-265
Mplex-5	MCW0222	6-FAM	220-226
	MCW0069	VIC	158-176
	MCW0284	VIC	235-243
	MCW0183	PET	296-326
	ADL0278	PET	114-126
	LEI0192	NED	244-370

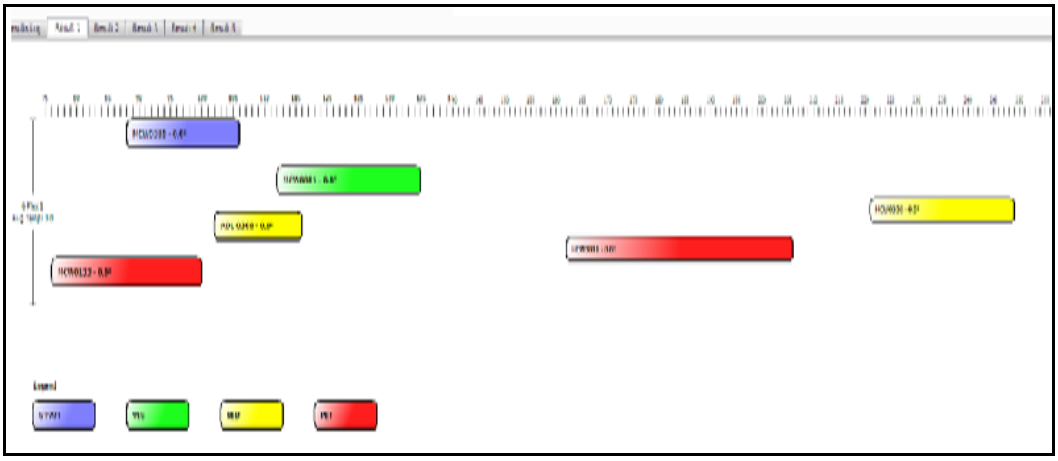
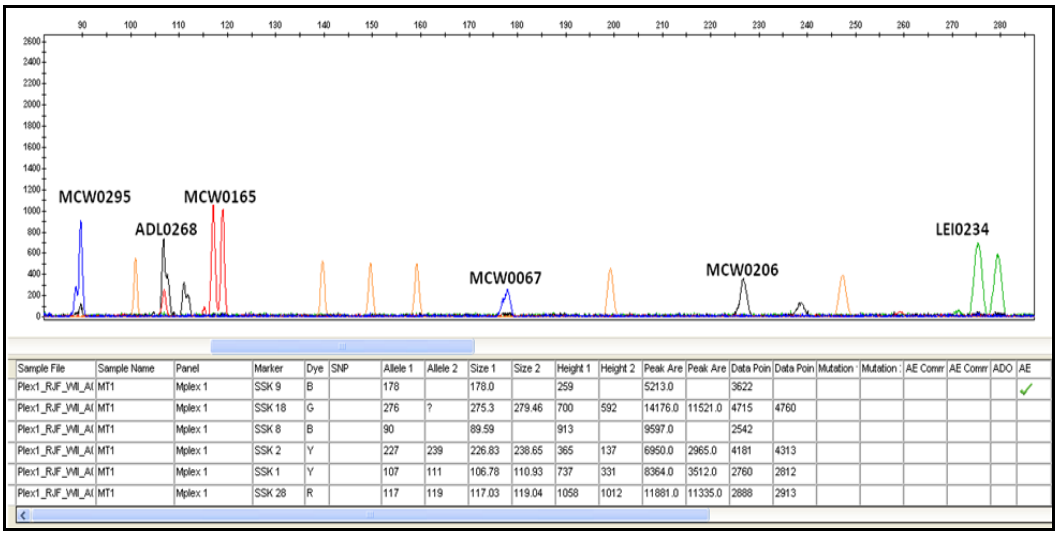


Figure 3.4 Generation of multiplex panel using program Multiplex Manager



Sample File	Sample Name	Panel	Marker	Dye	SNP	Allele 1	Allele 2	Size 1	Size 2	Height 1	Height 2	Peak Are	Peak Are	Data Poin	Data Poin	Mutation	Mutation	AE Comm	AE Comm	ADO	AE
Plex1_RJF_VMI_A(	MT1	Mplex 1	SSK 9	B		178		178.0		259		5213.0		3622							✓
Plex1_RJF_VMI_A(	MT1	Mplex 1	SSK 18	G		276	?	275.3	279.46	700	592	14176.0	11521.0	4715	4760						
Plex1_RJF_VMI_A(	MT1	Mplex 1	SSK 8	B		90		89.59		913		9597.0		2542							
Plex1_RJF_VMI_A(	MT1	Mplex 1	SSK 2	Y		227	239	226.83	238.65	365	137	6950.0	2965.0	4181	4313						
Plex1_RJF_VMI_A(	MT1	Mplex 1	SSK 1	Y		107	111	106.78	110.93	737	331	8364.0	3512.0	2760	2812						
Plex1_RJF_VMI_A(	MT1	Mplex 1	SSK 28	R		117	119	117.03	119.04	1058	1012	11881.0	11335.0	2888	2913						

Figure 3.5 Allele calling by Gene Mapper version 3.7 of multiplex panel 1

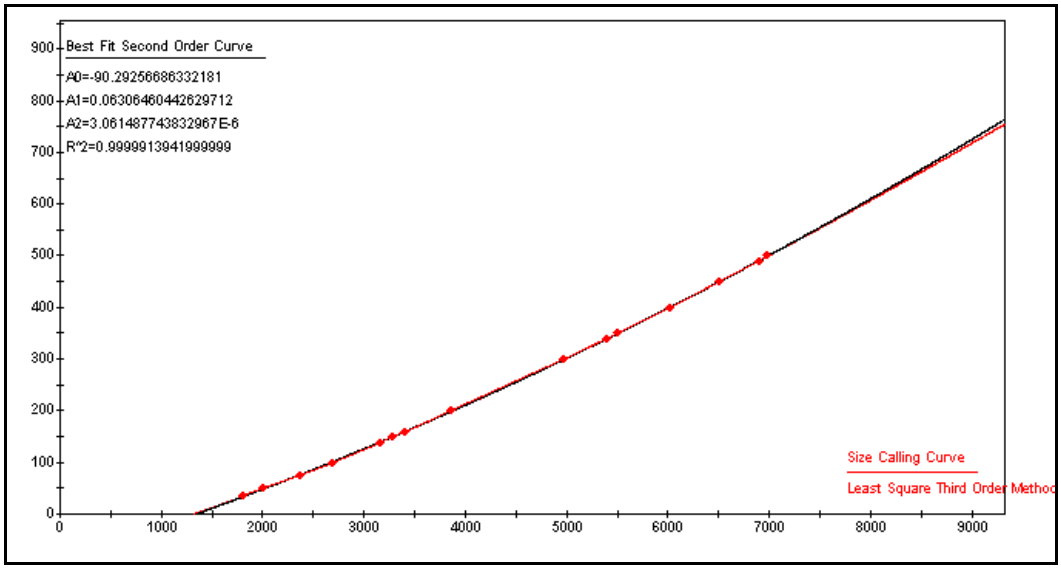


Figure 3.6 Regression Curve of Gene Scan LIZ 500 (-250) for allele sizing of RJF samples

3.5 RESULTS

A total of 385 samples (305 RJFs and 79 domestic chickens) were collected from 19 RJF range states of India and genotyped with 30 highly polymorphic microsatellite markers. Of these four microsatellite markers showed success rate < 80%, *i.e.* LEI0192 (69%), MCW0284 (69%), MCW0183 (71%) and MCW0014 (76%) were therefore excluded from further analysis. The remaining 26 loci showed success rate > 80% and subjected for further analysis.

3.5.1 Genetic Diversity Statistics and polymorphism at 26 loci

All 26 loci across six populations (5 RJF and one domestic chicken) were polymorphic and the diversity estimates have been presented in Table 3.5. In total, 628 alleles were examined across the 26 microsatellite loci. The number of observed alleles ranged from 49 (LEI0234) to 9 (MCW0103), with overall mean number of alleles per locus of 24.15 (±8.31 s.e.). The observed number of alleles for all loci exceeded the effective number of alleles, which varied from 12.40 (MCW0123) to 2.96 (MCW0216) with mean 6.50±2.71. The effective number of alleles depicted the number that should not be lost from the population with due course of time by chance. Observed (HO) and expected heterozygosity (HE) ranged from 0.23 (MCW0103) to 0.79 (MCW0069) and from 0.62 (MCW0103) and 0.92 (MCW0123), respectively. The mean observed heterozygosity was 0.52 (±0.13s.e) which was lower than the mean expected heterozygosity 0.82 (±0.08s.e) that points towards presence of population structure in the studied populations. Polymorphic Information Content (PIC) ranged 0.56 to 0.91 with an average of 0.80. PIC value for all 26 loci was higher than 0.5, which is normally considered as informative in population-genetic analyses (Botstein et al., 1980).

3.5.2 Population wise genetic diversity and polymorphism

Population wise genetic diversity estimates i.e. observed and effective number of alleles, observed and expected heterozygosity was calculated for all the six populations and the results are presented in Table 3.6 and 3.7. The mean values of observed & effective number of alleles, frequency of those alleles whose frequencies >5%, <25% and <50%, no. of private alleles, Shannon's information index and mean expected heterozygosity are presented in Figure 3.7.

In total, 179 private alleles were found in northern RJF population and only single private allele was observed in South-Eastern RJF population while no private allele was found in central RJF population (Figure 3.8).

Genetic diversity estimates of Northern RJF population (Table 3.6) were as follows: The observed number of alleles ranged from 9 (MCW0103) to 40 (LEI0234), with overall mean number of alleles per locus of 21.2 (±7.14). The observed number of alleles for all the

26 loci exceeded the effective number of alleles, which varied from 2.28 (MCW0103) to 11.82 (MCW0295) with mean (± s.e.) of 5.56±2.44. Observed heterozygosity was ranged from 0.18 (MCW0103) to 0.78 (MCW0069). Mean (± s.e.) observed heterozygosity over 26 loci was 0.49±0.16, which was lower than the expected heterozygosity 0.79±0.10. Expected heterozygosity in Northern RJF population ranged from 0.56 (MCW0103) to 0.92 (MCW0295).

Only two wild RJF samples could be collected from Central RJF population and these samples could not reflect the genetic diversity of the entire region. Therefore these samples were treated as "Ecotypes". Most of loci for these samples were found to be monomorphic or showed 4 alleles at maximum. This was treated as a sampling artifact. The mean observed and effective number of alleles was 1.92 ±0.89 and 1.81± 0.85 and mean observed and expected heterozygosity was 0.35 ± 0.42 and 0.47±0.39, respectively in Central RJF population.

Genetic diversity estimates of Eastern RJF population (Table 3.6) were as follows:

The number of alleles detected ranged from 4 (MCW0222 and MCW0103) to 17 (MCW0123) while effective number of alleles were ranged from 2.31 (MCW0216) to 12.12 (MCW0123). The observed and expected heterozygosity ranged from 0.14 to 0.91 and 0.58 to 0.94, respectively. Mean observed and expected heterozygosity was 0.49± 0.22 and 0.78± 0.09. The mean observed heterozygosity was lower than the expected values.

Genetic diversity estimates of South-Eastern RJF population (Table 3.7) were as follows: Altogether 134 alleles were found across 26 loci. The number of observed alleles ranged from 3 (six loci) to 9 (MCW0034), with overall mean number of alleles per locus of 5.15 (±1.85). The effective number of alleles varied from 1.29 (LEI0166) to 5.82 (MCW0034) with mean 3.41±1.34. The expected heterozygosity ranged from 0.32 (MCW0295) to 0.88 (MCW0034) with mean value 0.69 ±0.18. The mean observed and expected heterozygosity was 0.58 ± 0.23 and 0.69 ± 0.18 and expected heterozygosity value was higher than the observed values.

Genetic diversity estimates of North-Eastern RJF population (Table 3.7) were as follows: There were 199 alleles observed in North-Eastern RJF population and the number of alleles ranged from 3 (MCW0098) to 12 (LEI0094 and MCW0123) with an average of 7.6 ± 2.35 alleles per locus. The effective number of alleles ranged between 2.02 to 7.81 with mean 5.0 ± 1.65. Observed and expected heterozygosity ranged from 0.33 (MCW0222) to 0.88 (MCW0295 and MCW0248) and from 0.52 (MCW0098) and 0.90 (MCW0111 and LEI0094), respectively. Mean observed and expected heterozygosity was 0.61± 0.17 and 0.80± 0.09.

Genetic diversity estimates of pooled domestic chicken population (Table 3.7) were as follows: The number of observed alleles ranged from 4 (MCW0103) to 30 (LEI0094) with

mean number of alleles per locus 15.23± 6.41. The effective number of alleles ranged from 2.97 for locus MCW0103 to 16.05 for locus LEI0094 with mean 7.06 ±3.48. The observed heterozygosity ranged from 0.36 (MCW0103) to 0.86 (MCW0069). Mean observed heterozygosity over 26 loci was 0.60± 0.12, which was lower than the expected heterozygosity. The expected heterozygosity ranged from 0.67 (MCW0103) to 0.94 (LEI0094 and MCW0123) with overall mean 0.83 ± 0.08.

Table 3.5 Summary of genetic diversity statistics of 26 microsatellites over six populations (5 RJF & 1 Dom Chick.) in India.

Locus	N_a	N_e	I	H_o	H_e	PIC
ADL0268	26.00	8.03	2.42	0.50	0.88	0.86
MCW0206	30.00	6.41	2.33	0.49	0.85	0.83
LEI0166	13.00	3.90	1.60	0.43	0.74	0.70
MCW0020	19.00	4.14	1.82	0.58	0.76	0.73
MCW0037	14.00	4.77	1.76	0.47	0.79	0.76
ADL0112	22.00	6.19	2.19	0.49	0.84	0.82
MCW0295	32.00	11.93	2.75	0.43	0.92	0.91
MCW0067	19.00	5.23	2.02	0.46	0.81	0.79
MCW0104	29.00	6.19	2.28	0.65	0.84	0.82
MCW0111	25.00	7.29	2.29	0.76	0.86	0.85
MCW0034	22.00	5.78	2.24	0.52	0.83	0.81
MCW0222	11.00	3.34	1.49	0.37	0.70	0.66
LEI0094	35.00	9.10	2.77	0.62	0.89	0.88
MCW0216	24.00	2.96	1.66	0.40	0.66	0.64
MCW0081	31.00	11.36	2.80	0.60	0.91	0.91
MCW0330	22.00	7.40	2.31	0.58	0.87	0.85
LEI0234	49.00	8.36	2.80	0.58	0.88	0.87
MCW0103	9.00	2.63	1.22	0.23	0.62	0.56
MCW0098	21.00	3.95	1.83	0.50	0.75	0.72
MCW0069	23.00	6.06	2.07	0.79	0.84	0.82
MCW0016	27.00	9.44	2.59	0.57	0.90	0.89
MCW0078	24.00	5.95	2.04	0.69	0.83	0.81
MCW0123	33.00	12.40	2.90	0.70	0.92	0.91
MCW0165	25.00	7.62	2.36	0.33	0.87	0.86
MCW0248	19.00	4.23	1.85	0.40	0.76	0.74
ADL0278	24.00	4.28	2.01	0.45	0.77	0.75
Mean	**24.15**	**6.50**	**2.17**	**0.52**	**0.82**	**0.80**
SD	**8.31**	**2.71**	**0.44**	**0.13**	**0.08**	**0.09**

Here, N_a = Observed number of alleles; N_e = Effective number of alleles; I = Shannon's Information index; H_o = Observed heterozygosity and H_e = Expected heterozygosity

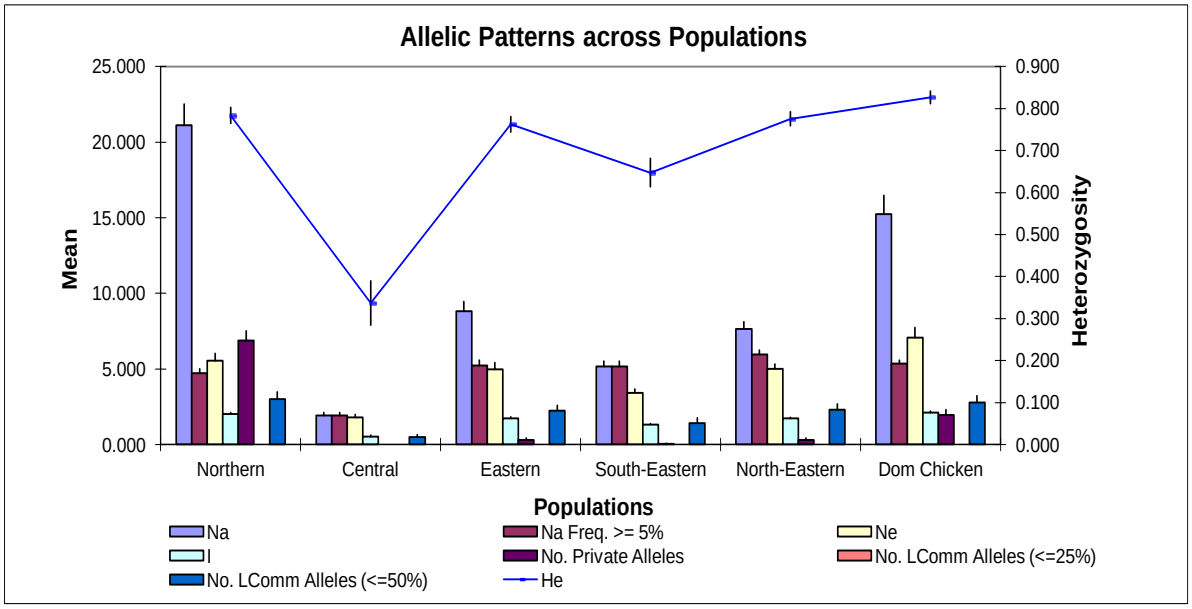


Figure 3.7 Graphical representations of allelic patterns across six populations (5 RJF & 1 domestic chicken) in India

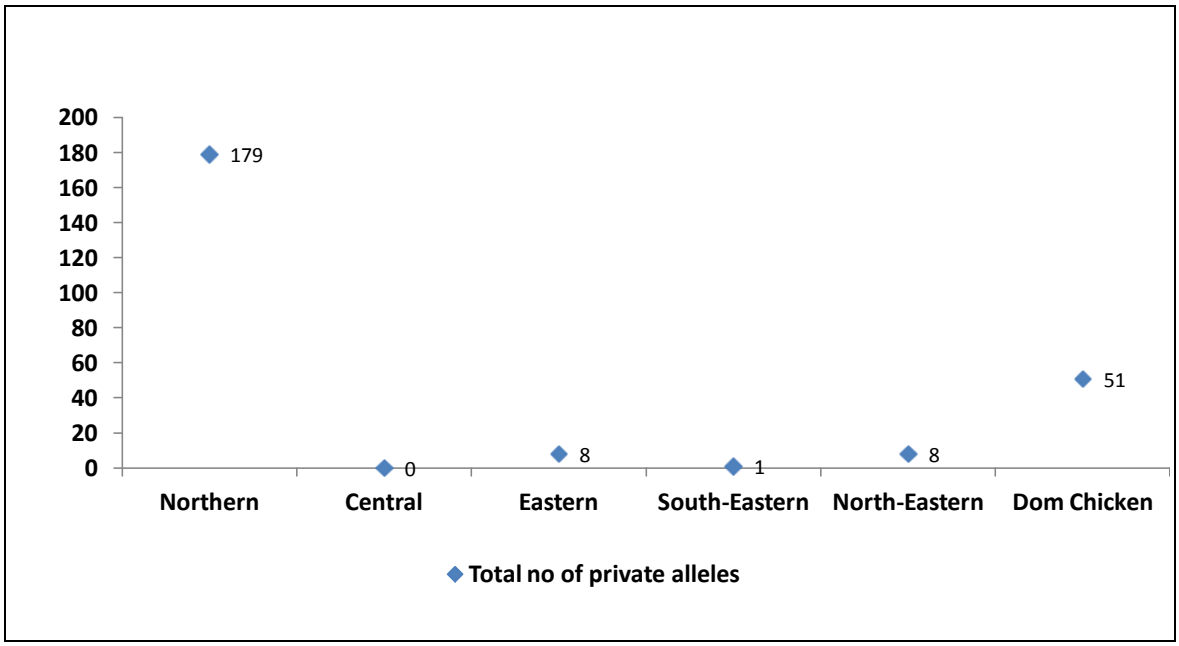


Figure 3.8 Distribution of private alleles in six populations (5 RJF & 1 Dom Chick.) across 26 microsatellite loci

Table 3.6 Genetic diversity statistics of six populations (5 RJF & 1 Dom Chick.) in India

Northern RJF population						Central RJF population					Eastern RJF population				
Locus	**N_a**	**N_e**	**I**	**H_o**	**H_e**	**N_a**	**N_e**	**I**	**H_o**	**H_e**	**N_a**	**N_e**	**I**	**H_o**	**H_e**
ADL0268	22.00	7.05	2.32	0.47	0.86	2.00	1.60	0.56	0.50	0.50	9.00	6.26	1.98	0.14	0.86
MCW0206	25.00	5.18	2.11	0.44	0.81	4.00	4.00	1.39	1.00	1.00	11.00	3.32	1.72	0.24	0.71
LEI0166	10.00	3.24	1.42	0.37	0.69	2.00	2.00	0.69	0.00	0.67	7.00	3.45	1.46	0.29	0.73
MCW0020	18.00	3.77	1.78	0.55	0.74	2.00	2.00	0.69	1.00	1.00	7.00	2.66	1.35	0.60	0.64
MCW0037	14.00	5.11	1.83	0.47	0.81	2.00	2.00	0.69	0.00	0.67	5.00	3.49	1.40	0.35	0.73
ADL0112	20.00	6.08	2.17	0.45	0.84	2.00	1.60	0.56	0.50	0.50	6.00	4.60	1.61	0.36	0.80
MCW0295	31.00	11.82	2.71	0.41	0.92	2.00	2.00	0.69	0.00	0.67	11.00	6.59	2.13	0.23	0.87
MCW0067	16.00	3.58	1.74	0.37	0.72	2.00	2.00	0.69	0.00	0.67	10.00	3.75	1.77	0.48	0.75
MCW0104	21.00	5.66	2.08	0.71	0.83	2.00	2.00	0.69	1.00	1.00	11.00	4.20	1.80	0.70	0.78
MCW0111	22.00	6.55	2.16	0.75	0.85	3.00	2.67	1.04	1.00	0.83	9.00	5.85	1.93	0.91	0.85
MCW0034	19.00	3.96	1.82	0.48	0.75	4.00	4.00	1.39	1.00	1.00	12.00	6.86	2.17	0.42	0.87
MCW0222	10.00	2.79	1.33	0.31	0.64	1.00	1.00	0.00	0.00	0.00	4.00	2.81	1.15	0.68	0.66
LEI0094	30.00	5.97	2.46	0.55	0.83	1.00	1.00	0.00	0.00	0.00	11.00	6.17	2.05	0.81	0.86
MCW0216	20.00	2.29	1.48	0.36	0.56	1.00	1.00	0.00	0.00	0.00	7.00	2.31	1.19	0.52	0.58
MCW0081	30.00	8.85	2.65	0.62	0.89	3.00	2.67	1.04	1.00	0.83	13.00	9.64	2.39	0.40	0.92
MCW0330	20.00	5.71	2.13	0.57	0.83	2.00	1.60	0.56	0.50	0.50	10.00	4.30	1.73	0.56	0.78
LEI0234	40.00	7.00	2.59	0.52	0.86	2.00	1.60	0.56	0.50	0.50	15.00	7.16	2.30	0.79	0.88
MCW0103	9.00	2.28	1.11	0.18	0.56	1.00	1.00	0.00	0.00	0.00	4.00	2.57	1.07	0.22	0.63
MCW0098	18.00	4.07	1.86	0.50	0.76	1.00	1.00	0.00	0.00	0.00	5.00	2.86	1.22	0.64	0.66
MCW0069	18.00	5.38	1.93	0.78	0.82	1.00	1.00	0.00	0.00	0.00	7.00	3.80	1.56	0.79	0.75
MCW0016	23.00	7.34	2.34	0.58	0.87	1.00	1.00	0.00	0.00	0.00	10.00	6.29	2.04	0.22	0.87
MCW0078	19.00	5.31	1.93	0.72	0.81	3.00	2.67	1.04	0.50	0.83	8.00	4.22	1.70	0.64	0.78
MCW0123	32.00	10.58	2.78	0.73	0.91	2.00	2.00	0.69	0.00	0.67	17.00	12.12	2.65	0.55	0.94
MCW0165	22.00	8.35	2.41	0.29	0.88	1.00	1.00	0.00	0.00	0.00	6.00	4.10	1.57	0.31	0.78
MCW0248	18.00	3.40	1.71	0.34	0.71	2.00	1.60	0.56	0.50	0.50	7.00	4.31	1.62	0.29	0.78
ADL0278	22.00	3.13	1.76	0.33	0.68	1.00	1.00	0.00	0.00	0.00	7.00	5.33	1.81	0.63	0.83
Mean	**21.12**	**5.56**	**2.02**	**0.49**	**0.79**	**1.92**	**1.81**	**0.52**	**0.35**	**0.47**	**8.81**	**4.96**	**1.74**	**0.49**	**0.78**
SD	**7.14**	**2.44**	**0.44**	**0.16**	**0.10**	**0.89**	**0.85**	**0.45**	**0.42**	**0.39**	**3.27**	**2.28**	**0.40**	**0.22**	**0.09**

Here, N_a = Observed number of alleles; N_e = Effective number of alleles; I = Shannon's Information index; H_o = Observed heterozygosity and H_e = Expected heterozygosity

Table 3.7 Genetic diversity statistics of six populations (5 RJF & 1 Dom Chick.) in India

South-Eastern RJF population						North-Eastern RJF population					Dom Chicken Population				
Locus	**N_a**	**N_e**	**I**	**H_o**	**H_e**	**N_a**	**N_e**	**I**	**H_o**	**H_e**	**N_a**	**N_e**	**I**	**H_o**	**H_e**
ADL0268	4.00	1.71	0.82	0.50	0.44	6.00	4.21	1.59	0.73	0.79	20.00	9.41	2.50	0.62	0.90
MCW0206	7.00	4.57	1.72	1.00	0.83	10.00	3.83	1.78	0.41	0.76	19.00	6.42	2.22	0.65	0.85
LEI0166	3.00	1.29	0.46	0.25	0.24	7.00	3.97	1.58	0.63	0.77	8.00	4.13	1.65	0.63	0.76
MCW0020	4.00	3.33	1.28	0.80	0.78	6.00	5.04	1.68	0.45	0.84	12.00	5.08	1.82	0.69	0.81
MCW0037	5.00	2.98	1.30	0.63	0.71	6.00	4.55	1.61	0.73	0.81	5.00	3.48	1.37	0.45	0.72
ADL0112	4.00	2.17	0.99	0.38	0.58	7.00	4.07	1.60	0.76	0.78	16.00	5.49	2.04	0.60	0.82
MCW0295	3.00	1.41	0.57	0.33	0.32	8.00	6.65	1.97	0.88	0.88	18.00	9.13	2.48	0.49	0.90
MCW0067	5.00	3.66	1.42	0.75	0.78	6.00	5.16	1.71	0.82	0.83	10.00	5.90	1.90	0.60	0.84
MCW0104	8.00	4.92	1.84	0.50	0.85	7.00	3.76	1.59	0.63	0.76	22.00	5.27	2.21	0.49	0.82
MCW0111	8.00	5.12	1.84	0.75	0.86	11.00	7.53	2.17	0.81	0.90	14.00	7.48	2.23	0.74	0.87
MCW0034	9.00	5.82	1.98	0.63	0.88	11.00	7.11	2.14	0.75	0.89	14.00	6.42	2.11	0.64	0.85
MCW0222	5.00	3.12	1.35	0.38	0.73	5.00	4.11	1.48	0.33	0.79	6.00	3.10	1.38	0.49	0.68
LEI0094	8.00	5.12	1.84	0.63	0.86	12.00	7.81	2.26	0.82	0.90	30.00	16.05	3.04	0.75	0.94
MCW0216	4.00	2.51	1.10	0.75	0.64	6.00	3.06	1.38	0.47	0.69	13.00	3.43	1.54	0.47	0.71
MCW0081	5.00	4.17	1.50	1.00	0.84	9.00	6.35	2.00	0.59	0.87	19.00	9.07	2.49	0.56	0.90
MCW0330	6.00	4.74	1.65	0.75	0.84	7.00	4.62	1.70	0.65	0.81	12.00	5.80	2.03	0.61	0.83
LEI0234	5.00	3.20	1.33	0.75	0.73	9.00	3.75	1.72	0.47	0.76	24.00	12.13	2.74	0.70	0.92
MCW0103	3.00	1.47	0.60	0.13	0.34	5.00	4.23	1.52	0.38	0.79	4.00	2.97	1.20	0.36	0.67
MCW0098	3.00	2.00	0.83	0.43	0.54	3.00	2.02	0.87	0.44	0.52	16.00	4.48	1.88	0.49	0.78
MCW0069	7.00	3.37	1.56	0.63	0.75	10.00	7.31	2.11	0.75	0.89	17.00	7.79	2.27	0.86	0.88
MCW0016	6.00	4.92	1.68	0.25	0.85	9.00	5.40	1.90	0.65	0.84	22.00	13.33	2.78	0.65	0.93
MCW0078	5.00	2.98	1.30	0.50	0.71	6.00	2.76	1.36	0.40	0.66	14.00	5.60	2.01	0.71	0.83
MCW0123	7.00	5.33	1.80	0.75	0.87	12.00	7.63	2.25	0.40	0.90	25.00	14.02	2.87	0.74	0.94
MCW0165	3.00	2.51	0.98	0.75	0.64	6.00	3.32	1.43	0.45	0.73	13.00	5.62	2.03	0.39	0.83
MCW0248	3.00	2.67	1.04	0.25	0.67	6.00	4.88	1.67	0.88	0.82	8.00	5.37	1.79	0.54	0.82
ADL0278	4.00	3.46	1.31	0.63	0.76	9.00	6.76	2.04	0.57	0.88	15.00	6.47	2.11	0.70	0.85
Mean	**5.15**	**3.41**	**1.31**	**0.58**	**0.69**	**7.65**	**5.00**	**1.74**	**0.61**	**0.80**	**15.23**	**7.06**	**2.10**	**0.60**	**0.83**
SD	**1.85**	**1.34**	**0.43**	**0.23**	**0.18**	**2.35**	**1.65**	**0.32**	**0.17**	**0.09**	**6.41**	**3.48**	**0.47**	**0.12**	**0.08**

Here, N_a = Observed number of alleles; N_e = Effective number of alleles; I = Shannon's Information index; H_o = Observed heterozygosity and H_e = Expected heterozygosity

3.5.3 Analysis of molecular variance (AMOVA)

The analysis of molecular variance was carried out to find out the differences among population and within populations. The AMOVA revealed a total of 6% variation among population while 94% variance was found within population (Figure 3.9). It means to specify

that the individuals within population contributed more to the variability than the individuals between the populations. Among population degree of freedom was 5 and percentage of variation contributed by these populations was close to 13%, whereas, the percentage of variation within population was 87%.

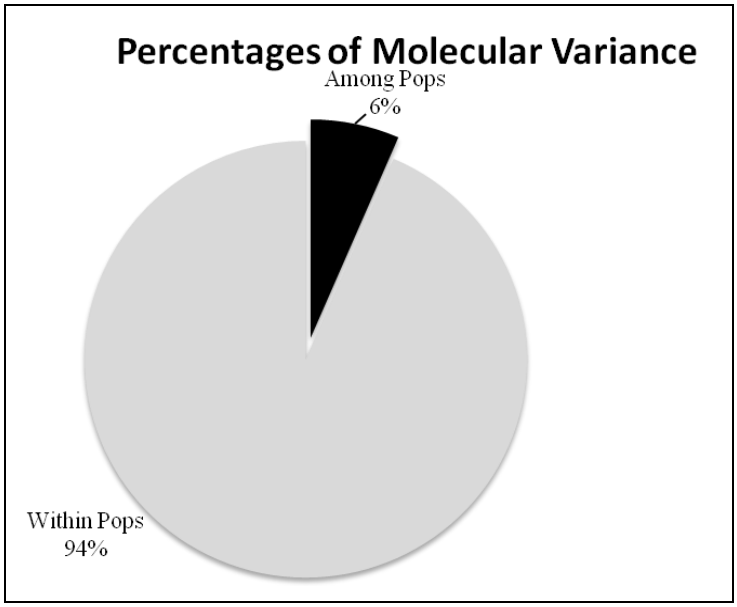


Figure 3.9 Analysis of Molecular Variance (AMOVA) of six populations (5 RJF & 1 Dom Chick.) over 26 loci

3.5.4 Summary of F- Statistics and gene flow among populations

The F statistics of 26 loci over the six populations was estimated. F_{ST} value represents the population differentiation and Nm value depicts the number of migrants between the populations. The population differentiation and number of migrants were calculated and presented in Table 3.8. Nm value was highest (10.84) and Fst value lowest (0.023) between Northern and Eastern RJF population, reflected maximum gene flow and Minimum population differentiation between Northern and Eastern RJF populations. Maximum population differentiation (0.215) or minimum gene flow (0.911) was between Central and Eastern RJF population. The overall, Nm values were quite high, suggesting the high gene flow among RJF populations.

3.5.5 Nei's genetic identity and genetic distance among populations

The Nei's standard genetic distance is basically a correlation among the allelic frequencies between the populations. The Nei's standard genetic distance and genetic identity is given in Table 3.9 (below diagonal & above diagonal). The maximum genetic distance and minimum genetic identity values were 0.9091 and 0.4029 between Northern

and Central RJF populations while the minimum genetic distance (0.17121) and maximum genetic identity (0.8419) values were found between Northern and Eastern RJF population. The pooled domestic chicken population showed highest value of genetic distance (0.9423) and lowest value of genetic identity (0.3897) with Central RJF population while lowest value of genetic distance (0.2557) and highest value of genetic identity (0.7744) with North-Eastern RJF population. A dendrogram was constructed using Nei's genetic distances data using unweighted pair group method with arithmetic mean (UPGMA) (Figure 3.10). Dendrogram showed that RJF populations formed three clusters (i) Northern & Eastern populations formed one group. (ii) Central & Southeastern Populations formed a group (iii) Northeastern RJF population formed a separate branch from rest of the four RJF populations. The domestic chicken population of pooled samples collected across the country was found to be grouped with North-Eastern RJF population.

Table 3.8 Population wise F_{ST} values and number of migrants

Population	Northern	Central	Eastern	South-Eastern	North-Eastern	Dom Chicken
Northern	*****	0.215	0.023	0.084	0.046	0.031
Central	0.920	*****	0.214	0.171	0.207	0.208
Eastern	10.846	0.911	*****	0.077	0.048	0.030
South-Eastern	2.741	1.209	3.010	*****	0.086	0.079
North-Eastern	5.239	0.958	4.960	2.651	*****	0.029
Dom Chicken	7.791	0.950	8.200	2.923	8.453	*****

Number of migrants (below diagonal) and Fst (via Frequency) values (above diagonal).

Table 3.9 Nei's Original Measures (1972) of Genetic Identity and Genetic distance

Population	Northern	Central	Eastern	South-Eastern	North-Eastern	Dom.Chicken
Northern	****	0.4029	0.8419	0.5758	0.6684	0.7454
Central	0.9091	****	0.4149	0.6331	0.4270	0.3897
Eastern	0.1721	0.8797	****	0.6262	0.6666	0.7729
South-Eastern	0.5521	0.4571	0.4681	****	0.5548	0.5757
North-Eastern	0.4029	0.8511	0.4056	0.5892	****	0.7744
Dom. Chicken	0.2939	0.9423	0.2576	0.5521	0.2557	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

3.5.6 Correspondence analysis

The three axis of the correspondence analysis contributed 15.53, 19.93 and 44.30 percent of the variance and cumulatively explained 79.76 percent of the variance. The three dimensional plot is shown in Figure 3.11 where each of the population is shown in a different color. Multifactorial correspondence analysis showed similar pattern of clustering RJF population as revealed by UPGMA dendrogram.

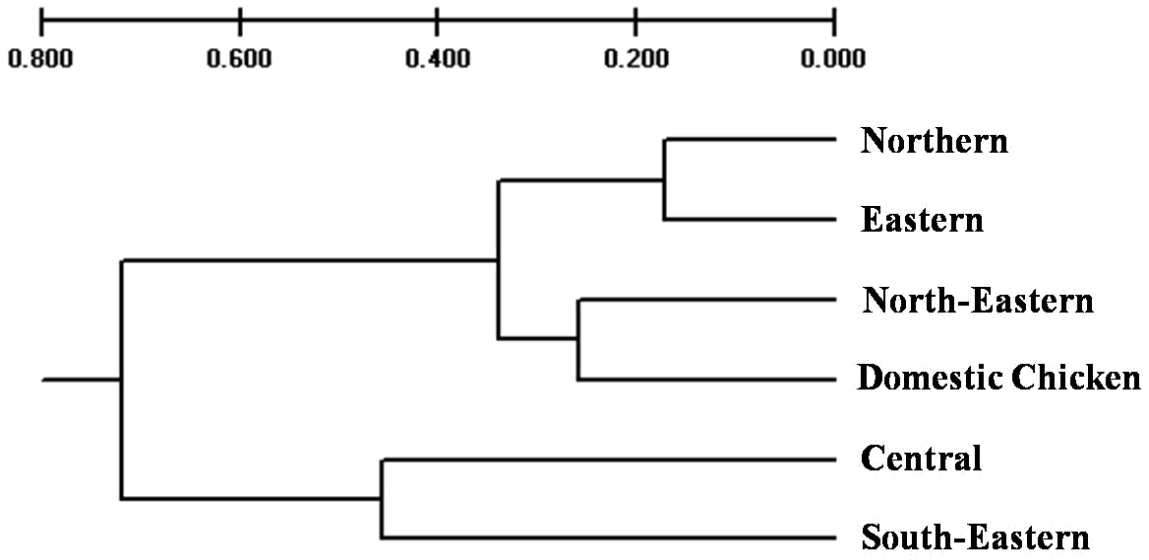


Figure 3.10 UPGMA dendogram based on Nei's original (1972) measures of genetic distance of six populations (5 RJF & 1 domestic chicken)

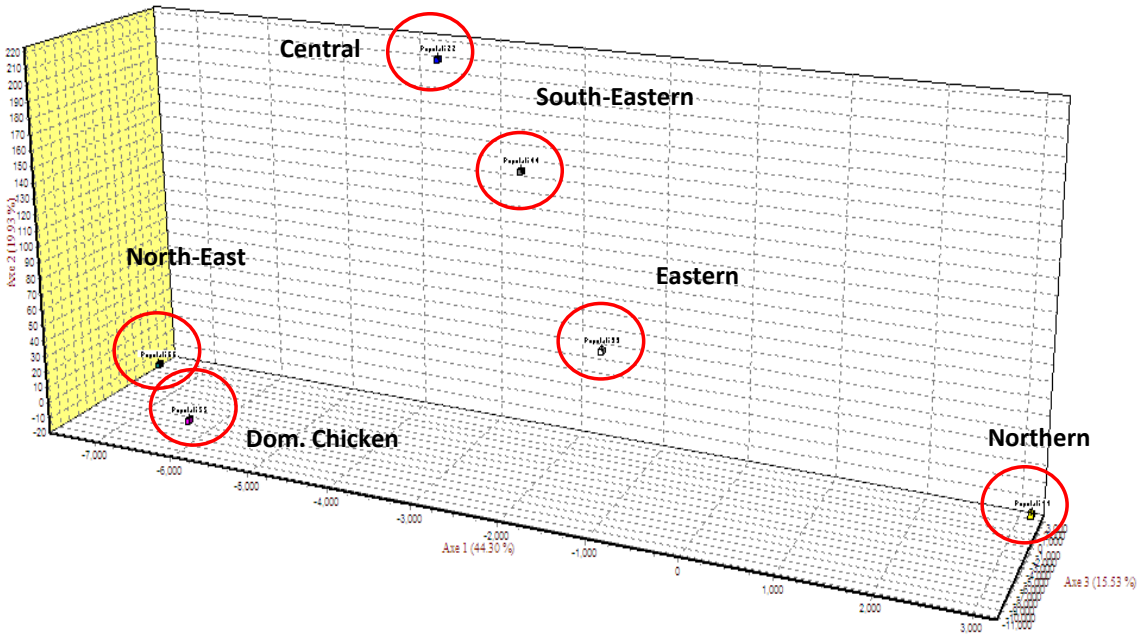


Figure 3.11 Correspondence analysis of five RJF populations and one domestic chicken population

3.6 DISCUSSION

The RJF is threatened with genetic endangerment throughout its distribution range. Habitat loss, fragmentation and poaching are the cause of reduction in the distribution range of wild RJF populations in India. The phylogenetic relationship among the RJF populations requires knowledge of genetic variations that can be effectively measured within and between the populations using DNA based molecular tools and the present study revealed the existing genetic diversity, population differentiation and phylogenetic analysis among RJF populations of India as no genetic information was available on RJF genetics at a landscape level in India prior to the present study.

3.6.1 Allelic patterns and heterozygosity statistics in RJF and Doestic chicken populations based on microsatellite markers

The five RJF and one domestic chicken population were found to be highly polymorphic for 26 microsatellite loci. The PIC value for the present study ranged from 0.56 to 0.91 while it was 0.02 to 0.74 in the study conducted by Wen-Bin et al., (2007) with the same panel of microsatellite markers. All the 26 microsatellite loci used in the present study showed PIC value higher than 0.5 and thus these loci were informative. The high PIC values for these loci make them fit for diversity analysis. The PIC values closely resemble the values of the expected heterozygosity and this can be attributed to the large number of alleles being at very low frequencies.

The population wise genetic diversity measures are presented in Table 3.6 and 3.7. The North-Eastern RJF population had highest observed heterozygosity followed closely by domestic chicken population. The observed heterozygosity was lowest in Central RJF population. These values were significantly less than the expected heterozygosity in all the populations meaning deficiency of heterozygotes. This could be attributed to non random union of gametes and existence of population structure. The rich heritage of genetic diversity in northeastern RJF population identified in the present study is consistent with the domestication history of domestic chicken and also corroborate the findings of Gongora et al. 2008. The high genetic diversity in this population is important for genetic resources conservation and utilization. The information from the DNA markers together with phenotypic performance and population history provides reliable guidelines that can be used in developing practical strategies for conservation purposes.

The FST values and the effective number of migrants exchanged between populations are given in Table 3.8. The calculated FST estimates provided a measure of population differentiation among RJF populations. The maximum population differentiation was between Central and Eastern RJF population and this was because of sharing of less number of alleles between the populations. The minimum population differentiation was

between Northern and Eastern RJF population as there was high value of effective number of migrants between these two populations. It also reflected a high gene flow between the Northern and Eastern RJF population. The mean number of effective migrants per generation was calculated on the basis of FST values and was quite high. These results demonstrated that there was quite high gene flow among RJF populations.

The analysis of molecular variance was carried out to find the differences among and within populations. The AMOVA revealed that the individuals within populations contributed more to the variability than the individuals between the populations.

3.6.2 Relationships among RJF populations in Northern India

The relationship among the populations can be examined using the genetic distances. The genetic distances can be estimated based on the allele frequency data and number of repeats. Both the methods are in vogue for studying the evolutionary relationships of closely related populations. The separation of the populations and subsequently the changes in the allele frequency are based on the reproduction isolation, drift leading to fixation of alleles. The fixation of alleles in the descendent populations due to drift is in relation of initial allele frequencies in the unified population before splitting. Another reason for the change in allele frequency is attributable to inbreeding among the individuals of the same population. The most common genetic distances based on allele frequency data are Nei's Standard genetic distances-Ds, (Nei, 1972), Chord distance-Dc (Cavalli Sforza and Edwards, 1967), Nei's-DA (Nei, 1983) and Reynold distance (Reynold et al., 1983). These genetic distances are useful for ascertaining out the genetic relationship among the populations for allozyme analysis and other classical markers where each mutation gives rise to a new allele, IAM, (Kimura and Crow, 1964). This model seems to apply approximately to classical genetic markers such as protein and blood group polymorphism (Nei, 1987). In the present study, microsatellite data has been utilized in finding the relationship among RJF populatios in Northern India.

The efficiency of constructing the phylogenetic trees by means of genetic distances depends on the linear relationships with time (Nei et al., 1983, Tajima and Takezaki, 1994). The computer simulation studies (Takezaki and Nei, 1996) revealed that DA and DC are the most efficient in obtaining the correct tree topology in several conditions they examined. This has been revealed despite the fact that DA and DC are based on geometric distances of the population represented on a multidimensional hyper sphere and are independent of the mutational models (Nei, 1987). The results obtained in the present study were very similar to those reported by Nei et al., (1983) and suggested that different distance measures should be used for the reconstruction of a topology and estimating the Branch lengths. In

general, DA and DC are superior for construction of phylogenetic trees (Vijh et al., 2005 a, b).

Nei's genetic distances showed central RJF population is most genetically distant (MGD = 0.942) and least genetically identical (MGI=0.3897) with domestic chicken while Northeastern RJF population is least genetically distant (MGD =0.255) and most genetically identical (MGI = 0.7744) with domestic chicken population. Dendrogram indicated that Northern and eastern population formed one group that shows the connectivity between the two populations through Nepal. Northeastern RJF population formed a separate branch from rest of the four RJF populations; it may be because of the sub species *G.g. spadicus*. That is distributed here only. Though, there is habitat connectivity among eastern, central and southeastern population, the central and southeast population here formed one separate group. The domestic chicken samples collected across India, clustered along with northeastern RJF population.

The rich heritage of genetic diversity in northeastern RJF population was identified in the present study is consistent with the domestication history of domestic chicken and also corroborate the findings of Gongora et al. 2008. Therefore, North-Eastern RJF population may be presumed as one centers of domestication in Indian subcontinent.

Plate 5: (a) Red Junglefowl and domestic fowl interaction at Nandini WLS, JK

(b) RJF eggs collected from the wild and hatched along with domestic fowl eggs in a village in Odisha

Plate 6: Sampling and tagging of Red Junglefowl in captivity

4.0 INTERACTION OF WILD RED JUNGLEFOWL WITH DOMESTIC FOWL

"*The whole trend of ecological thought at present is towards a realization of the importance of community, and in this respect we are becoming increasingly aware of the social interactions between species and the whole complex of the ecological association*".

Fraser F. Darling

4.1. Introduction

A major component of a species' environment is the presence of other individuals either of the same species or different (Thompson 1999). The nature of such an interaction and the associated dynamics is critical not only in the context of ecological communities but also in determining the structure and hierarchy of oneself in the group, a so called "sociality" index. The nature and strength of this sociality has a profound influence in shaping the evolution of the species. One of the most important issues in such group living organism with respect to reproduction is dominant-codominant structure. It is this hierarchy that will influence male-male interactions and male-female interactions in the selection criteria for mates.

In many species, the role accorded to females (utilisation of male gametes) with respect to sexual selection and thereby the consequences of co-evolution in male traits and the nature of female attention to and assessment of males are of considerable importance (Janetos 1980; Bradbury and Andersson 1987; Real 1990, 1991; Andersson 1994; Andersson and Iwasa 1996). Although male–male competition and female selection often operate in unison, part of the variance in male reproductive success is often explained by variation in a male's ability to compete for copulation (Darwin 1868, Andersson 1994) and by variation in fertilisation opportunities (Birkhead and Pizzari 2002; Pizzari and Birkhead 2002). However, it is well established that females that attend to and choose mates during courtship (Kirkpatrick 1982, Bateson 1983) also consistently prefer to be inseminated by and use the sperms of certain males (Andersson 1994, Birkhead and Pizzari 2002); this may not always be the case (Moore and Moore 1999).

In polygamous species, where males typically do not provide paternal care, resources and where their contribution to reproduction is assumed to be limited to the provision of semen (Kirkpatrick and Ryan 1991), has led to the hypothesis that female preference may be explained by genetic benefits (Bradbury and Gibson 1983, Kirkpatrick and Ryan 1991, Birkhead and Parker 1997). Preference for socially dominant males may thus be driven by the pursuit of alleles associated with superior fitness. However, there is some

evidence that social dominance can be transmitted from parents to offspring in some species (Craig et al., 1965; Moore 1990), few studies have found convincing evidence that genetic benefits explain female partner choice (Wilkinson et al., 1998, David et al., 2000) while others have produced more ambiguous results (Griffith et al., 1999, Holland and Rice 1999, Brooks 2000, Holland 2002) or have identified environmental mechanisms confounding results consistent with genetic benefits (Sheldon 2000). Theoretically, males of polygamous species may influence female reproductive success directly even without providing paternal care (Reynolds and Gross 1990, Sheldon 1994), and the few studies that have tested this idea have focused on the possibility that female preference evolves to reduce direct costs such as sexual harassment (Sæther et al., 1999).

As mating strategies change due to the change in the value of copulation over time, selection on males to mate at a higher rate than females often results in male harassment of females and counteracting female responses. One potential explanation of this result is that while the mating propensity of individual males may increase with more males in a population, male interference and inhibition may also increase as more males compete over fewer copulation opportunities. This mechanism may be particularly relevant for social species such as the junglefowl, where socially dominant males are able to interrupt or altogether inhibit the sexual investment of subordinate males (Koyama and Kamimura 2000, Pizzari 2001).

In their natural habitats RJF are extremely cautious and difficult to observe for any length of time (Collias and Collias 1967, 1996, Hakansson and Jensen 2005). Hence, very few studies have been undertaken on the natural behaviour in the wild. Most inferences in the behaviour of the species have been drawn from birds housed in various zoological parks, pheasantries or at research stations outside their native distributional range. In such ambience, where there is a relaxation of natural selection pressure Schutz et al., (2001) has shown a modification of the natural behaviour towards less energy demanding strategies, such behavioural differences are a cause of concern (Hakansson and Jensen 2005). There are instances where in mating is forced within individuals primarily done so as to improve vigour in the domestic fowl. In free ranging domestic fowl both sexes are sexually promiscuous because of some degree of mating propensity by males and females wherein males often coerce females into copulation (McBride et al. 1969, Thornhill 1988, Collias and Collias 1996, Ebenhard 1996, Etches 1996, Pizzari 2001) and the propensity of the female to mate with multiple males (Ligon and Zwartjes 1995) made us to address the issues of interactions in the wild. Thereby, one of the main objective was to understand such interactions between the wild and domestic fowl populations in light of hybridization and to verify whether domestic males do dominate and mate with the wild RJF hens given that domestic males are larger in size and to check whether there is a difference in the tolerance

levels with respect to space utilisation during the breeding and non-breeding season between RJF and domestic fowls.

4.2. METHODS

4.2.1 Study Population

The study was carried out on free ranging populations of wild RJF in India from October 2009- May 2011. Opportunistic sightings using an Ad libitum (Altman 1974) method was used to gather information about interactions. None of the individuals were marked hence observation were recorded till the flocks were at sight. New sets of observations were considered from different locations of the same sight. All observations for the present study were at the forest-village (fringe) interface.

4.2.2. Interactions

For each mixed group observed date, time, number of individuals within the gender was noted down for wild RJF and domestic group. Two behavioural patterns were noted namely; agnostic and cordial behaviour. These were used for individual interactions between male-male and male-female that were estimated using a focal animal sampling rule with continuous recording (Altmann 1974). These observations continued until any one (wild or domestic) group was out of sight. Only when wild RJF and domestic populations were within 10 m or less of each other it was considered as a mixed flock for the present study.

4.2.3. Analytical

Observations were broadly divided into breeding (February- June) and non-breeding (July-January) season. Due to low sample sizes, observations were pooled for all sites for the breeding and non-breeding season and inferences are drawn in percentages and proportions. Care must be taken as these results are generalised and it may be an artifact of the sample size and not represent the ideal situation.

4.3. RESULTS AND DISCUSSION

There were 13 sites (3 breeding, 10 nonbreeding) from where observations were recorded of mixed groups. All observations were in pre-dawn hours fifty-one encounters totalling to approximately 500 minutes of observation were recorded with 75 males, 90 females of wild RJF and 112 males and 144 female domestic fowls. There were 41 sightings comprising of 445 mins of observation during the non-breeding season (June-February) while 10 observations of approximately 49 minutes that were recorded in the breeding season (March–May), there was no observation of mating either between wild RJF cockerel and domestic hens and vice-versa. This could be due to the highly sensitive behaviour of the

wild RJF (Collias and Collias 1967) or there could be a shift in the mating pattern due to the strong female biased group size as reported by Lovlie and Pizzari (2007). Hence, inferences are drawn with respect to group sizes and social spacing during the breeding and non-breeding season.

4.3.1 Breeding season

On 10 occasions comprising 49 mins of observation we recorded flocks in close proximity of domestic fowls. In all there were 77 individuals recorded of which 33 represented wild RJF (12 males, 21 females) and 44 were domestic fowls (21 males, 23 females). The maximum group size recorded were 10 individuals where both groups had five individuals each, in the wild group there were three females while the domestic female group had two individuals. RJF are sedentary and philopatric species (Collias and Collias 1967) and at all the three sites where we recorded flocks of wild RJF, individuals were near to agricultural lands (agro-forest interface). All recording were approximately for 5 minutes except once when a single male individual was seen with a flock for more than 5 minutes. Though the wild RJFs were sighted there seems to be some spatial avoidance as the distance between the groups were more than 20m to each other. On all occasions, the wild flock retreated back into the woodland patches, while the domestic flocks continued foraging hence, we could not consider any interaction during this period.

4.3.2 Non-breeding season

During the non-breeding season, there were 41 sightings comprising of 445 mins of observation. In all there were 344 individuals recorded, out of which 63 male and 69 female were wild RJF and 91 male and 121 female were domestic fowls. The maximum group size comprised of 13 individuals that included five wild (2 males and 3 females) and eight domestic fowls (3 males, 5 females). On three occasions when the group was 10m apart there was a single wild male seen interacting with the domestic fowls. From the 10 sites, the maximum number of interactions with mixed flocks were from Sariyanj village in Himachal Pradesh (n=16) while observations at the remaining nine sites ranged from one to eight.

4.3.3 Male-Male Interaction

In all there were 41 interactions between the wild RJF and domestic fowl, the males of wild and domestic were mostly recorded at the periphery of the group. On 12 occasions, when the groups were closer to each other (<5m apart) the domestic male fowl chased the wild RJF away, even when the domestic males (n=2) were less than the wild RJF. Similarly, when groups were 10m apart on 21% of the time (n= 29), males were chased. These include three occasions when only a single RJF male was recorded. Though we have considered that the dominant domestic male to have chased away the dominant RJF male, there is no way in

identifying that the male chased away could be a subdominant wild RJF as it is reported that there are no morphological differences associated between the dominant and subdominant males across groups (Wilson et al., 2009). Furthermore, studies indicate that these traits are a function of time, given that the individual has been associated with the group (Cornwallis and Birkhead 2008) hence, dominant-subdominant is a group and not across a group phenomena.

4.3.4 Male-Female Interaction

There seemed to be greater levels of tolerance in interactions between the sexes. All interactions (n=41) were cordial and the females (wild and domestic) moved freely within the mixed flocks without being harassed by the wild RJF or the domestic group. On three occasions a single female (wild) was observed feeding along with a domestic population. The females in these cases were seen in the near vicinity of a settlement and after disturbance the female RJF flew back into the forest patch.

4.4 Limitations

One of the major limitations in our interpretation of the data is the representation of observations from a particular area and we could not identify dominant male of the flock. As this study was initiated to be undertaken with free-ranging individuals, opportunities for such observations were rather scarce and restricted to areas were wild RJF were recorded in patches of forest interspersed with human habitation. Most of these areas are at the periphery of PAs or in Reserve Forest areas, with an agro-forest interface. The species is extremely shy and hence limitations in tracking and habitualising the flocks led us to use *Ad libutum* method. Furthermore, the occurrences of such events were few and this is a reflection in our low sample size. Though much is written about the mate selection behaviour of male and female RJF, all these studies have been undertaken in captivity and there are studies showing self-organized social assortment in captive groups of males and females (Smith et al. 2002, White et al. 2002) these patterns can emerge from the birds behaviour even thought they might not be imposed by the experiments. While, there are only anecdotal evidences of female breeding in the wild (Collias and Collias, 1967) and no information with regards to mate preferences. This information is very critical especially while addressing issues related to evolution of female choice and hybridisation. Studies need to address preferences, preference mechanisms and physiological state of individual females from birth through breeding in order to understand the choices undertaken by the RJF hen.

It is known that male domination has an immense influence on the structure and behaviour of other members within the flock. We could not single out the dominant male of the flocks in all these interactions. Due to our inability of identifying dominant males we generalised our dominant individual male to be the largest with the most prominent crown to be the dominant male of the group for both wild RJF and domestic fowl.

5.0. CONSERVATION ACTION PLAN

I believe sustainable use is the greatest propaganda in wildlife conservation at the moment.
- Steve Irwin

5.1. INTRODUCTION

During the 3rd Meeting of the National Board for Wildlife (NBWL) that was held on 19th June 2006 under the Chairmanship of the Honourable Prime Minister, a Specialist Group (SG) was constituted to prepare an Action Plan for RJF.

The SG discussed various aspects of RJF conservation in India along with an outline wherein major thrust areas that needed attention were identified. Within the given ambit Central and State Government, institutions and Non- governmental organisations were earmarked to take up issues identified.

As proposed by the SG the issues mainly discussed were:

(i) status and distribution: although the general distribution range for RJF was known, limits to the range and the current status of the species were not known.

(ii) *In situ* conservation: need to monitor trends in population of the species within PA and areas outside the PA network.

(iii) *Ex situ* conservation: Introgression and hybridisation of the species is a concern and hence screening of the captive population was required and

(iv) Research: Further insights and research into breeds and genetic traits responsible for immunity especially Avian influenza.

The SG thus observed that the ongoing WII's RJF project (this study) would be in an enhanced capacity and a better position to address certain thrust areas at its completion. Hence, information generated from the WII's RJF project would supplement and formulate parts of the conservation action plan for the species. An extract of the RJF Action plan is placed as Appendix - 2.

5.2.a Status in the wild

In regards to the status and distribution, the present study suggests that the RJF is still recorded in 21 States and 205 Districts, with a representation in 34 NPs and 135 WLSs, covering a probably range of approximately 354,978 km^2 (Chapter 2.0). There still exists a strong ethno-cultural bond where the wild males are used to invigorate the domestic stock in order to enhance the first generation individuals. These individuals are used in the context of cultural and religious relevance in the sport. The downside of this association is the introgression of genes which has been speculated to affect the species (Peterson and Brisbin 1999) and the supplement of protein in either form (meat or egg) in the diet of

indigenous ethnic groups. WII's RJF study made a comprehensive assessment of the genetic diversity of wild RJF population in India (Chapter 3.0).

5.2.b Genetic management of captive RJF in India

In India, the loss of genetic diversity and maintenance of the pure RJF populations in zoos/captive centres have been issues of major concern because of the inbreeding and cross-breeding of RJF with domestic chicken either intentionally or unintentionally. Possibly, hybridization in captivity occurs either during setting up the founder population or during the birds exchange programs of the various centres. At few places, domestic hens are used as foster mothers and kept along with RJF to incubate RJF eggs. These practices increase the chances of silent breeding of RJF with domestic chicken which goes unreported. However, there is a paucity of information with zoo/captive centers regarding the origin of the founder stock, per year hatching/mortality rate, population genetic diversity, population genetic structure and effective population size of RJF. Such information is vital for decision making in conservation breeding programs, including the exchange or shifting the breeding birds among different zoos/captive centers to enhance the genetic diversity and overcome the possible effects of inbreeding. Since, threat of hybridization of RJF with domestic chicken is real and to address the level of admixture in birds being reared in their enclosures is beyond the capacity of the zoo authorities due to lack of facilities, infrastructure and expertise. We therefore sampled the birds from 14 captive centers/zoos and addressed the issue of hybridization using molecular markers.

5.3. MATERIALS AND METHODS

5.3.a. Sample collection and DNA isolation

The RJFs were live-trapped in their enclosures with the help of zoo keepers and approximately 500 µl blood samples was taken from the wing vein and stored in DNA zol BD (U.S. Patent- 5,945,515). Sampling in zoos/captive centers was carried out directly by Veterinarian or under supervision of a Veterinarian. Extreme precautions were taken and birds were released soon after the sampling to their respective enclosures. In total, 220 RJF samples were collected across 14 captive centers/zoos (Table 5.1). All the birds sampled in captivity were marked by putting metal rings or color coated bands. Genomic DNA was extracted from the blood following the protocol as proposed by Mackey *et al.,* (1996).

5.3.b. Polymerase chain reaction and microsatellite genotyping

Twenty-three microsatellite markers (AVIANDIV, Weigend S Coordinator *et al.,* 1998-2000) were used for the present study. The selected markers were: ADL0268, MCW0206, LEI0166, MCW0037, ADL0112, MCW0295, MCW0067, MCW0104, MCW0111, MCW0034,

MCW0222, LEI0094, MCW0216, MCW0081, MCW0330, LEI0234, MCW0103, MCW0098, MCW0069, MCW0016, MCW0078, MCW0248 and MCW0278. The PCR amplifications were carried out as described elsewhere (Mukesh *et al.*, 2011). The fragment analysis was performed on ABI 3130 Genetic Analyzer (Applied Biosystem, USA) with GeneScan 500 (-250) LIZ as the internal lane size standard. The data was collected and allele sizing was done using Gene Mapper version 3.7 software (Applied Biosystem, USA).

5.3. c Clustering and inferring populations

The population genetic structure and identification of admixed individuals were performed using Bayesian method as implemented in STUCTURE version 2.2 software (Pritchard *et al.,* 2000a). The program STRUCTURE implements a model based clustering method for inferring population structure using genotype data consisting of unlinked markers. Bayesian clustering analyses groups individuals into genetic clusters that minimize Hardy–Weinberg and linkage disequilibrium. Briefly, a model was assumed in which there were K populations (K=2-14), each of which is characterized by a set of allele frequencies at each locus. We specified the admixture model with a burn-in period of 40,000 and 60,000 Markov chain Monte Carlo repetitions and a model of correlated allele frequencies. Ten independent replicates at each 'K' levels were run. The real K value for the four captive RJF populations was determined by calculating ad hoc quantity (ΔK) as proposed by Evanno *et al*., 2005.

5.3.d Identification of admixed birds

All the domestic chicken samples collected across the country were pooled and defined a single population. This pooled domestic chicken population was run with each captive stock separately to identify the hybrids using structure analysis. The individuals were assigned to one or more clusters on the basis of their q values, if the q value was equal or larger than the probability threshold of 0.800 then the individuals were assigned to a single cluster while individuals with q value lower than 0.800, were assigned to more than one clusters.

5.4. RESULTS AND DISCUSSION

5.4.1. *In situ* conservation

There needs to be an increased effort within states to curb hunting and egg collection of this species which continues unabated, especially in central and northeastern states. While, states that have restricted distribution *viz;* Sikkim , Andhra Pradesh and Maharashtra increased attention is needed.

1. Sikkim: RJF is present in two districts and one PAs. The species is reported only from Kitam WLS and forest patches adjoining it (present study). The species was

recorded all along the Rangit River from Naya Bazar upto Melli and in the forest patches leading to Kitam village.

2. Andhra Pradesh: Papikonda WS was the only PA where this species was recorded. In Papikonda WS, RJF is known to occur in certain areas and forest tracts (forest areas between Rampachodavaram and Maranmalli and at Maranmalli the road that leads to Badrachalam, also locals have reported the species at Krupan and Wangasar areas. The present study did not record any presence of RJF at Rajahmundry, Eturnagaram WLS and Kawal WLS.

3. Maharashtra: Districts of Chhindwara and Gondiya have reports for this species, but due to logistic difficulties surveys were not conducted within this area.

As this study could not cover all possible areas we suggest that there needs to be increased efforts to understand whether the species is prevalent within forested tracts outside the dominion of the PA network, especially Punjab, Haryana, Bihar and Uttar Pradesh where the present distribution is highly fragmented with growing pressures on the existing PA of these states.

Genetic analysis of wild RJF population revealed that there are three distinct clusters viz; north and eastern, central and southeastern and northeatern. nei's genetic distances showed central RJF population is most genetically distant and least genetically identical with domestic chicken population (Chapter 3.0). Northeastern RJF population formed a separate branch from rest of the four RJF populations; it may be because of th subspecies *G.g. spadiceus.*

5.4.2.a Identification of real K value and population assignment of the individuals

The log-likelihood [L(K)] values appeared to be an increasing function of K for all examined K values (Figure 5.1A). Therefore, true K value could not be determined through log likelihood values. Accordingly, another ad hoc quantity (ΔK) was used to overcome the difficulty in determining the real population structure (Table 5.2 and Figure 5.1 A&B). The real K value in the present study was found to be K =3 while successive increase in the K values did not split the three major groups into additional clusters. The graphical clustering of populations and the contribution of the individuals in a cluster are shown in figure 5.3. All birds sampled from Chail, Patna and Vizag zoo were assigned to cluster 1 and birds from Shimla, Gopalpur, Lucknow and Padmaja Naidu zoo were assigned to cluster 3 (Table 5.3). Since, all the birds were assigned to single clusters, therefore, these stocks did not show any admixture while birds of Nainital, Renuka and Kufri zoo themselves showed a fair degree of admixture which reflected mixing among these three stocks. This may be because of the founder stock of these populations having been taken from a single source population or arbitrary exchange of birds would have taken place among these zoos/captive

centers in the past. No admixed individual was observed in Morni, Delhi and Tata zoo and individual assignment in these populations was 91.66, 94.73 and 75%, respectively. In Tripura stock, no samples was assigned to any of these three clusters and all seven individuals were remained unassigned. The global performance of STRUCTURE assigning individuals (at K=3) was 169/220=76.81% while 8.63% individuals remained unassigned (Figure 5.4).

5.4.2.b Identification of admixed birds in zoos/captive centers

No prior population information was fed and Bayesian analysis for clustering individuals was carried out to detect the admixed individuals in the captive stocks. Each captive stock was run independently with the pooled population of domestic chicken collected across the country. The analysis parameters were kept similar as used to detect the real population structure. Since, there could be a possibility of having two populations at maximum. Therefore, admixed individuals were addressed at K=2 and 10 independent replicates were carried out. Individuals showing q value less than the probability threshold of 0.80 were considered hybrids as they were assigned to more than one clusters.

Altogether, 95.45% birds (210/220) were found to be relatively pure and 4.55 % birds (10/220) were found admixed with domestic chicken that were being reared in different zoos/captive centers. The zoo-wise illustration of the admixed individuals has been presented in the figure 5.5 (A-N) using structure generated bar plots. The captive birds showed q value less than the threshold value 0.80 were admixed with the domestic chicken and these admixed birds are represented with superimposing In total, 50% (2/4) birds of Nainital zoo, 18.18% (2/11) birds of Renuka Pheasantry, 7.14% (1/14) birds of Shimla Pheasantry, 5% (1/20) birds of Dhauladhar Nature Park, Gopalpur and 4.16 % (4/96) birds of Pheasant Breeding Centre, Morni was found admixed with domestic chicken. The individuals were traced back and the IDs of admixed birds are presented in table 5.4.

As a recommendation by the SG, enhanced capabilities to handle *ex situ* captive breeding for the species would be initiated. Presently, the current captive status of the birds, suggest that these populations are mainly from one or two clusters with the exception of the birds housed at Sepahijala Zoo, that belong to subspecies *G.g. spadiceus*. (Mukesh et al., 2011).

5.5. Recommendations

- As this study could not survey all areas within RJF's distribution range, we suggest that there is a need to increase efforts to understand whether the species is prevalent within forested tracts outside the PA network, especially Bihar, Haryana, Punjab, Sikkim and Uttar Pradesh where the present distribution is highly fragmented with growing pressures on the existing PA of these States.

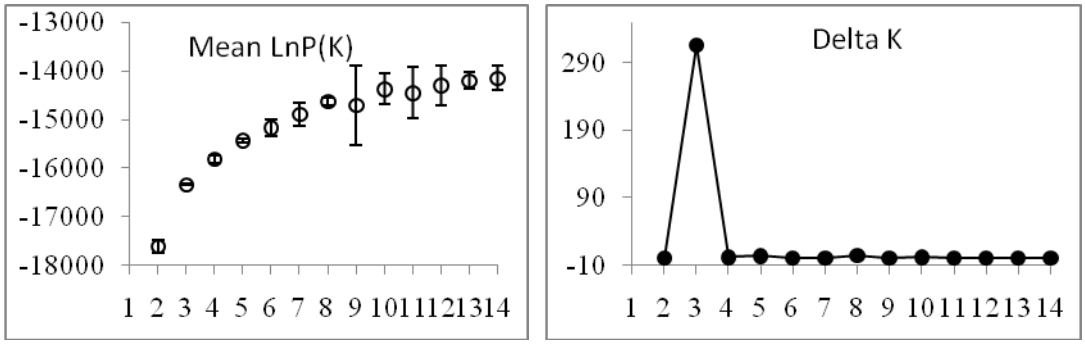
- Similarly, in the States of Andhra Pradesh, Jammu & Kashmir and Maharashtra, extensive field surveys should be carried out to ascertain the presence/absence and exact distribution limits of RJF as these States encompass the limits or edges of the distribution range of this species.
- Special focus surveys/studies are required at range overlaps between *G.g. murghi* and *G.g. spadiceus* (northeastern States) and also between RJF and Grey Junglefowl (central India).
- Based on our samples collected from zoos/captive centres (Table 5.1), admixed birdwere identified (Table 5.4). These admixed individuals (hybrids between RJF and domestic chicken) that are kept in zoos/captive centres should be removed from these captive stocks to avoid any further hybridisation. They should not be exchanged with any other zoos/captive centres and should not be released back into the wild. The list of individual birds in the zoos/captive centres that have been identified as 'not admixed' have been provided to these centres.
- For RJF individuals in zoos/captive centres that were not sampled during the study or born or added after the sampling, similar genetic analysis should be carried out. Such individuals should not be used /exchanged for any breeding programme.
- As there are chances of silent breeding between RJF and domestic chicken, hence the use of domestic hens as foster parents should be avoided.

Table 5.1 Sampled centres with number of sampled individuals.

Zoological Park / Pheasantries	Samples
Chail Pheasantry, Solan, Himachal Pradesh	7
Renuka Zoo, Sirmur, Himachal Pradesh	11
Himalayan Nature Park, Kufri, Himachal Pradesh	18
Shimla Pheasantry, Shimla, Himachal Pradesh	14
Dhauladhar Nature Park, Gopalpur, Himachal Pradesh	20
Morni, Haryana	96
Pt.G.B. Pant High Altitude Zoo, Nainital, Uttarakhand	4
Lucknow Prani Udyan, Uttar Pradesh	6
National Zoological Park, New Delhi	19
Sanjay Gandhi Biological Park, Patna, Bihar	2
Tata Steel Zoological Park, Jamshedpur, Jharkhand	8
Padmaja Naidu Himalayan Zoological Park, Darjeeling, West Bengal	4
Sepahijala Zoological Park, Sepahijala, Tripura	7
Indira Gandhi Zoological Park, Visakhapatnam, Andhra Pradesh	4

Table 5.2. Inferring the value of K, the real number of populations, for RJF samples collected from 14 captive centres across India.

K	Mean L(K)	SD L(K)	L'(K)	L''(K)	Δ K
2	-17616.13	123.96	0	0	0
3	**-16339.83**	**2.38**	**1276.30**	**755.95**	**316.62**
4	-15819.48	71.82	520.35	133.25	1.85
5	-15432.38	37.95	387.10	127.20	3.35
6	-15172.48	174.76	259.90	16.16	0.09
7	-14896.42	245.77	276.06	8.82	0.03
8	-14629.18	81.78	267.24	345.70	4.22
9	-14707.64	826.03	-78.46	409.39	0.49
10	-14376.71	311.89	330.93	405.76	1.30
11	-14451.54	530.91	-74.83	224.77	0.42
12	-14301.6	414.78	149.94	47.08	0.11
13	-14198.74	173.64	102.86	58.74	0.33
14	-14154.62	253.09	44.12	0	0



A B

Figure 5.1. Graphical representation of the true number of groups in predefined RJF populations where the Mean L(K) (±SD) over 10 runs for each K value is plotted in A, and the ad hoc quantity (ΔK) is represented in B.

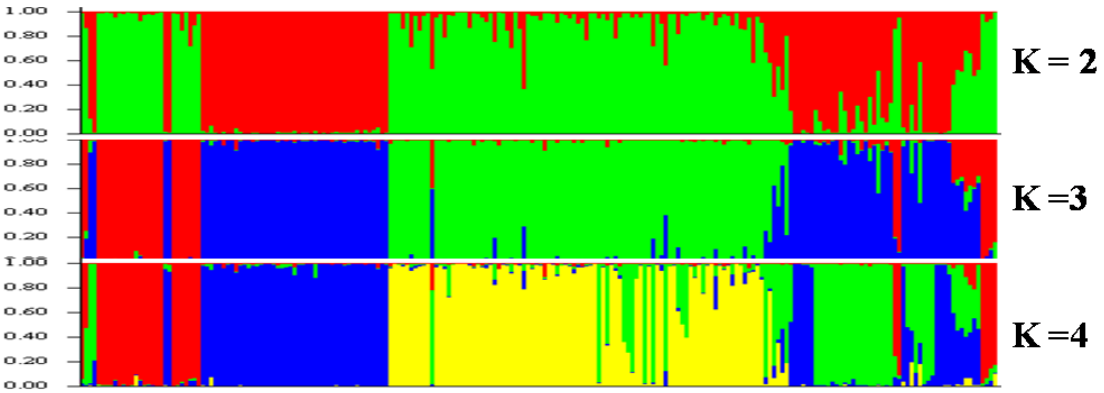


Figure 5.2 Bayesian analysis for Individuals using STRUCTURE software

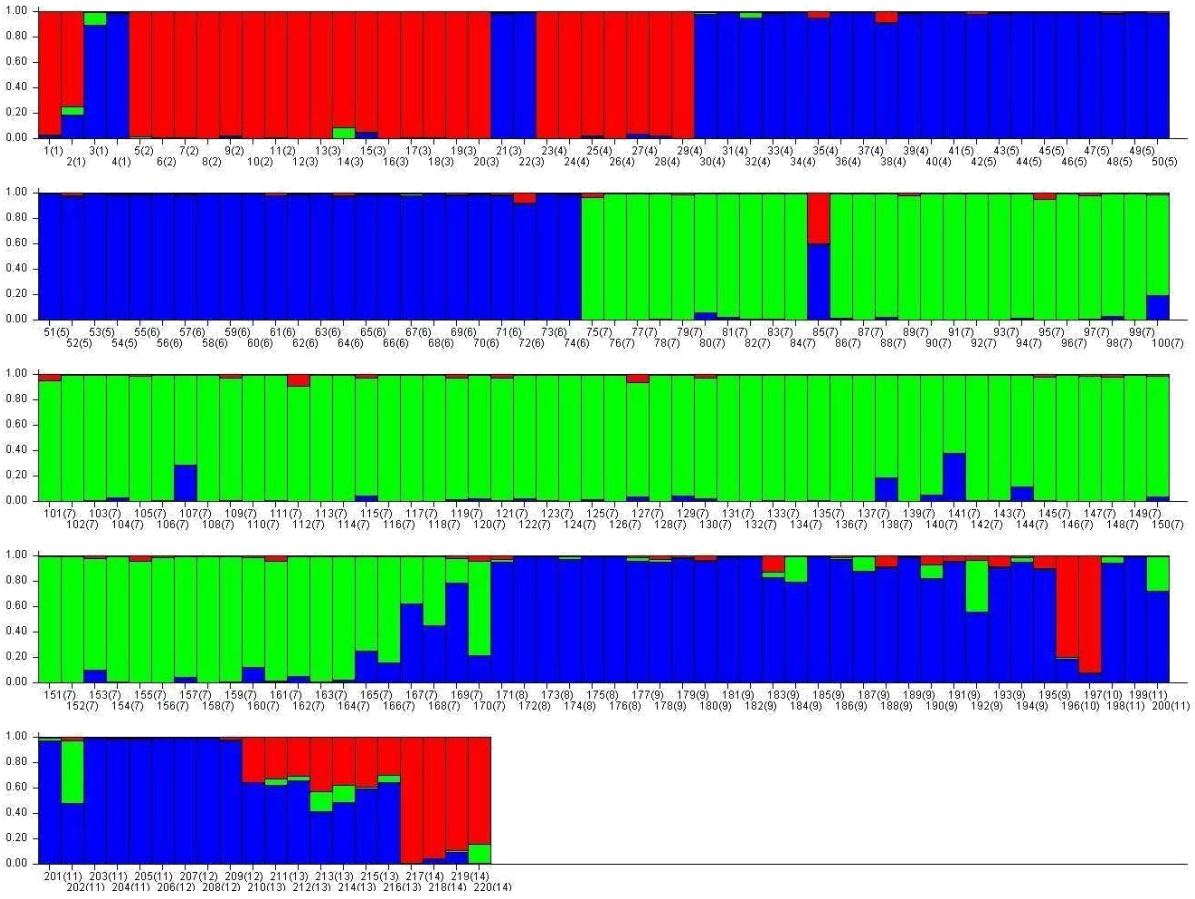


Figure 5.3 Relative ancestory output of individuals using STRUCTURE Software (X-axis) denotes Individuals and Y-axis denotes the relative ancestory)

Table 5.3 Bayesian clustering analysis of 14 captive RJF populations

Captive centre	Cluster 1	Cluster 2	Cluster 3	Unassigned individuals	Assignment to a single cluster (%)
Nanital Zoo (4)	0.431 (1)	0.042 (0)	0.527 (2)	1	0

Chail Pheasantry (7)	0.989 (7)	0.005 (0)	0.006 (0)	0	100
Renuka Pheasantry (11)	0.803 (9)	0.01 (0)	0.187 (2)	0	0
Kufri Pheasantry (18)	0.392 (7)	0.006 (0)	0.602 (11)	0	0
Shimla Pheasantry (14)	0.006 (0)	0.004 (0)	0.99 (14)	0	100
Gopalpur Zoo (20)	0.009 (0)	0.005 (0)	0.986 (20)	0	100
Morni (96)	0.013 (0)	0.929 (88)	0.058 (0)	8	91.66
Lucknow Zoo (6)	0.006 (0)	0.01 (0)	0.984 (6)	0	100
Delhi Zoo (19)	0.034 (0)	0.055 (0)	0.911 (18)	1	94.73
Patna Zoo (2)	0.855 (2)	0.011 (0)	0.135 (0)	0	100
Tata Zoo (8)	0.006 (0)	0.107 (0)	0.887 (6)	2	75
Padmanaidu Zoo (4)	0.007 (0)	0.004 (0)	0.989 (4)	0	100
Tripura (7)	0.353 (0)	0.064 (0)	0.583 (0)	7	0
Vizag Zoo (4)	0.919 (4)	0.042 (0)	0.039 (0)	0	100

- Values in bracket are indicate individual birds

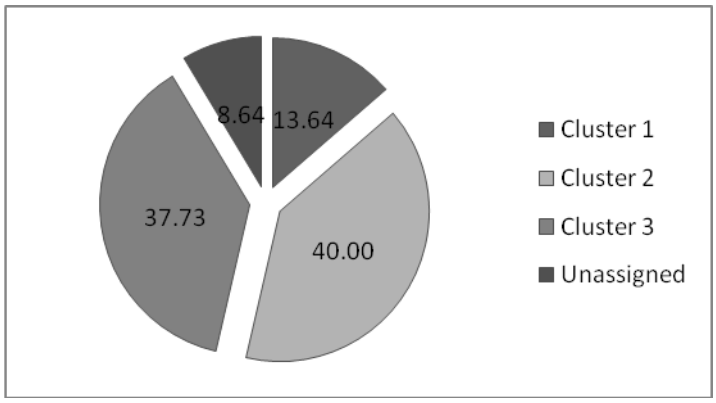
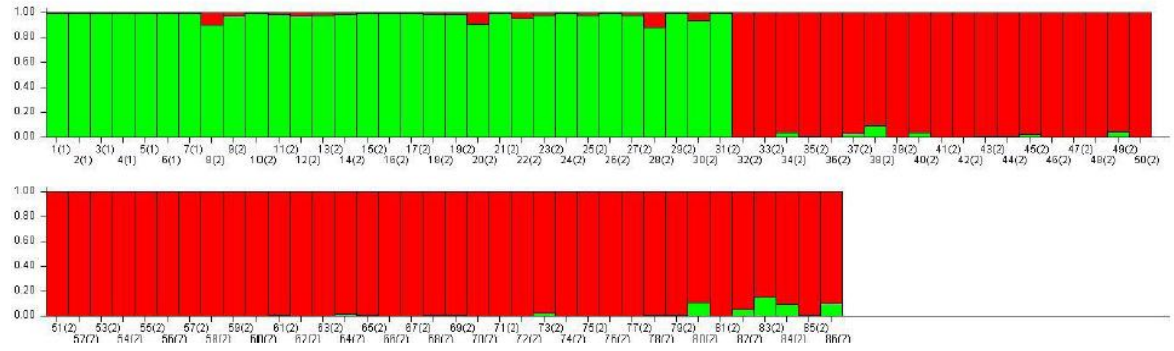
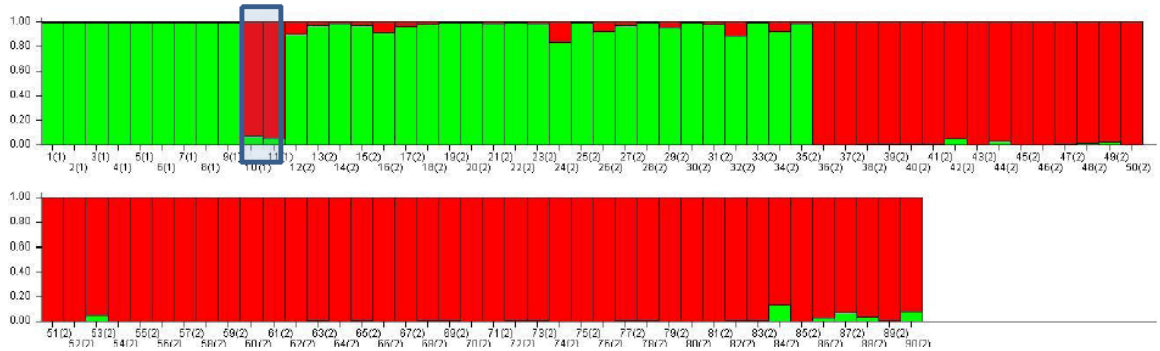


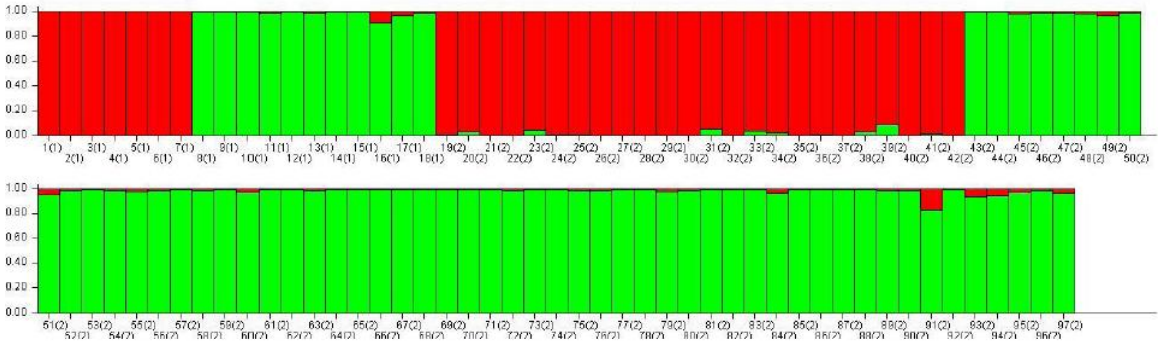
Figure 5.4 Global percentage of STRUCTURE for assigning populations



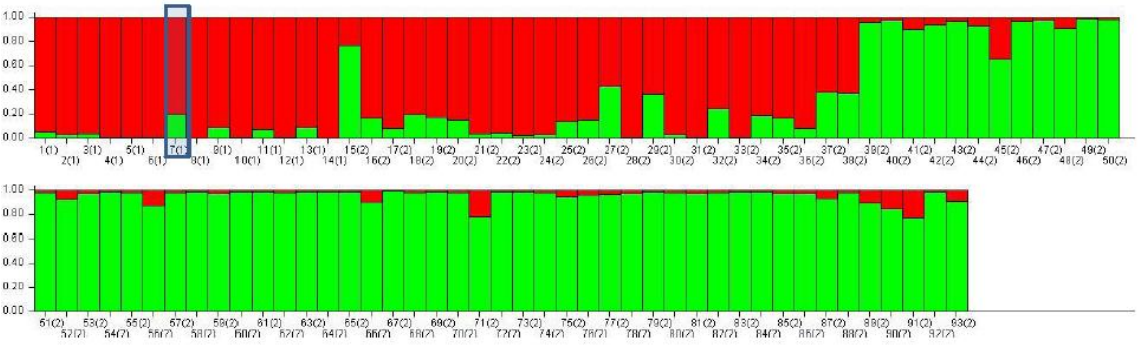
A- Identification of hybrids in the captive stock of Chail pheasantry, Himachal Pradesh



B- Identification of hybrids in the captive stock of Renuka pheasantry, Himachal Pradesh

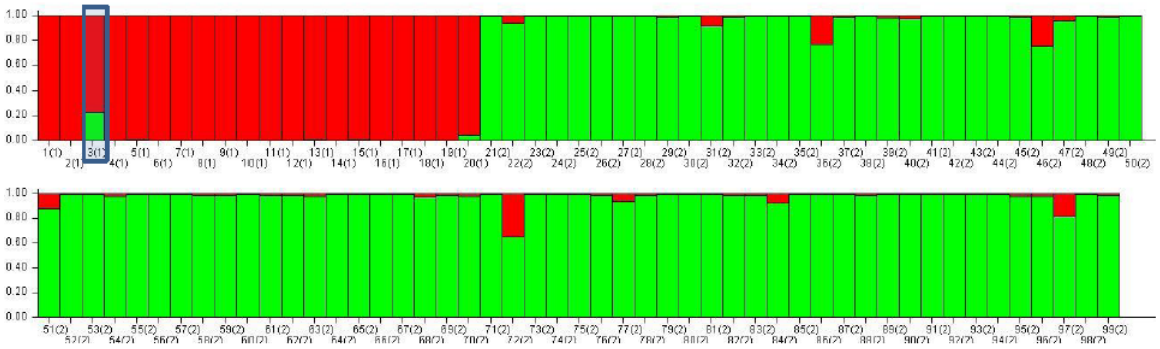


C- Identification of hybrids in the captive stock of Kufri zoo, Himachal Pradesh

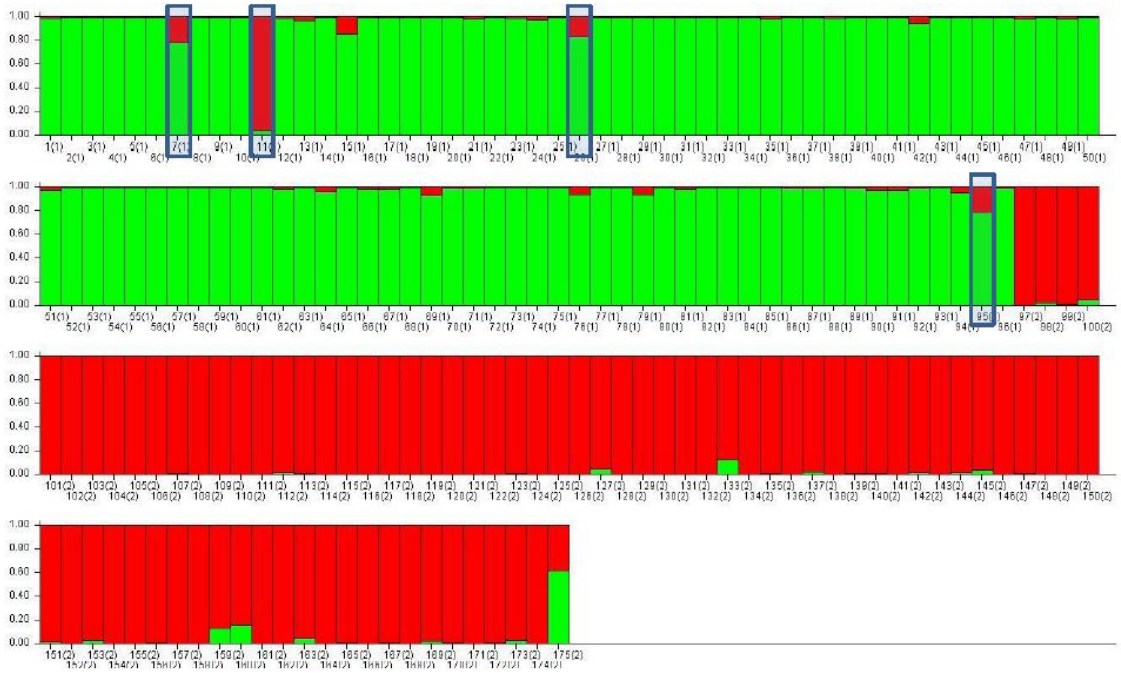


D- Identification of hybrids in the captive stock of Shimla pheasantry, Himachal Pradesh

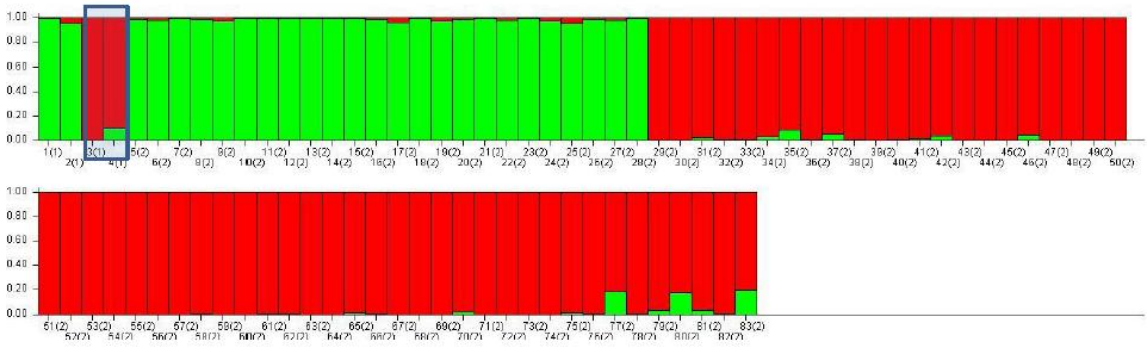
Figure 5.5 Admixture analysis and Identification of hybrids in different zoos/captive centers (Cont'd.)



E- Identification of hybrids in the captive stock of Gopalpur zoo, Himachal Pradesh

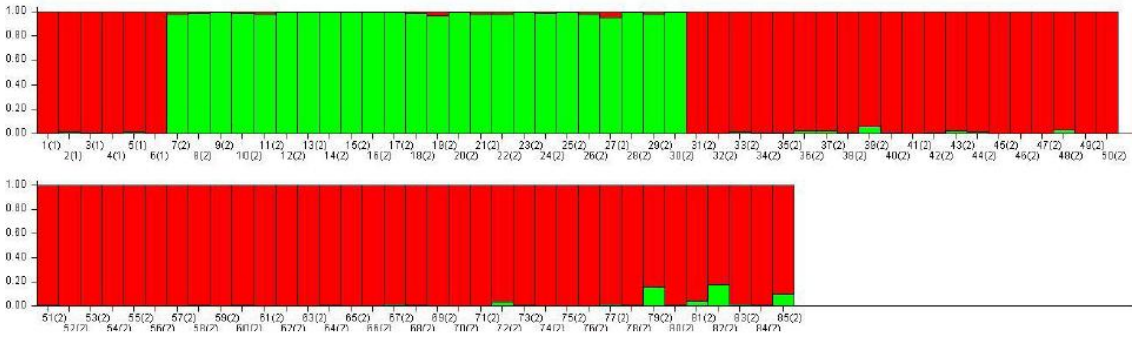


F- Identification of hybrids in the captive stock of Morni RJF breeding center, Haryana

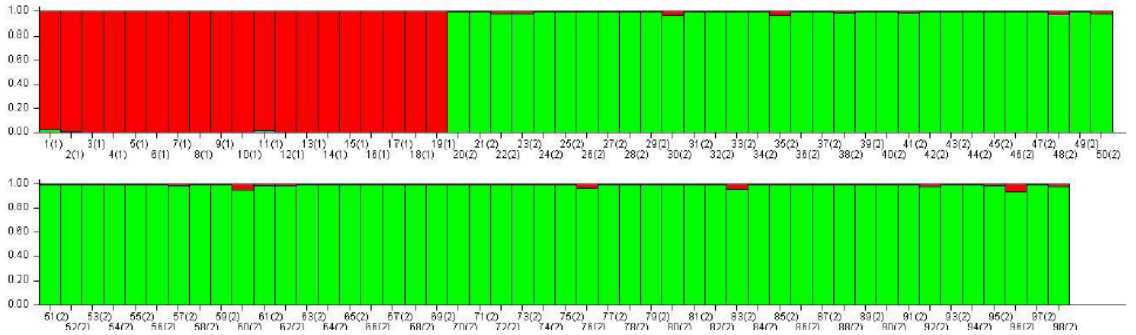


G- Identification of hybrids in the captive stock of Nainital zoo, Uttarakhand

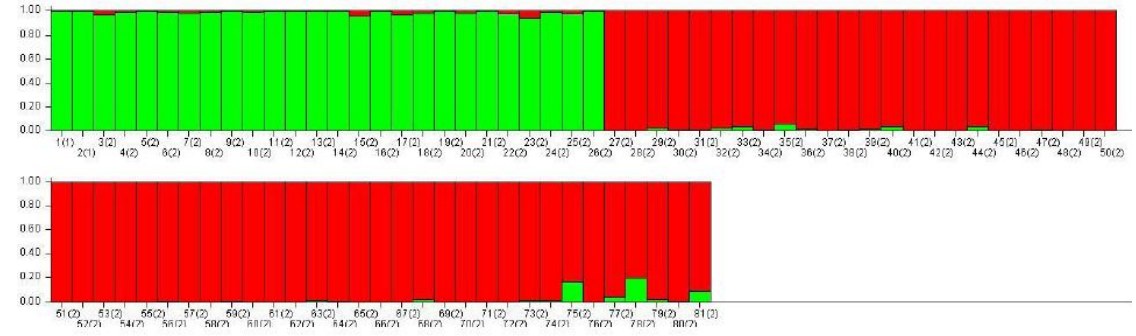
Figure 5.5 Admixture analysis and Identification of hybrids in different zoos/captive centers (Cont'd.)



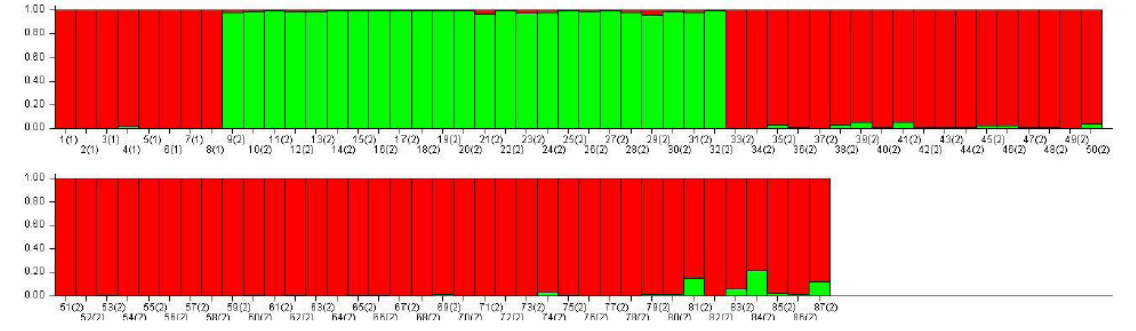
H- Identification of hybrids in the captive stock of Lucknow zoo, Uttar Pradesh



I- Identification of hybrids in the captive stock of Delhi zoo, New Delhi

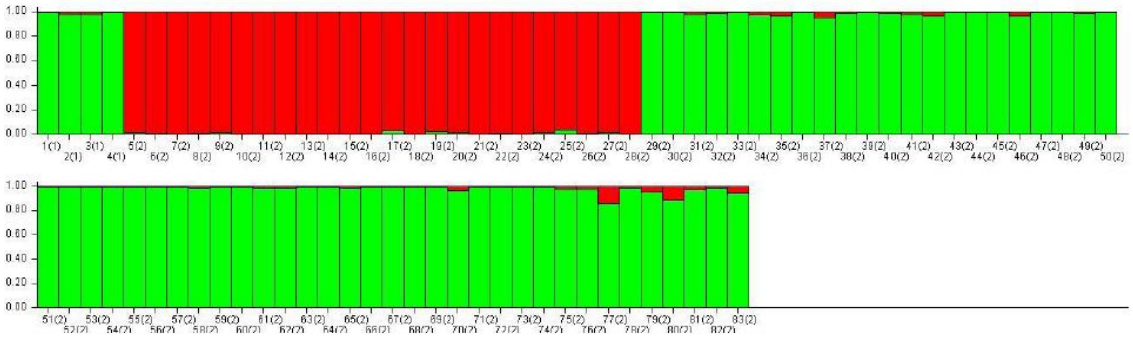


J- Identification of hybrids in the captive stock of Patna zoo, Bihar

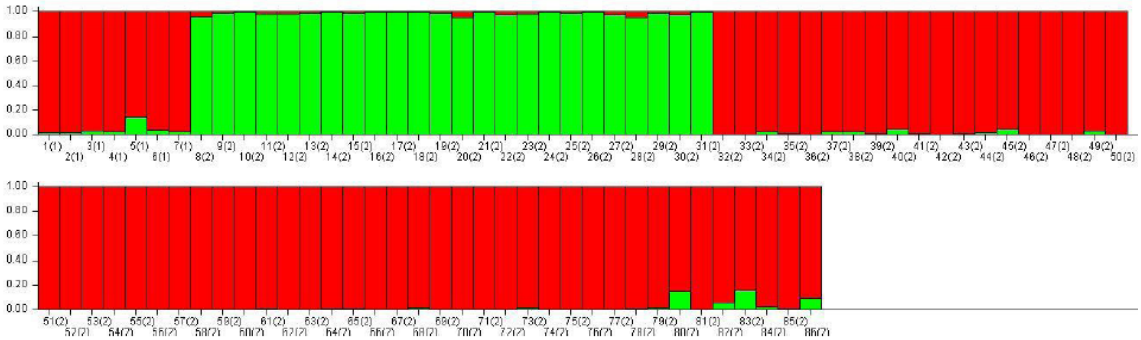


K- Identification of hybrids in the captive stock of Tata zoo, Jharkhand

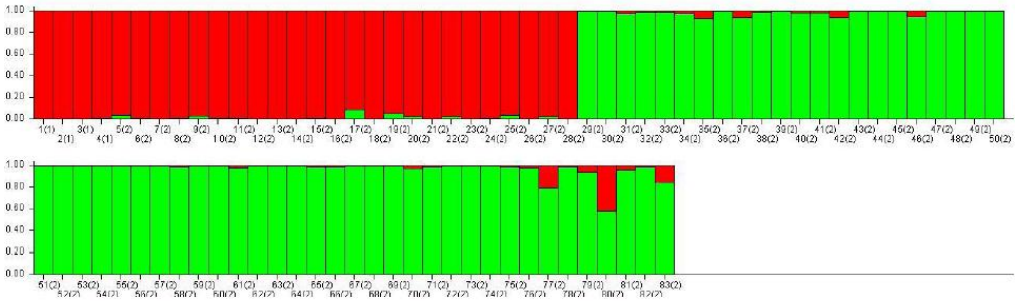
Figure 5.5 Admixture analysis and Identification of hybrids in different zoos/captive centers (Cont'd.)



L- Identification of hybrids in the captive stock of Padmaja Naidu zoo, West Bengal



M- Identification of hybrids in the captive stock of Sepahijala zoo, Tripura



N- Identification of hybrids in the captive stock of Vizag zoo, Visakhapatnam

Figure 5.5 Admixture analysis and Identification of hybrids in different zoos/captive centers. (Birds with q value <0.80 were considered as admixed and they are shown with a superimposing light shade blue box).

Table 5.4- List of admixed individuals which are being reared in various captive centres

State	Place	Sex	Specimen ID	Unique Identity
Uttarakhand	Nainital Zoo (4)	M	MT 216 MT 217	RN- 1525, RN- 1515
Himachal Pradesh	Renuka Pheasantry (11)	F	MT 177 MT 178	S-29 S-30
	Shimla Pheasantry (14)	M	MT 151	S-8
	Gopalpur Zoo (20)	F	MT 183	S-35
Haryana	Morni (96)	F	MT 293 MT 297 MT 385	Pan 1, Berwala, F1 H Pan 5, Berwala, F5-L Ring -G12418 Walking Aviary, Morni, F 3-3
		M	MT 312	Pan 5, Berwala, M5-E Ring -G12447

REFERENCES

Ahlawat, S.P.S., J. Sunder., A. Kundu., R. N. Chatterjee., R. B. Rai., B. Kumar., S. Senani., S. K. Saha., and S. P. Yadav. 2004. Use of RAPD-PCR for genetic analysis of Nicobari fowl of Andamans. British Poultry Science 45: 194–200.

Aiyadurai, A. 2007. Hunting in a biodiversity hotspot. A survey on hunting practices by indigenous communities in Arunachal Pradesh, North-east India. Rufford Maurice Lang Foundation. Report. Pp44.

Ali, S., and S. D. Ripley. 1983. Handbook of the birds of India and Pakistan. Compact Edition. Delhi. Oxford University Press. Pp 737+113 plates.

Allendorf, W. F., R. F. Leary., P. Spruell., and K. J. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. Trends in Ecology and Evolution 16: 613-622.

Altman, J. 1974. Observational study of behavior: sampling methods. Behaviour **49:** 227–267.

Anderson, R. P., M. Gomez-Laverde., and A. T. Peterson. 2002. Geographical distributions of spiny pocket mice in South America: Insights from predictive models. Global Ecology and Biogeography 11: 131-141.

Andersson, L., C. S. Haley., H. Ellegren., S. A. Knott., M. Johansson., K. Andersson., L. Andersson-Eklund., I. Edfors-Lilja., M. Fredholm., I. Hansson., J. Hakansson., and K. Lundstrom. 1994. Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science 263: 1771-1774.

Andersson, M. 1994. *Sexual Selection*. Princeton University Press: Princeton. Pp 599.

Andersson, M and Y. Iwasa. 1996. Sexual selection. Trends in Ecology and Evolution 11: 53–58.

AVIANDIV; Weigend, S (Coordinator), M.A.M. M. Groenen., A. Tixier-Boichard., J. Vignal., J. Hillel., K. Wimmers., T. Burke., and A. Maki-Tanila. 1998-2000. Development of Strategy and Application of molecular tools to assess biodiversity in chicken genetic resources. EC Contract No. BI04-CT98-0342; http://aviandiv.tzv.fal.de/primer_table.html

Baker, E. C. S. 1920. The game birds of India, Burma and Ceylon Part 20. Journal of Bombay Natural History Society 27: 417-430.

Bartholomé, E., and A. S. Belward. 2005. GLC2000: a new approach to global land cover mapping from Earth observation data. International Journal of Remote Sensing 26: 1959–1977.

Bateson, P. 1983. Mate choice. Cambridge University Press, Cambridge. Pp 462.

Beebe, W. 1922. A Monograph of the Pheasants. Vol I – IV. H.F. & G Witherby, London, England.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N. and F. Bonhomme. 2004. GENETIX 4.05. (http://www.univmontp2.fr/~genetix/genetix/genetix.htm).

Beuzen N.D., M. J. Stear., and K. C. Chanj. 2000. Molecular markers and their use in animal breeding. Veterinary Journal 160: 42-52.

Beveridge, H. 1909. Jehangir the Emperor of Hindustan the Tuzuk-I-Jahangiri. Stephen Austen and Sons Limited. Hertford, Britian. 483 Pp.

Birkhead, T. R., and T. Pizzari. 2002. Postcopulatory sexual selection. Nature Review Genetics 3: 262-273.

Birkhead, T. R. and G. A. Parker. 1997. Sperm competition and mating systems. Pg 329-334. In: Behavioural ecology: and evolutionary approach (Krebs JR, Davies NB, eds). Oxford: Blackwell. Pp 432.

Botstein D., White R. L., Skolnick M. and R. W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics. 32: 314–331.

Bradbury, J. W. and M. B. Andersson. 1987. Sexual Selection: Testing the Alternatives. New York: J. Wiley. Pp 308.

Bradbury, J. W. and R. M. Gibson. 1983. Leks and mate choice. Pg 109-138. In: Mate choice (Bateson eds). Cambridge University Press, Cambridge Pp 462.

Brooks, R. 2000. Negative genetic correlation between male sexual attractiveness and survival. Nature 406: 67–70.

Brisbin, I. L. 1995. Conservation of the wild ancestors of domestic animals. Conservation Biology 9: 1327-1328.

Brisbin, I. L. Jr., A.T. Peterson., R. Okimoto, and G. Amoto. 2002. Characterisation of the genetic status of populations of Red Junglefowl. Journal of Bombay Natural History Society 99: 217-223.

Brisbin, I. L. Jr., and A. T. Peterson. 2007. Playing chicken with Red Junglefowl: identifying phenotypic markers of genetic purity in Gallus gallus. Animal Conservation 10: 429–435.

Brooks, T. M., R. A. Mittermeier, G. A. B. da Fonseca., J. Gerlach., M. Hoffmann., J. F. Lamoreux., C. G. Mittermeier., J. D. Pilgrim., and A. S. L. Rodrigues. 2006. Global Biodiversity Conservation Priorities. Science 313: 58-61.

Brumfield, R. T., P. Beerli., D. A. Nickerson., and S. V. Edwards. 2003. The utility of single nucleotide polymorphisms in inferences of population history. Trends in Ecology and Evolution 18: 249-256.

Bruford, M.W. and R. K. Wayne., 1993. Microsatellites and their application to population genetic studies. Current Opinion in Genetics and Development, 3: 939-943

Bump, G., and W. H. Bohl. 1961. Red Junglefowl and Kalij pheasants. U.S. Fish & Wildlife, Washington, DC, Special Scientific Report. 62.

Burt, D.W. 2005. Chicken genome: Current status and future opportunities. Genomics Research 15:1692-1698.

Cavalli-Sforza, L. L. and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 32: 550-570

Channell, R., and M. V. Lomolino. 2000. Trajectories to extinction: spatial dynamics of the contraction of ranges. Journal of Biogeography 27: 167-179.

Chappell, M. A., M. Zuk., T. H. Kwan, and T. S. Johnsen. 1995. Energy cost of an avian vocal display: crowing in red junglefowl. Animal Behaviour 49: 255-257.

Chen G., W. Bao., J. Shu., C. Ji., M. Wang., H. Eding., F. Muchadeyi., and S. Weigend. 2008. Assessment of population structure and genetic diversity of 15 Chinese indigenous chicken breeds using microsatellite markers. Asian Australian Journal of Animal Science 21: 331–339.

Cheng H.H. and L. B. Crittenden. 1994. Microsatellite markers for genetic mapping in the Chicken. Poultry Science 73: 539-546.

Clements, G.R., C. J. A. Bradshaw., B. W. Brook., and W. F. Laurance. 2011. The SAFE index: using a threshold population target to measure relative species threat. Frontiers in Ecology and Environment doi: 10.1890/100177.

Collias, N. E., and E. C. Collias. 1967. A field study of the red junglefowl in North-central India. Condor 69: 360–386.

Collias, N. E., and E. C. Collias. 1996. Social organization of a red junglefowl, Gallus gallus, population related to evolution theory. Animal Behaviour **51:** 1337–1354.

Collias, N. E., E. C. Collias, and R. I. Jennrich. 1994. Dominant red junglefowl (Gallus gallus) hens in an unconfined flock rear the most young over their lifetime. Auk **111:** 863–872.

Coombs, J. A., B. H. Letcher, and K. H. Nislow, 2008. CREATE: a software to create input files from diploid genotypic data for 52 genetic software programs. Molecular Ecology Resources 8:578-580.

Coppolillo, P., H. Gomez., F. Maisels., and R. R. Wallace. 2004. Selection criteria for suites of landscape species as a basis for site-based conservation. Biological Conservation 115: 419-430.

Cornwallis, C. K., and T. R. Birkhead. 2008. Plasticity in reproductive phenotypes reveals status-specific correlations between behavioral, morphological, and physiological sexual traits. Evolution 62: 1149-1161.

Craig, J. V., L. L. Ortman, and A. M. Guhl. 1965. Genetic selection for social dominance ability in chicks. *Animal Behaviour* 13: 114–131.

Crawford, R.D. 1990. Origin and history of poultry species. Pg 1-41. In: Poultry breeding and genetics. (Crawford, R.D. ed.). Elsevier, Amsterdam. Pp. 1123.

Crooijmans R. P. M. A., A. J. A. Van Kampen, J. J. Van der Poel., and M. A. M. Groenen. 1993. Highly polymorphic microsatellite markers in poultry. Animal Genetics 24: 441-443.

Crooijmans R. P. M. A., P. A. M. Van Oers, J. A. Strijk, J. J. Van der Poel. and M. A. M. Groenen. 1996. Preliminary linkage map of the chicken *Gallus domesticus* genome based on microsatellite markers: 77 new markers mapped. Poultry Science 75: 746-54.

Crooijmans R. P. M. A., A. F. Groen., A. J. A. Van Kampen., S. Van der Beek., J. J. Van der Poel. and M. A. M. Groenen. 1996. Microsatellite polymorphism in commercial broiler and layer lines estimated using pooled blood samples. Poultry Science 75: 904-909.

Cuc, N. T. K., F. C. Muchadeyi., U. Baulain.,H. Eding., S. Weigend., and C. B. A. Wollny. 2006. An assessment of genetic diversity of Vietnamese H'mongs Chickens. International Journal of Poultry Science 5: 912- 920.

Darwin, C. 1868. The variation of Animals and Plants under Domestication. John Murray, London. Pp 576.

David, P., T. Bjorksten., K. Fowler., and A. Pomiankowski. 2000. Condition dependent signalling of genetic variation in stalk-eyes flies. Nature 406: 186–188.

Dawkins, M. S. 1989. Time budgets in red junglefowl as a baseline for the assessment of welfare in domestic fowl. Applied Animal Behaviour Science 24: 77–80.

De Marchi, M., C. Targhetta., B. Contiero., and M. Cassandro. 2003. Genetic traceability of chicken breeds. Agriculturae Conspectus Scientificus 68: 255-259.

Delacour, J. 1977. The Pheasants of the World. Spur Publications and the World Pheasant Association. Pp 395.

Diamond, J. 2002. Evolution consequences and future of plant and animal domestication. Nature 418: 700-707.

Dodgson, J.B., H. H. Cheng., and R. Okimoto., 1997. DNA marker technology: A revolution in animal genetics. Poultry Science 76: 1108-1114.

Dowling, T. E., and C. L. Secor. 1997. The role of hybridisation and introgression in the diversification of animals. Annual Review of Ecology and Systemmatics 28: 593-619.

Dudik, M., Phillips, S. J., and R. E. Schapire. 2007. Maximum entropy density estimation with generalised regularisation and an application to species distribution modelling. Journal of Machine Learning Research 8: 1217-1260.

Dunnington E.A., O. Gal., Y. Plotsky., A. Haberfeld., T. Kirk., A. Goldberg., U. Lavi ., A. Cahaner., P. B. Siegel., and J. Hillel. 1990. DNA Fingerprints of chickens selected for high and low body weight for 31 generations. Animal Genetics 21: 221-231.

Dunnington E.A., L. C. Stallard., J. Hillel., and P. B. Siegel. 1994. Genetic diversity among commercial chicken populations estimated from DNA fingerprints. Poultry Science 73: 1218-1225.

Ebenhard, W. G. 1996. Female Control: Sexual Selection by Female Cryptic Choice. Princeton University Press: Princeton. Pp 501.

Elith, J., C. H. Graham., R. P. Anderson., M. Dudik., S. Ferrier., A. Guisan., R. J. Hijmans., F. Huettmann., J. R. Leathwick., A. Lehmann., J. Li., L. G. Lohmann., B. A. Loiselle., G. Manion., C. Moritz., M. Nakamura., Y. Nakazawa., J. M. Overton., A. T. Peterson., S. J. Phillips., K. S. Richardson., R. Scachetti-Pereira., R. E. Schapire., J. Soberon., S. Williams., M. S. Wisz., and N. E. Zimmermann. 2006. Novel methods improve prediction of species distribution from occurrence data. Ecography 29: 129-151.

Elith, J., and J. R. Leathwick. 2009. Species distribution models. Ecological explanation and prediction across space and time. Annual Reviews of Ecology Evolution and Systematics. 40: 677-697.

Elith, J., M. Kearney., and S. Phillips. 2010. The art of modelling range shifts. Methods in Ecology and Evolution 1: 330-342.

Ellegren, H. 2000. Microsatellite mutations in the germline: implications for evolutionary inference. Trends in Genetics 16: 551-558.

Eriksson J, G. Larson., U. Gunnarsson., B. Bed'hom., M. Tixier-Boichard., L. Strömstedt., D. Wright., A. Jungerius., A. Vereijken., E. Randi., P. Jensen., and L. Andersson. 2008. Identification of the yellow skin gene reveals a hybrid origin of the domestic chicken. PLoS Genetics 4: e1000010.

Etches, R. J. 1996. Reproduction in Poultry CAB International, Oxford. Pp 318.

Evanno, G., S. Regnaut., and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611-2620.

FAO.2004a. Secondary guidelines for development of national farm animal genetic resources management plans. Measurement of domestic animal diversity (MoDAD): Recommended microsatellite markers, Rome., Italy.

Fernandes, M., Mukesh., S. Sathyakumar, R. Kaul., R. S. Kalsi., and D. Sharma. 2009. Conservation of Red Junglefowl in India. International Journal of Galliformes Conservation 1: 94-101.

Fielding, A. H., J. F. Bell. 1997. A review of methods for the assessment of prediction error in conservation presence/absence models. Environmental Conservation 24: 38-49.

Fuller R. A. and P.J. Garson. (eds) 2000. Pheasants: status survey and conservation action plan 2000-2004. WPA/Birdlife/SSC Pheasant Specialist Group. IUCN, Gland. Switzerland and Cambridge, U.K. and the World Pheasant Association, Reading, U.K.

Fumihito, A., T. Miyake., S. Sumi., M. Takada., S. Ohno., and N. Kondo. 1994. One subspecies of the red junglefowl Gallus gallus gallus. suffices as the matriarchic ancestor of all domestic breeds. Proceedings of the National Academy of Sciences 91:12505-12509.

Fumihito, A., T. Miyake., M. Takada., R. Shingu., T. Endo., T. Gojobori., N. Kondo., and S. Ohno. 1996. Monophyletic origin and unique dispersal patterns of domestic fowls. Proceedings of the National Academy of Science. 93: 792-6795.

Gardner, J. P. A. 1997. Hybridisation in the sea. In: Advances in Marine Biology. 31 (Blaxter, J.H.S. and Southward, A. J. eds.) Academic Press. Pp 78.

Gaston, K. J. 2009. Geographical range limits: achieving synthesis. Proceeding of the Royal Society Biological Science 276: 1395-1406.

Gaston, K. J. 2011. Common Ecology. BioScience 61: 354-362.

Geist, H. J. and E. F. Lambin. 2002. Proximate causes and underlying driving forces of tropical deforestation. BioScience 52: 143-150.

Githiru, M., E. Matthysen., L. Lens., and L. A. Bennun. 2007. Can a common bird species be used as a surrogate to draw insights for the conservation of a rare species? A case study from the fragmented Taita Hills, Kenya. Oryx 41: 239-246.

Glaubitz J.C., 2004. CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Molecular Ecology Notes 4: 309-310.

Gongora, J., N. J. Rawlence., V. A. Mobegi., H. Jianlin., J. A. Alcalde., J. T. Matus., O. Hanotte., C. Moran., J. J. Austin., S. Ulm., et al., 2008. Indo-European and Asian origins for Chilean and Pacific chickens revealed by mtDNA. Proceedings of the National Academy of Sciences 105: 10308-10313.

Graham, C. H., S. Ferrier., F. Huettman., C. Moritz., and A. T. Peterson. 2004. New developments in museum-based informatics and applications in biodiversity analysis. Trends in Ecology and Evolution 19: 497-503.

Grant P. R., and B. R. Grant. 1992. Hybridisation of bird species. Science 256: 193-197.

Griffith, S. C., I. P. F. Owens., and T. Burke. 1999. Environmental determination of a sexually selected trait. Nature 400: 358-360.

Groen A.F., R. P. M. A. Crooijmans., A. J. A. Van Kampen, S. Van der Beek., J. J. Van der Poel., and M. A. M. Groenen. 1994. Microsatellite polymorphism in commercial broiler and layer lines. In: Proceedings of the 5th World Congress on Genetics Applied to Livestock Production, Guelph, 7-12 August, 1994, Vol. 21, University of Guelph, Ontario, pp. 94-97.

Groenen M. A. M., H. H. Cheng., N. Bumstead., B. Benkel., E. Briles., D. W. Burt., T. Burke., J. Dodgson., J. Hillel., S. Lamont., F. A. Ponce de Leon., G. Smith., M. Soller., H. Takahashi., and A. Vignal. 2000. Consensus linkage map of the chicken genome. Genome Research 10: 137-147.

Gueye, E. F., 1998. Village egg and fowl meat production in Africa. World's Poultry Science 54: 73-86.

Guisan, A., and W. Thuiller. 2005. Predicting species distribution: offering more than simple habitat models. Ecology Letters 8: 993-1009.

Guisan, A., and N. E. Zimmermann. 2000. Predictive habitat distribution models in ecology. Ecological Modelling 135: 147-186.

Hakansson, J. and P. Jensen. 2005. Behavioural and morphological variation between captive junglefowl populations of red junglefowl *Gallus gallus* possible implications for conservation. Biological Conservation 122: 431-439.

Hanotte, O., and H. Jianlin. 2005. Genetic characterization of livestock populations and its use in conservation decision-making. The Role of Biotechnology. Villa Gualino, Turin, Italy – 5-7 March, 2005.

Hedrick, P.W. 1975. Genetic similarity and distance: Comments and comparisons. Evolution 29: 362-366.

Helback, H. 1959. Domestication of food plants in the old world. Science.130: 365-372.

Hijmans, R. J., S. E. Cameron., J. L. Parra., P. G. Jones., and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25: 1965-1978.

Hillel J., Y. Plotzky., O. Gal., A. Haberfeld., U. Lavi., E. A. Dunnington., P. B. Siegel., A. J. Jeffreys., and A. Cahaner. 1989. DNA fingerprint in chickens. In: Proceedings of the 31st British poultry breeders roundtable, 20-22 September 1989, Reading.

Hillel J., R. Avner., C. Baxter-Jones., E. A. Dunnington., A. Cahaner., and P. B. Siegel. 1990. DNA fingerprints from blood mixes in chickens and turkeys. Animal Biotechnology 2: 201-204.

Hillel, J., M. A. M. Groenen., M. Tixier-Boichard., A. B. Korol., L. David., V. M. Kirzhner., T. Burke., A. Barre-Dirie., R. P. M. A. Crooijmans., K. Elo., M. W. Feldman., P. J. Freidlin., A. Maki-Tanila., M. Oortwijn., P. Thomson., A. Vignal., K. Wimmers., and S. Weigend. 2003. Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. Genetics Selection Evolution 35: 533–557.

Holland, B. 2002. Sexual selection fails to promote adaptation to a new environment. Evolution 56: 721–730.

Holland, B. and W. R. Rice. 1999. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. Proceedings of the National Academy of Science. 96: 5083–5088.

Hume, A. O., and C. H. T.Marshall. 1879. The game birds of India, Pakistan, Bangladesh, Burma and Srilanka (including Nepal, Bhutan and Tibet) Vol I-III. Bhavana Books and Prints, New Delhi.

Hutchinson, G. E. 1957. Concluding remarks. Cold Spring Harbour Symposium. 22: 415-427.

Irina, G. M., M. N. Romanov., A. A. Nikiforov., A. A. Sevastyanova., and S. K. Semyenova. 2003. Evolutionary relationships of red junglefowl and chicken breeds. Genetic Selection Evolution 35: 403-423.

Janetos, A. C. 1980. Strategies of female mate choice: a theoretical analysis. Behaviour Ecology and Sociobiology 7:107-112.

Jaime, G., N. J. Rawlence., V. A. Mobegi., H. Jianlin., J. A. Alcalde., J. T. Matus., O. Hanotte., C. Moran., J. J. Austin., S. Ulm., A. J. Anderson., G. Larson., and A. Cooper. 2008. Indo-European and Asian origins for Chilean and Pacific Chickens revealed by mt DNA. Proceedings of the National Academy of Sciences 105: 10308-10313.

Javed, S., and A. R. Rahmani. 2000. Flocking and habitat use pattern of the Red Junglefowl Gallus gallus in Dudwa National Park, India. Journal of Tropical Ecology 41: 11-16.

Jensen, P. 2005. Genomics: The chicken genome sequence. Heredity 94: 567–568.

Jetz, W., D. S. Wilcove, and A. P. Dobson. 2007. Projected impacts of climate and land-use change on the global diversity of birds. PLoS Biology 5:1211–1219.

Johnsen, T.S., and M. Zuk. 1996. Repeatability of mate choice in female Red Junglefowl. Behavioural Ecology 7:243-246.

Johnsgard, P. A. 1986. The Pheasants of the World. Oxford University Press, New York. Pp300.

Johnson, K., R. Thornhill., J. D. Ligon., and M. Zuk. 1993. The direction of mothers and daughters preferences and the heritability of male ornaments in Red Jungle fowl (Gallus gallus). Behavioral Ecology 4: 254-259.

Kalinowski, S.T., M. L. Taper., and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099-1006

Kalsi, R. S. 1992. Habitat selection of Red Junglefowl at Kalesar Reserve Forest, Haryana, India. Pg 80-82. In: (Jenkins, David. eds) and others Proceedings of the Fifth International Symposium on Pheasants in Asia. 28th September – 4th October 1992, Lahore. Pakistan.

Kanginakudru, S., M. Murlidhar., R. D. Jakati., and J. Nagaraju. 2008. Genetic evidence from Indian red junglefowl corroborates multiple domestication of modern day chicken. BMC Evolutionary Biology 8: 174.

Kaul, R., J. N. Shah., and B. Chakrabarty. 2004. An assessment of important physical traits shown by some captive Red Junglefowl in India. Current Science 87: 1498-1499.

Kavaca M., F. G. Karaca., C. Patel., and M. G. Emara. 1999. Preliminary analysis of microsatellite loci in commercial broiler chickens, In: Plant and Animal Genome VII Conference, 17-21 January, 1999 San Diego, CA, USA.

Khatib H., A. Darvasi., Y. Plotski., and M. Soller. 1994. Determining relative microsatellite allele frequencies in pooled DNA samples. PCR Methods and Applications 4: 13-18.

Kaya, M. and M. A. Yildiz. 2008. Genetic diversity among Turkish native chickens, Denizli and Gerze, estimated by microsatellite markers. Biochemical Genetics 46:480–491.

Kimura. M., and J. F. Crow. 1964. The number of allele that can be maintained in a finite population. Genetics 4: 725-38.

Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. Evolution 36:1-12.

Kirkpatrick, M. and M. J. Ryan. 1991. The evolution of mating preference and the paradox of the lek. Nature 350: 33-38.

Koyama, S. and S. Kamimura. 2000. Influence of social dominance and female odour on the sperm activity of male mice. Physiological Behaviour. 71: 415–422.

Lambeck, R. J. 1997. Focal species: a multi-species umbrella for nature conservation. Conservation Biology 11: 849–856.

Lee, E.-J., H. Mannen., M. Mizutani., and S. Tsuji. 2000. Genetic analysis of chicken lines by amplified fragment length polymorphism (AFLP). Animal Science Journal 71: 231-238.

Leemans, R., and S. Serneels. 2004. Understanding land-use change to reconstruct, describe or predict changes in land cover. GeoJournal 61:305–307.

Lenton, S. M., J. E. Fa., and J. P. D. Val. 2000. A simple non-parametric GIS model for predicting species distribution: endemic birds in Bioko Island, West Africa. Biodiversity and Conservation 9: 869 – 885.

Levin, S. A. 1992. The problem of pattern and scale in ecology. Ecology 73: 1943-1967.

Lewis, R. 2002. SNPs as windows on evolution. The Scientist 16: 16.

Ligon, J. D., R. Thornhill., M. Zuk., and K. Johnson. 1990. Male-male competition in Red junglefowl and the multiple roles of testosterone in sexual selection. Animal Behaviour 40: 367-373.

Ligon, J. D., and P. W. Zwartjes. 1995. Female Red Junglefowl choose to mate with multiple males. Animal Behaviour 49: 127-135.

Liu, Yi-Ping., Wu, Gui-Sheng., Yao,Yong-Gang., Miao, Yong-Wang., G. Luikart., M, Baig., A, Beja-Pereira., Ding, Zhao-Li., M. G. Palanichamy., and Ya-Ping. Zhang. 2006. Multiple origins of chickens: out of Asian jungles. Molecular Phylogenetics and Evolution 38: 12–19.

Loiselle, B., C. A. Howell., C. A., C. H. Graham., J. M. Goerck., B. Thomas., K. G. Smith., and P. H. Williams. 2003. Avoiding pitfalls of using species-distribution models in conservation planning. Conservation Biology 17: 1-10.

Lovlie, H. and T. Pizzari. 2007. Sex in the morning or evening? Females adjust daily mating patterns to the intensity of sexual harassment. The American Naturalist 170: E1-E13.

Ma, C-X., M. Lin., R. C. Littell., T. Yin., and R. Wu. 2004. A likelihood approach for mapping growth trajectories using dominant markers in a phase-unknown full-sib family. Theoretical and Applied Genetics 108: 699-705.

Mackey K., P. Williams., S. Seim., and P. Chomczynski. 1996. The use of DNAzol® for the rapid isolation of genomic DNA from whole blood. Biomedical Products Supplement 13-15.

Mackey K., A. Steinkamp., and P. Chomczynski. 1997. DNA extraction from small blood volumes and single –tube DNA extraction and amplification using blood filter cards. Molecular Biotechnology 9: 1-5.

Madge, G., and P. J. K. McGowan. 2002. Pheasants, Patridges & Grouse including buttonquails, sandgrouse and allies. Christopher Helm, an imprint of A & C Black publishers Ltd, London. Pp 488.

Margules, C. R., and R. L. Pressey. 2000. Systematic conservation planning. Nature 405: 243-253.

McBride, G., I. P. Parer and F. Foenander. 1969. The social organisation and behaviour of the feral domestic fowl. Animal Behaviour Monograph **2:** 125–181.

McGowan, P. J. K., and P. J. Garson. 1995. Status survey and conservation action plan 1995-1999. Pheasants. IUCN and World Pheasant Association, Switzerland and U.K. Pp 116.

Mckinney, M. 2007. Selectivity During Extinctions in Palaeobiology II. In: (Briggs, D. E. and Crowther, P. R. eds.) Blackwell Science Ltd, Malden, MA, USA. doi: 10.1002/9780470999295.ch42.

Meadows, J.R., O. Hanotte., C. Drögemüller., J. Calvo., R. Godfrey., D. Coltman., J.F. Maddox., N. Marzanov., J. Kantanen., and J. W. Kijas., 2006. Globally dispersed Y- chromosomal haplotypes in wild and domestic sheep. Animal Genetics 10: 444-453.

Miller, M. P. 1997. Tools for population genetic analyses (TFGPA) 1.3: a windows program for the allozyme and molecular population genetic data.

Mina, N.S., B. L. Sheldon., B. H. Yoo., and R. Frankham. 1991. Heterozygosity at protein loci in inbred and outbred lines of chickens. Poultry Science 70: 1864-1872.

Mohd-Azmi, M.L., A. S. Ali., and W. K. Kheng. 2000. DNA fingerprinting of red junglefowl, village chicken and broilers. Asian-Australasian Journal of Animal Sciences 13: 1040-1043.

Moiseyeva, I. G. 1998. Ancient evidence for the origin and distribution of domestic fowl. In: Proceedings of the 10th European Conference "The poultry industry towards the 21st centuary", Vol 1. Jerusalem, 21-28 June 1998, pp 244-245.

Moore, A. J. 1990. The evolution of sexual dimorphism by sexual selection – the separate effects of intrasexual selection and intersexual election. Evolution 44: 315–331.

Moore, A. J. and P. J. Moore. 1999. Balancing sexual selection through opposing mate choice and male competition. Proceedings of the Royal Society of London, Series Biological. 266: 711–716.

Morejohn, G. V. 1953. A gene for yellowish-white down in the red junglefowl. Journal of Heredity 44: 46-52.

Morejohn, G. V. 1955. Plumage colour allelism in the Red Junglefowl and related domestic forms. Genetics 40: 519-530.

Morejohn, G. V. 1968a. Study of plumage of the four species of the genus Gallus. Condor 70: 56-65.

Morejohn, G. V. 1968b. Breakdown of isolation mechanisms in two species of captive junglefowl. Evolution 22: 576-582.

Msofee, P. L. M., Minga, U. M., Olsen, J. E., Yongolo, M. G. S., Juul-madesen, H.R., Gwakisa, P. S. and Mtambo, M. M. A., 2001. Phenotypes including immunocompetence in scavenging local chicken ecotypes in Tanzania. Tropical Animal Health and Production 33: 341-354.

Msoffe, P. L. M., M. M. A. Mtambo., U. M. Minga., J. E. Olsen., H. R. Juul-Madesen, P. S. Gwakisa., S. K. Mutayoba., and A. M. Katule. 2004. Productivity and reproductive performance of the free-range local domestic fowl ecotypes in Tanzania. Livestock Research for Rural Development 16: *67.*

Mukesh., R.S. Kalsi., R.P. Mandhan and S. Sathyakumar. 2011. Genetic diversity studies of Red Junglefowl across its distributional range in northern India. Asian Journal of Biotechnology 3: 293-301.

Nagula, V., V. Vasudeva Rao., C. Srinivasulu. and V. Manohar Reddy. 1997. Birds of Karimnagar (E) Forest Division. A potential corridor connecting Eturnagaram WLS and Indravati National Park. Pavo 35: 39-52.

Nei. M. 1972. Genetic distance between populations. American Naturalist 106:283-292.

Nei. M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Nei M., F. Tajima., and Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. Journal of Molecular Evolution 19: 153-170.

Nikiforov, A.A., I. G. Moiseyeva., and I. A. Zakharov. 1998. Position of Russian chicken breeds in the diversity of Eurasian breeds. Genetika 34: 850-851.

Nishibori, M., T. Shimogiri., T. Hayashi., and H. Yasue. 2005 Molecular evidence for hybridization of species in the genus Gallus except for *Gallus varius*. Animal Genetics 36: 367–375.

Niu D., Y. Fu., J. Luo., H. Ruan., X. P. Yu., G. Chen., and Y. P. Zhang. 2002. The origin and genetic diversity of chinese native chicken breeds. Biochemical Genetics 405: 163-174.

Noss. R. 1996. Protected areas: how much is enough? In: Wright, R.G. (Ed.), National Parks and Protected Areas. Blackwell Science, Cambridge, MA, Pp. 91–120.

Okumura, F., T. Shimogiri., K. Kawabe., S. Okamoto., M. Nishibori., Y. Yamamoto., and Y. Maeda. 2006. Gene constitution of South-East Asian native chickens, commercial chickens and jungle fowl using polymorphisms of four calpain genes. Animal Science Journal 77: 188-195.

Olowofeso, O., J. Y. Wang, K. Z. Xie., and G. Q. Liu. 2005. Phylogenetic scenario of port-city chickens in china based on two-marker types. International Journal of Poultry Science 4: 206-212.

Osman, S., M. Sekino., M. Nishibori., Y. Kawamoto., K. Kinoshita., Y. Yamamoto., and M. Tsudzuki. 2005a. Genetic variability and relationships of Japanese native chickens assessed by means of microsatellite profiling approach - focusing on the Oh-Shamo (Japanese Large Game) and its related breeds. Journal of Poultry Science 41: 94-109.

Osman, S. A. M., M. Sekino., M. Nishibori., Y. Yamamoto., and M. Tsudzuki. 2005b Genetic variability and relationships of native Japanese chickens assessed by microsatellite DNA profiling - focusing on the breeds established in Kochi Prefecture, Japan. Asian-Australasian Journal of Animal Science 18: 755-779.

Ottaviani, D., G. J. Lasinio., and L. Boitani. 2004. Two statistical methods to validate habitat suitability models using presence-only data. Ecological Modelling 179: 417-443.

Pandey, A. K., M. S. Tantia., D. Kumar., B. Mishra., P. Chaudhary., and R. K. Vijh. 2002 Microsatellite analysis of three poultry breeds of India. Asian-Australasian Journal of Animal Science 15: 1536-1542.

Parker, T.H., and D. L. Ligon. 2002. Dominant male red junglefowl (Gallus gallus) test the dominance status of other males. Behavioural Ecology and Sociobiology 53: 20-24.

Peakall, R. and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes. 6: 288-295.

Pearson, R. G., W. Thuiller., M. B. Araujo., E. Martinez-Meyer., L. Broton., C. McClean., L. Miles., P. Segurado., T. P. Dawson., and D. C. Lees. 2006. Model based uncertainty in species range prediction. Journal of Biogeography 33: 1704-1711.

Peterson, A.T., and I. L. Brisbin. Jr. 1999. Genetic endangerment of wild Red Junglefowl Gallus gallus? Bird Conservation International 8: 387-394.

Peterson, A. T., M. Papes., and J. Soberon. 2008. Rethinking receiver operating characteristics analysis applications in ecological niche modeling. Ecological Modelling 213: 63-72.

Petrides, G.A., 1946. Snares and Deadfalls, The Journal of Wildlife Management, 10, 3:234-238.

Phillips, S. J., R. P. Anderson., and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190: 231-259.

Phillips, S. J., and M. Dudik. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography 31: 161-175.

Piepho, H. P., and G. Koch. 2000. Codominant analysis of banding data from a dominant marker system by normal mixtures. Genetics 155: 1459-1468.

Pizzari, T. 2001. Indirect partner choice through manipulation of male behaviour by female fowl, Gallus gallus domesticus. Proceedings of the Royal Society of London, Series Biological **268** : 181–186.

Pizzari, T. and T. R. Birkhead. 2002. The sexually-selected sperm hypothesis: sex-biased inheritance and sexual antagonism. Biological Reviews 77: 183-209.

Ponsuksili S., K. Wimmers., F. Schmoll., P. Horst., and K. Schellander. 1999. Comparison of multilocus DNA finngerprints and microsatellites in an estimate of genetic distance in chicken. Journal of Heredity 90: 656-659.

Prakash, V., D.J. Pain., A. A. Cunningham., P.F. Donald., N. Prakash., A. Verma., R. Gargi., S. Sivakumar., and A. R. Rahmani. 2003. Catastrophic collapse of Indian white-backed *Gyps bengalensis* and long-billed *Gyps indicus* vulture populations. Biological Conservation 109: 381-390.

Pritchard, J. K., M. Stephens., and P. J. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Ramesh, K., S. Sathyakumar and G.S. Rawat. 2008. Methods of capture and radiotracking of Western tragopan *Tragopan melanocephalus* J.E. grey 1829. in the Greathimalayan national park, india. Journal of bombay natural history society 105: 127-132.

Randi, E. 2008. Detecting hybridisation between the wild species and their domestic relatives. Molecular Ecology 17: 285-293.

Rasmussen, P.C., and J. C. Anderton. 2005. Birds of South Asia. The Ripley Guide. Vol 1-2. Smithsonian Institution and Lynx Edicions, Washington, D.C. and Barcelona.

Raxworthy, C. J., E. Martinez-Meyer., N. Horning., R. A. Nussbaum., G. E. Schneider., M. A. Ortega-Huerta., and A. T. Peterson. 2003. Predicting distributions of known and unknown reptile species in Madagascar. Nature 426: 837-841.

Raymond, M., and F. Rousset. 1995. Genepop, version 1.2: population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248–249.

Real, L. 1990. Search theory and mate choice. I. Models of single- sex discrimination. The American Naturalist 136: 376-404.

Real, L. 1991. Search theory and mate choice. II. Mutual inter- action, assortative mating, and equilibrium variation in male and female fitness. The American Naturalist 138: 901-917.

Reynolds, J. D., and M. R. Gross. 1990. Costs and Benefits of Female Mate Choice: Is There a Lek Paradox? The American Naturalist 136: 230-243.

Rhymer, M. J., and D. Simberloff. 1996. Extinction by hybridisation and introgression. Annual Review of Ecology and Systematics 27: 83-109.

Romanov, M. N., and S. Weigend. 2001. Analysis of genetic relationships between various populations of domestic and junglefowl using microsatellite markers. Poultry Science 80: 1057-1063.

Rosenberg, N. A., T. Burke., M. W. Feldman., P. J. Freidlin., A. M. Groenen., J. Hillel., A. Mäki- Tanila., M. Tixier-Boichard., A. Vignal., K. Wimmers., and S. Weigend. 2001. Empirical evaluation of genetic clustering methods using multilocus genotypes from twenty chicken breeds. Genetics 159: 699-713.

Rotenberry, J. T., K. L. Preston., and S. T. Knick. 2006. GIS based niche modeling for mapping species habitat. Ecology 87: 1458-1464.

Rotta, C. T., R. J. Fletcher. Jr., J. M. Evans., and R. L. Hutto. 2011. Does accounting for imperfect detection improve species distribution models? Ecography 34: 659-670.

Rouget, M., D. M. Richardson, R. M. Cowling, J. W. Lloyd, and A. T. Lombard. 2003. Current patterns of habitat transformation and future threats to biodiversity in terrestrial ecosystems of the Cape Floristic Region, South Africa. Biological Conservation 112: 63–85.

Sæther, S. A., P. Fiske., and J. A. Kalas. 1999. Pushy males and choosy females: courtship disruption and mate choice in the lekking great snipe. Proceedings of the Royal Society of London, Series Biological 266: 1227-1234.

Schierwater, B., and A. Ender. 1993. Different thermostable DNA polymerases may amplify different RAPD products. Nucleic Acids Research 21: 4647-4648.

Schutz, K.E., B. Forkman., and P. Jensen. 2001. Domestication effects on foraging strategy, social behaviour and different fear responses: a comparison between the red junglefowl (Gallus gallus) and a modern layer strain. Applied Animal Behaviour Science 74: 1–14.

Scott, J.M., P. J. Heglund., M. L. Morrison. et al (Eds) 2002. Predicting Species Occurrence: Issues of accuracy and scale. Covelo CA. Island Press. Pp 840.

Sharma, A. K. 1998. Identification and evaluation of genetic diversity among the native and exotic chicken breeds using DNA fingerprinting and RAPD markers. Ph.D. Thesis submitted to Deemed University, Indian Veterinary Research Institute, Izatnagar.

Sharma, A.K., Singh, R.K., Kumar, S. and Sharma, D. 2001. Genetic differentiation of selected and control lines of White Leghorn and Rhode Island Red using DNA markers. Indian Journal of Poultry Science 36: 5-8.

Sharma, D. and Singh, R.V., 2001. Random amplified polymorphic DNA polymorphism in Rhode Island Red chicken. Indian Journal of Poultry Science 36: 1-4.

Sharma, D. 2006. Annual progress report: Comparative Genomic Studies on wild and domesticated avain species. Submitted to the Department of Biotechnology, New Delhi.

Sheldon, B. C. 1994. Male phenotype, fertility and the pursuit of extra-pair copulations by female birds. Proceedings of the Royal Society of London, Series Biological 257: 25–30.

Sheldon, B. C. 2000. Differential allocation: tests, mechanisms and implications. *Trends in Ecology and Evolution* **15**: 397–402.

Shen, X.J., S. Ito., M. Mizutani., and Y. Yamamoto. 2002. Phylogenetic analysis in chicken breeds inferred from complete cytochrome-b gene information. Biochemical Genetics 40: 129-141.

Simberloff, D. 1986. The proximate causes of extinction. Patterns and processes in the history of life. (Raup, D. M., and D. Jablonski. eds) 259-276. Springer-Verlag, Berlin.

Smith, V. A., A. P. King., and M. J. West. 2002. Learning in a social context: affiliation patterns in a captive flock of brown-headed cowbirds (Molothrus ater). Animal Behaviour 63: 23–35.

Soberon, J. 2007. Grinnellian and Eltonian niches and geographical distribution of species. Ecology Letters 10: 1115-1123.

Stebbins, G. L. 1950. Variation and evolution in plants. Columbia University Press. Pp 643.

Syvänen, A. C. 2001. Accessing genetic variation: genotyping single nucleotide polymorphisms. Nature Reviews Genetics 2: 930-942.

Tadelle, D., T. Million., Y. Alemu., and K. J. Peters. 2003. Village chicken production systems in Ethiopia: 2. Use pattern and performance valuation and chicken products and socio-economic functions of chicken. Livestock Research of Rural Development, 15(1).

Tajima. F., and N. Takezaki. 1994. Estimation of evolutionary distance for reconstructing molecular phylogenetic trees. Molecular Biology and Evolution 11: 278-286.

Tautz, T., M. Trick., and G. A. Dover. 1986. Cryptic simplicity in DNA is a major source of DNA variation. Nature 332: 652-658.

Takezaki, N., and M. Nei. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144: 389–399.

Tautz. D. 1989. Hypervariability of simple sequences as a general source of polymorphic markers. Nucleic Acids Research 17: 6463-6471.

Thompson, J. N. 1999. The evolution of species interaction. Science 284: 2116-2118.

Thornhill, R. 1988. The jungle fowl hen's cackle incites male competition. Verhandlungen der Deutschen Zoologischen Gesellschaft 81**:** 145–154.

Thuiller, W. 2003. BIOMOD- optimising predictions of species distribution and projecting potential future shifts under global change. Global Change Biology 9: 1353-1362.

Tsoar, A., O. Allouche., O. Steinitz., D. Rotem., and R. Kadmon. 2007. A comparative evaluation of presence-only methods for species distribution. Diversity and Distribution 13: 397-405.

Vanhala T., M. Tuiskula-Haavisto., K. Elo., J. Vilkki., and A. S. O. Maki-Tanila. 1998.. Evaluation of genetic variability and genetic distances between eight chicken lines using microsatellite markers. Poultry Science 77: 783-790.

Van Marle- Koster, E. and L. H. Nel. 2000. Genetic characterization of indigenous South African chicken populations: Evaluation and selection of polymorphic microsatellite markers. South African Journal of Animal Science 30: 1-6.

Van Oosterhout, C., W. F. Hutchinson., D. P. M. Wills., and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4: 535-538.

Van Zeveren, A., Y. Bouquet., A. Van De Weghe., and W. Coppieters. 1990. A genetic blood marker study on four pig breeds. I-Estimation and comparison within-breed variation. II- Genetic relationship between the populations. Journal of Animal Breeding and Genetics 107: 104- 118.

Vaughan, I. P., and S. J. Ormerod. 2005. The continuing challenges of testing species distribution models. Journal of Applied Ecology 42: 720-730.

Vijh, R. K., B. Mishra., R. Arora., P. Chaudhary., U. Sharma., and M. S. Tantia. 2005a. Comparative evaluation of three buffalo populations using microsatellite markers. Indian Journal of Animal Science 75:289–296.

Vijh, R. K., B. Mishra., R. Arora., U. Sharma., and M. S. Tantia. 2005b. Estimation of genetic distances using allele frequencies at microsatellite loci in buffalo populations. Indian Journal of Animal Sciences 75:282-288.

Weber J. L. and P. E. May. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. American Journal of Human Genetics 44: 388-396.

Weigend, S., and M. N. Romanov. 2001. Current strategies for the assessment and evaluation of genetic diversity in chicken resources. World's Poultry Science Journal 57: 275-288.

West, B., and B. X. Zhou. 1989. Did chickens go north? New evidence for domestication. Journal of Archaeological Science 15: 515–533.

White, D. J., A. P. King., and S. D. Duncan. 2002. Voice recognition technology as a tool for behavioural research. Behavioural Research Methods Inst Comp 34:1-5.

Woodroffe, R., and J. R. Ginsberg. 1998. Edge effects and the extinction of populations inside protected areas. Science 280: 2126–2128.

Wilkinson, G. S., D. C. Presgraves., and L. Crymes. 1998. Male eye span in stalk-eyed flies indicates genetic quality by meiotic drive suppression. Nature 391: 276-279.

Wilson, D.R., X. J. Nelson., and C. S. Evans. 2009. Seizing the opportunity: Subordinate male fowl respond rapidly to variation in social context. Ethology 115: 996-1004.

Xu, R.F., K. Li., G. H. Chen., B. Y. Z. Qiang., D. L. Mo., B. Fan., C. C. Li., M. Yu., M. J. Zhu., T. A. Xiong., and B. Liu. 2005. Investigation of PCR-RFLPs within Major Histocompatibility Complex B-G genes using two restriction enzymes in eight breeds of Chinese indigenous chickens. Asian-Australian Journal of Animal Science 18: 942-956.

Ya-Bo, Y., W. Jin-Yu., D. M. Mekki., T. Qing-Ping., L. Hui-Fang., G. Rong., G. Qing- Lian., Z. Wen-Qi., and C. Kuan-Wei. 2006. Evaluation of Genetic Diversity and Genetic Distance Between Twelve Chinese Indigenous Chicken Breeds Based on Microsatellite Markers, International Journal of Poultry Science 5: 550-556.

Yamashita, H., S. Okamoto., Y. Maeda., and T. Hashiguchi. 1994. Genetic relationships among domestic and junglefowls revealed by DNA fingerprinting analysis. Japanese Poultry Science 315: 335-344.

Yeh Francis, C., R. C. Yang., T. B. J. Boyle., Z. H. Ye., and J. X. Mao. 1999. POPGENE version 1.32, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

Yu, Y. B., J-Y. Wang., D. M. Mekki., Q-P. Tang., H-F. Li., R. Gu., Q-L. Ge., W-Q. Zhu., and K-W. Chen. 2006. Evaluation of genetic diversity and genetic distance between 12 chinese indigenous chicken breeds based on microsatellite markers. International Journal of Poultry Science 5: 550-556.

Zelder, A. M., D. G. Bradley., E. Emshwiller., and B. D. Smith. 2006a. Documenting domestication new genetics and archaeological paradigms. University of California Press. Pp 361.

Zelder, A. M. 2006b. Central questions in the domestication of plants and animals. Evolutionary Anthropology 15: 105-117.

Zhou H., and S. J. Lamont. 1999. Microsatellite markers to estimate genetic differences among pedigree and inbred chicken lines of commercial and exotic origin, In: Proceedings from Jay Lush to Genomics: Visions for Animal Breeding and Genetics. 1999. Iowa USA. Pp. 175 abstract.

Zuk, M., K. Johnson., R. Thornhill., and J. D. Ligon. 1990a. Mechanisms of mate choice in Red Junglefowl. Evolution 44: 477-485.

Zuk, M., R. Thornhill., K. Johnson., and J. D. Ligon. 1990b. Parasites and mate choice in Red Junglefowl. American Zoologist 30: 235-244.

Zuk, M., R. Thornhill., J. D. Ligon., K. Johnson., S. Austad., S. Ligon., N. Thornhill., and C. Costin. 1990c. The role of male ornaments and courtship behavior in female choice of red junglefowl. The American Naturalist 136: 459-473.

Zuk, M., K. Johnson., R. Thornhill., and J. D. Ligon. 1990d. Parasites and male ornaments in free-ranging and captive Red Junglefowl. Behaviour 114: 232-248.

Zuk, M., J. D. Ligon., and R. Thornhill. 1993. Effects of experimental manipulation of male secondary sex characters on female mate preference in Red Junglefowl. Animal Behaviour 44: 999-1006.

Zuk, M., T. S. Johnsen., and T. MacLarty. 1995a. Endocrine-immune interactions, ornaments and mate choice in red junglefowl. Proceeding of the Royal Society of London 260: 205-210.

Zuk, M., S. L. Popma., and T. S. Johnsen. 1995b. Male courtship displays, ornaments, and female mate choice in captive Red Junglefowl. Behaviour 132: 821-836.

http://www.birdlife.org/ accessed on July 1, 2011.
http://www.worldclim.org/bioclim.htm
http://www.gbif.net accessed on September 3, 2010
http://www.gdem.aster.ersdac.or.jp/
http://iucn.org.
http://ies.jrc.ec.europa.eu/global-land-cover-2009
http://www.cs.princeton.edu/~schapire/maxent/
http://aviandiv.tzv.fal.de/primer_table.html. AVIANDIV
http://www.absc.usgs.gov/research/genetics/sampling.pdf
http://www.fao.org/dad-is
http://www.fao.org/DOCREP/ARTICLE/AGRIPPA/X9500E01.HTM
http://www.gallus.forestry.uga.edu/genetics/protocols/pdf/feather.pdf
http://www.ualberta.ca/~fyeh/..

APPENDIX -1. List of protected areas with Red Junglefowl in India.

Name of State and Protected Area	Area (sq km)	District (s)	Biogeographic Zone / Biotic Province
Andhra Pradesh			
Papikonda NP	1012.86	East & West Godavari, Khammam	6 (06D)
Arunachal Pradesh			
Mouling NP	483.00	Upper Siang	2 (02D)
Namdapha NP	1807.82	Changlang	2 (02D)
D'Ering Memorial (Lali) WLS	190.00	Upper Siang	2 (02D)
Dibang WLS	4149.00	Dibang Valley	2 (02D)
Eagle Nest WLS	217.00	West Kameng	2 (02D)
Itanagar WLS	140.30	Papum Pare	2 (02D)
Kamlang WLS	783.00	Lohit	2 (02D)
Kane WLS	31.00	West Siang	2 (02D)
Mahao WLS	281.50	Dibang Valley	2 (02D)
Pakke (Pakhui) WLS	861.95	East Kameng	2 (02D)
Sessa Orchid WLS	100.00	West Kameng	2 (02D)
Tale Valley WLS	337.00	Lower Subansiri	2 (02D)
Yordi-Rabe Supse WLS	397.00	West Siang	2 (02D)
Assam			
Dibru-Saikhowa NP	340.00	Tinsukia,Dibrugarh	9 (09A)
Kaziranga NP	858.98	Golaghat, Nagaon, Sonitpur	9 (09A)
Manas NP	500.00	Barpeta, Bongaigaon	9 (09A)
Nameri NP	200.00	Sonitpur	9 (09A)
Amchang WLS	78.64	Kamrup	9 (09A)
Barail WLS	326.25	Cachar Karimgang	9 (09A)
Barnadi WLS	26.22	Darrang	9 (09A)
Burachapori WLS	44.06	Sonitpur	9 (09A)
Chakrashila WLS	45.56	Dhubri	9 (09A)
Dihing Patkai WLS	111.19	Dibrugarh, Tinsukia	9 (09A)
East Karbi Anglong WLS	221.81	Karbi-Anglong	9 (09A)
Garampani WLS	6.05	Karbi-Anglong	9 (09A)
Hollongapar Gibbon WLS	20.98	Jorhat	9 (09A)
North Karbi Anglong WLS	96.00	Karbi-Anglong	9 (09A)
Lawkhowa WLS	70.14	Nagaon	9 (09A)
Marat Longri WLS	451.00	Karbi-Anglong	9 (09A)
Nambor WLS	37.00	Karbi-Anglong	9 (09A)
Nambor Doigrung WLS	97.15	Karbi-Anglong	9 (09A)
Porbitora WLS	38.81	Marigaon	9 (09A)
Sonai Rupai WLS	220.00	Sonitpur	9 (09A)
Bihar			
Valmiki NP	335.65	Pashchim Champaran	7 (07B)
Bhimbandh WLS	681.99	Munger	6 (06B)
Gautam Budha WLS	138.34	Gaya	6 (06B)
Kaimur WLS	1342.00	Rohtas	6 (06A)
Udaipur WLS	8.87	Pashchim Champaran	7 (07B)
Valmiki WLS	545.15	Pashchim Champaran	7 (07B)

Chhattisgarh			
Indravati (Kutru) NP	1258.37	Dantewada	6 (06C)
Kanger Valley NP	200.00	Bastar	6 (06C)
Achanakmar WLS	551.55	Bilaspur	6 (06A)
Badalkhol WLS	104.45	Jashpur	6 (06B)
Bhairamgarh WLS	138.95	Dantewada	6 (06C)
Bhoramdev WLS	163.80	Kawardha	6 (06A)
Sarangarh-Gomardha WLS	277.82	Raigarh	6 (06C)
Pamed Wild Buffalo WLS	262.12	Dantewada	6 (06C)
Semarsot WLS	430.36	Surguja	6 (06B)
Sitanadi WLS	553.36	Dhamtari	6 (06C)
Tamor Pingla WLS	608.53	Surguja	6 (06B)
Udanti Wild Buffalo WLS	247.59	Raipur	6 (06C)
Haryana			
Kalesar NP	46.82	Yamuna Nagar	4 (04A)
Abubshehar WLS	115.30	Sirsa	4 (04A)
Kalesar WLS	54.06	Yamuna Nagar	4 (04A)
Morni Hills (Khol-Hi-Raitan) WLS	48.83	Panchkula	4 (04A)
Himachal Pradesh			
Simbalbara NP	27.88	Sirmaur	2 (02B)
Chail WLS	109.00	Solan	2 (02B)
Churdhar WLS	66.00	Sirmaur	2 (02B)
Daranghati WLS	176.12	Shimla	2 (02B)
Darlaghat WLS	6.00	Solan	2 (02B)
Dhauladhar WLS	1154.86	Kangra	2 (02A)
Gobind Sagar WLS	100.00	Bilaspur	2 (02A)
Majathal WLS	57.55	Solan	2 (02B)
Pong Dam Lake WLS	307.00	Kangra	2 (02A)
Renukaji WLS	4.50	Sirmaur	2 (02B)
Shilli WLS	2.00	Solan	2 (02B)
Shri Nainadevi WLS	123.00	Bilaspur	2 (02B)
Jammu & Kashmir			
Jasrota WLS	25.75	Kathua	4 (04A)
Nandni (Nandini) WLS	33.34	Jammu	4 (04A)
Ramnagar Rakha WLS	31.50	Jammu	4 (04A)
Surinsar Mansar WLS	55.50	Jammu	4 (04A)
Trikuta WLS	31.77	Jammu	2 (02A)
Bahu Con R	19.75	Jammu	2 (02A)
Jharkhand			
Betla NP	226.33	Latehar	6 (06B)
Dalma WLS	193.22	East Singhbhum, Saraikela	6 (06B)
Hazaribagh WLS	186.25	Hazaribagh	6 (06B)
Gautam Budha	121.14	Koderma, Hazaribagh	6 (06B)
Koderma WLS	177.35	Koderma	6 (06B)
Lawalong WLS	211.03	Chatra	6 (06B)
Mahauadanr WLS	63.26	Latehar	6 (06B)
Palamau WLS	752.94	Latehar	6 (06B)
Palkot WLS	182.83	Gumla, Simdega	6 (06B)

Parasnath WLS	49.33	Giridih	6 (06B)
Topchanchi WLS	12.82	Dhanbad	6 (06B)
Madhya Pradesh			
Bandhavgarh NP	448.85	Umaria,Katni	6 (06A)
Kanha NP	940.00	Mandla, Balaghat, Dindori	6 (06A)
Madhav NP	375.22	Shivpuri	4 (04B)
Pench (Priyadarshini) NP	292.85	Seoni, Chhindwara	6 (06D)
Sanjay NP	466.88	Sidhi	6 (06A)
Bagdara WLS	478.00	Sidhi	6 (06A)
Panpatha WLS	245.84	Umaria	6 (06D)
Pench WLS	118.47	Seoni, Chhindwara	6 (06A)
Phen WLS	110.74	Mandla	6 (06A)
Sanjay Dubri WLS	364.59	Sidhi	6 (06A)
Maharashtra			
Nawegaon NP	133.88	Gondia	6 (06D)
Nagzira WLS	152.81	Bhandara	6 (06D)
Manipur			
Yangoupokpi Lokchao WLS	184.40	Chandel	9 (09B)
Meghalaya			
Balphakram NP	220.00	South Garo Hills	9 (09B)
Nokrek Ridge NP	47.48	East, West & South Garo Hills	9 (09B)
Nongkhyllem WLS	29.00	Ri Bhoi	9 (09B)
Siju WLS	5.18	South Garo Hills	9 (09B)
Mizoram			
Murlen NP	100.00	Champhai	9 (09B)
Phawngpui Blue Mountain NP	50.00	Lawngtlai	9 (09B)
Dampa WLS	500.00	Mamit	9 (09B)
Khawnglung WLS	35.00	Serchhip	9 (09B)
Lengteng WLS	60.00	Champhai	9 (09B)
Ngengpui WLS	110.00	Lawngtlai	9 (09B)
Pualreng WLS	50.00	Kolasib	9 (09B)
Tawi WLS	35.75	Aizawl	9 (09B)
Thorangtlang WLS	50.00	Serchhip	9 (09B)
Tokalo WLS	250.00	Saiha	9 (09B)
Nagaland			
Intanki NP	202.02	Dimapur	9 (09B)
Fakim WLS	6.41	Tuensang	9 (09B)
Puliebadze WLS	9.23	Kohima	9 (09B)
Rangapahar WLS	4.70	Dimapur	9 (09B)
Odisha			
Bhitarkanika NP	145.00	Kendrapara	8 (08B)
Simlipal NP	845.70	Mayurbhanj	6 (06B)
Usakothi WLS	304.03	Sambalpur	6 (06B)
Baisipalli WLS	168.35	Nayagarh	6 (06C)
Balukhand Konark WLS	71.72	Puri	8 (08B)
Bhitarkanika WLS	525.00	Kendrapara	8 (08B)
Chandaka Dampara WLS	175.79	Khurda, Cuttack	6 (06B)
Debrigarh WLS	346.91	Sambalpur	6 (06B)

Hadgarh WLS	191.06	Keonjhar, Mayurbhanj	6 (06B)
Karlapat WLS	147.66	Kalahandi	6 (06C)
Khalasuni WLS	116.00	Sambalpur	6 (06B)
Kotagarh WLS	399.50	Phulbani	6 (06C)
Kuldiha WLS	272.75	Balesore	6 (06B)
Lakhari Valley WLS	185.87	Gajapati	6 (06C)
Nandankanan WLS	14.16	Khurda	6 (06C)
Satkosia Gorge WLS	745.52	Angul, Boudh, Cuttack	6 (06B)
Simlipal WLS	1354.30	Mayurbhanj	6 (06B)
Sunabeda WLS	500.00	Nuapada	6 (06C)
Punjab			
Abohar WLS	186.50	Firozpur	
Kathlaur Kushlian WLS	7.58	Gurdaspur	
Takhni-Rehampur WLS	3.82	Hoshiarpur	
Sikkim			
Kitam WLS	6.00	South Sikkim	2 (02C)
Tripura			
Clouded Leopard NP	5.08	West Tripura	9 (09B)
Bison (Rajbari) NP	31.63	South Tripura	9 (09B)
Gumti WLS	389.54	South Tripura	9 (09B)
Rowa WLS	0.86	North Tripura	9 (09B)
Sepahijala WLS	13.45	West Tripura	9 (09B)
Trishna WLS	163.08	South Tripura	9 (09B)
Uttar Pradesh			
Dudhwa NP	490.00	Lakhimpur-Kheri	7 (07A)
Katerniaghat WLS	400.09	Bahraich	7 (07A)
Kishanpur WLS	227.00	Lakhimpur-Kheri, Shahjahanpur	7 (07A)
Sohagibarwa WLS	428.20	Maharajganj	7 (07A)
Sohelwa WLS	452.47	Shravasti,Balrampur	7 (07A)
Uttarakhand			
Corbett NP	520.82	Nainital, Pauri Garhwal	7 (07A)
Rajaji NP	820.42	Dehradun, Pauri Garhwal, Haridwar	7 (07A)
Sonanadi WLS	301.18	Pauri Garhwal	7 (07A)
Asan Wetland Con. Res.	4.44	Dehradun	7 (07A)
Jhilmil Jheel Con. Res.	37.84	Haridwar	7 (07A)
West Bengal			
Buxa NP	117.10	Jalpaiguri	7 (07B)
Gorumara NP	79.45	Jalpaiguri	8 (08B)
Sunderban NP	1330.10	North & South 24-Paraganas	8 (08B)
Haliday Island WLS	5.95	South 24-Paraganas	7 (07B)
Jaldapara WLS	216.51	Jalpaiguri	8 (08B)
Lothian Island WLS	38.00	South 24-Paraganas	7 (07B)
Mahananda WLS	158.04	Darjeeling & Jalpaiguri	8 (08B)
Sajnakhali WLS	362.40	South 24-Paraganas	2 (02C)
Senchal WLS	38.88	Darjeeling	4 (04A)

APPENDIX 2-Conservation Action Plan drafted by the Specialist Group of the Government of India

Conservation Action Plan
Red Junglefowl
(*Gallus gallus*)

Drafted by:

The Specialist Group Constituted by the Ministry of Environment & Forests, Government of India, 2008

1.0 Introduction

The wild Red Junglefowl (Gallus gallus) is one of the most important species to mankind due to the economic and cultural significance. First domesticated in Mohenjodaro and Harappa in the Indus valley around 2500-2100 B.C., the Red Junglefowl (RJF) is believed to be the ancestor of all domestic chickens in the world. In other words, the present day multi-billion dollar poultry industry is based on the wild RJF and may have to depend on it in the future as well. Over 120 million broiler chicken and several million eggs are produced annually in India. Exotic high yielding breeds have replaced our native breeds. Poultry epidemics, such as the one in Hong Kong in 1998 and the 'bird flu' in India and other parts of S.E. Asia in the recent past, could spell doom to the poultry industry and the only fall back option for mankind would eventually be the 'wild' RJF.

It has been well recognised that the populations of domestic animals and their wild ancestors provide a valuable source of genetic diversity that may be exploited to develop animal models for quantitative traits of biological and medical interest1. Conservation of genetically pure wild forms or their representatives have great potential to make significant contribution to the study of some economically important genetic traits of the domestic form.

The RJF has widespread distribution and its five sub-species are spread in the Indian sub-continent eastwards across Myanmar, South China, Indonesia, to Java. In India, two sub-species of RJF occur, the type specimen, Gallus gallus murghii and Gallus gallus spadiceus. While the former is distributed in the north and central part of India, extending eastwards to Odisha and West Bengal, the sub-species spadiceus is confined to the north-eastern parts of India.

Recently, fears have been expressed that the wild RJF populations may be genetically contaminated leading to an inference that there may not be any pure RJF populations in the wild3. It is believed that the wild RJF populations have been hybridised with domestic, feral and/or domestic stock, especially near the villages causing an introgression of domestic genes into the wild populations. Other concerns include the reports of the decline in the distribution range and the populations of RJF in many parts of India.

II. The Specialist Group and its Mandate

Keeping the above in view, during the 3rd Meeting of the National Board for Wildlife that was held on 19th June 2006 under the Chairmanship of the Hon'ble Prime Minister, a Specialist Group was constituted to prepare an Action Plan for the Conservation of RJF.

The Specialist Group had discussions on various aspects of RJF conservation in India and identified the following five major action points

A. Evaluation of Current Status and Distribution of RJF in India

Although the general distribution range of RJF in India is known, the exact distribution limits of the two subspecies and their current status is not known. There is an urgent need to evaluate the current status and distribution of RJF in India.

Most of the required information on this aspect is expected to be available in the next two years as the Wildlife Institute of India (WII), Dehradun, has initiated a research project in September 2006 entitled "Conservation of Red Junglefowl in India" that seeks to provide more information on the status and distribution of RJF in India and ways to safeguard pure wild populations. The objectives of this project are: (i) assessing the status and distribution of RJF in India; (ii) identification of pure RJF populations by molecular genetic studies; and (iii) propose conservation action plan for the identified RJF populations. Some of the preliminary findings of this research project are presented below.

In India, the historical distribution of RJF was from northern Andhra Pradesh in the south, through Odisha to West Bengal in the east, north-east Indian states, and central to north India up to Jammu.5 This historical distribution would cover 300 Districts in 21 range states of the present day India. Based on literature, questionnaire surveys and reliable personal communication, information on the presence / absence of RJF in these districts of India was collected. The RJF is now reported to occur in 151 districts in the 21 range states (Table 1, Fig. 1). Information on the presence / absence of RJF from about 50% of the districts in its distribution range is unknown. The RJF distribution range is restricted due to geographical barriers, environmental and elevation gradients. Hence, in states such as Jammu and Kashmir, Himachal Pradesh, Uttarkhand, Sikkim, Uttar Pradesh, Madhya Pradesh and Andhra Pradesh, there are a few areas where RJF is present. Within central India, the RJF is not reported beyond the west bank of river Pench.

Of the 255 PAs that occur within the RJF distribution range in India, 149 PAs (26 NPs and 123 WSs) have reported presence of RJF in their areas (Table 2, Fig. 2), But there are no population estimates for RJF in these PAs. Information on the presence / absence of RJF from ca. 33% of PAs is unknown. However, WII's ongoing research project on the RJF will provide such information to the central and the concerned state governments as and when it will be available.

As of March 2007, there are 209 individuals (74:80:55) RJF in captivity in the different zoos and pheasantries in the states of Andhra Pradesh, Delhi, Haryana, Himachal Pradesh, and Uttar Pradesh.

Based on WII's recent surveys, it appears that intensive efforts have to be made to ascertain the presence of RJF in northern parts of Andhra Pradesh, which is the southern distribution for this species. Further, report of RJF presence has to be verified in areas where its distribution range overlaps with that of the Grey Junglefowl (*Gallus sonneratii*).

B. In situ Conservation of Red Junglefowl

To safeguard wild RJF populations, it is necessary to monitor the presence/absence and abundance of this species in different parts of the distribution range within as well as outside PA network by the Forest or Wildlife Departments of the Range States with the help of Research Institutions/ Organizations, Wildlife NGOs, Universities, and Nature/Bird Watchers Clubs.

Wild RJF populations need to be protected from poaching, habitat loss or habitat degradation, and from hybridisation with domestic/feral chicken.

C. Ex situ Conservation Needs for Red Junglefowl

The captive populations of RJF in the different zoos and pheasantries have to be screened through genetic studies and the hybridised individuals have to be removed from the existing captive stock.

Conservation breeding of 'genetically pure' RJF has to be initiated on priority basis and such captive stocks have to be scientifically managed and maintained for future use such as research and reintroductions. To begin with, four RJF range states, viz., Andhra Pradesh, Assam, Delhi, Haryana, and Himachal Pradesh have been identified to either initiate or strengthen their existing ex-situ conservation facilities for RJF representing the wild populations in their respective state or region. Specific proposals along with budget requirements are under preparation for initiating or strengthening existing ex-situ facilities for conservation breeding of RJF. A budget estimate for establishing a Conservation Breeding Facility for RJF in the five zones is placed as Annexure-I.

D. Research on Red Junglefowl

Field research on all aspects of ecology and behaviour of RJF and hybridisation threats to RJF in forest-village interface landscapes in particular has to be carried out.

Laboratory investigations on RJF to be accorded high priority particularly on aspects such as the genetic traits responsible for immunity to diseases that have great utility for the improvement and safeguard of the Poultry Industry.

Assessment of the diseases resistance ability of RJF by (a) measuring the general disease resistance parameters such as response to sheep Red blood cell; and (b) Challenge study with live virus such as RD / Avian Influenza etc

For measuring the genetic purity of RJF populations, specific molecular markers (micro-satellite markers and SNPs) for RJF will be identified. Similarly, the domestic chicken specific markers (microsatellite markers and SNPs) will be identified to detect the contamination of RJF with domestic chicken.

Wildlife Institute of India
P.O Box # 18, Chandrabani, Dehradun - 248001, Uttarakhand. INDIA
Email : wii@wii.gov.in Website : www.wii.gov.in