

Dedicated to

My Parents,

My Mentor, Dr. Min Bahadur

And

My Husband

DECLARATION

I declare that the thesis entitled "**Cytogenetic Divergence in the Indian Pygmy Field Mice *Mus terricolor*, Blyth of The Dooars and Terai regions of West Bengal**" has been prepared by me under the guidance of Dr. Min Bahadur, Professor of DEPARTMENT OF ZOOLOGY, UNIVERSITY OF NORTH BENGAL. No part of this thesis has formed the basis for the award of any degree of fellowship previously.

MAHUA RUDRA
DEPARTMENT OF ZOOLOGY
UNIVERSITY OF NORTH BENGAL
RAJA RAMMOHUNPUR
DARJEELING, INDIA

DATE : JULY, 2013

CERTIFICATE

I certify that Ms. MAHUA RUDRA has prepared the thesis entitled “**Cytogenetic Divergence in the Indian Pygmy Field Mice *Mus terricolor*, Blyth of The Dooars and Terai regions of West Bengal**” for the award of Ph.D. degree of the University of North Bengal, under my guidance. She has carried out the work at the Department of Zoology, University of North Bengal.

Dr. MIN BAHADUR
DEPARTMENT OF ZOOLOGY
UNIVERSITY OF NORTH BENGAL
RAJA RAMMOHUNPUR
DARJEELING, INDIA

DATE : JULY, 2013

Acknowledgement

I take this opportunity to express my deep sense of gratitude and indebtedness to those who helped me in carrying out this investigation.

I am immensely indebted and sincerely grateful to my honourable supervisor Dr. Min Bahadur, Associate Professor, Genetics and Molecular Biology Laboratory, Department of Zoology, University of North Bengal who introduced me to the exciting field of research and extended the fullest possible help and unrequited encouragement. His invaluable suggestion, guidance and supervision throughout my work help me to solve problems and to take correct decisions during hard times of my research work.

I am indebted to my respected teachers Prof. Ananda Mukherjee, Prof. Joydeb Pal, Prof. Sudip Barat and Prof. Tapas Kumar Chaudhury Department of Zoology, NBU for rendering instrument facilities and guiding by their valuable suggestion during the work.

I am indebted to Dr. Soumen Bhattacharjee (Associate professor) for his technical suggestion during my works.

I extend my thanks to the Head, Department of Zoology, NBU for ensuring necessary facilities in the department.

I express my thank to Asstt. Professor Dhiraj Saha, who like my friend and brother always helped me to take decisions.

I also thank Mr. Tilak Saha (Assistant Professor), newly joined faculty member, who helped me in different way.

My parents, brother and sister have been constant source of inspiration throughout my doctoral study, without their support and encouragement it seems quite impossible to finish such hard work.

I am grateful to my husband Mr. Jatindra Das for his continuous encouragement and moral support.

I like to thank Mr. Ratan and Mr. Kalicharan, who helped me during field study and to collect specimens from field. Thanks are also due to non-teaching staffs of the Deptt. of Zoology for their assistance in animal maintenance and in other fields. I specially thanks

to Mr. Surath Kundu for his cordial assistance during the works goes in central instrument room of Zoology.

I am also thankful to all administrative staffs for their help during my Ph.D. work.

I am indebted to Dr. Sumanta Bagchi, Dr. Arindam Das, Dr. Tamal Majumdar, Dr. Hadida Yasmin, Mr. Rudraprasad Roy and Mrs. Soma Das and the research scholars of Zoology Department for extending their helping hands. My special thanks to Mr. Gautam Debnath, Mr. Bappaditya Ghosh and Ms. Susmita Dutta my colleague of Genetics and Molecular biology Laboratory, for their untiring help and support at the end of my work.

I especially like to express my thanks to Dr. Biswanath Chatterjee, NIH, Bethesda, USA and Dr. Bikash Mitra to support me and for their help in sequencing of the mtDNA.

At the end I thank all those who have helped or associated with my work directly or indirectly.

MAHUA RUDRA
DEPARTMENT OF ZOOLOGY
UNIVERSITY OF NORTH BENGAL
RAJA RAMMOHUNPUR
DARJEELING, WEST BENGAL,
INDIA

DATE : JULY, 2013

ABBREVIATIONS

APD	:	Alipurduar
BDN	:	Bidhan Nagar
CBH	:	Cooch Behar
GDH	:	Garidhura
KGM	:	Kumargram
MLB	:	Malbazar
MNG	:	Maynaguri
NGK	:	Nagraka
NXL	:	Naxalbar
RBD	:	Rohimabad
mtDNA	:	mitochondrial DNA
Mya	:	million years ago
RFLP	:	Restriction fragment length polymorphism
PCR	:	Polymerase Chain Reaction
NOR	:	Nucleolus Organizer region
rDNA	:	Ribosomal DNA
FISH	:	Fluorescence in situ hybridization
UBF	:	Upstream Binding Factor
SL1	:	Promoter selectivity factor
Topo I	:	DNA topoisomerase I
RP 1	:	RNA polymerase 1
IGS	:	intergenic spacer
SatDNA	:	Satellite DNA
F-statistics	:	Statistics of Fixation Index
Nm	:	Gene flow
I	:	Nei's Genetic identity
A _E	:	Effective number of allele

H_o	:	Observed heterozygosity
H_E	:	Expected heterozygosity
D	:	Nei's Genetic Distance
HVR I	:	Hypervariable Region I
HVR II	:	Hypervariable Region II
dNTPs	:	deoxynucleotides
ddNTPs	:	dideoxynucleotides
UPGMA	:	Unweighted pair group method with arithmetic mean
π	:	Nucleotide diversity
NJ	:	Neighbour joining analysis
MP	:	Maximum parsimony

PREFACE

Indian subcontinent has been proposed as the place of origin of genus *Mus* and is the habitat of largest number of *Mus* species. The Indian pygmy field mice, *Mus booduga* and *Mus terricolor* (= *M. dunni*) are sibling species of India and occur in the same habitat. They heavily infest wheat and paddy fields, however, show differences in site preference for burrows. Both have $2n=40$ chromosomes. *M. terricolor*, in comparison to conserved karyotype of *M. booduga* with all acrocentric chromosomes, possesses divergent karyotypes with large submetacentric X and large acrocentric Y- chromosomes which is a unique feature of the species. Divergent karyotypes in *M. terricolor* are due to the presence of variable number of heterochromatic short arms on the autosomes. This species is a chromosomal complex with three distinct chromosome types I, II and III having apparently non-overlapping distribution.

M. terricolor chromosome type I, distributed in Northern India; Goa, Pune and Kolhapur in Western India; Jhansi in the Central India and as far as Alipurduar in Eastern India, have all acrocentric autosomes with minute heterochromatic short arms. The chromosome type II has been shown to be distributed in Mysore and Erode possessing two submetacentric autosome pairs 1 and 3 with heterochromatic short arms while the other autosomes are either telocentric or acrocentric with C-band positive minute arms. The populations of *M. terricolor* from Chennai (Madras), Tirupati, Pondicherry and Madurai are chromosome type III having autosome pairs 1, 3 and 6 with heterochromatic short arms. The large submetacentric X and the large acrocentric Y are identical in all types of *M. terricolor*. The short arm of X and the entire Y in *M. terricolor* are heterochromatic. Studies have also shown qualitative as well as quantitative differences in heterochromatin between *Mus terricolor* and its sibling species, *Mus booduga*.

The karyotype differentiation due to acquisition of heterochromatin in *Mus terricolor* complex indicates that it is still in the process of evolutionary divergence and has been considered as an incipient species. Therefore, *Mus terricolor* complex provide a novel model system to investigate the role of heterochromatin in evolutionary differentiation of species.

In this investigation a study on cytogenetic, allozyme and mitochondrial DNA of *M. terricolor* type I has been carried out from different populations of Terai and the Dooars of Darjeeling foot hills. The Section-3 of this dissertation deals with intra and inter population variation of constitutive heterochromatin and nucleolus organizing regions (NORs), the Section-4 is devoted to electrophoretic study of some enzyme and non-enzyme protein loci, the Section-5 deals with the analyses of sequence variation of control region of mtDNA and conclusion of the entire study has been included in the Section-6.

LIST OF TABLES

Table 3.1 Populations, collection sites, geographical coordinates and number of <i>M. booduga</i> and <i>M. terricolor</i> collected and studied from each population.	26
Table 3.2 C-band variation in different populations of <i>M. terricolor</i> .	40
Table 3.3 Ag-NOR banding sites in autosomes and sex chromosomes of <i>M. terricolor</i> of Terai and the Dooars regions.	50
Table 4.1 Ingredients and their proportions for separating and stacking gels.	70
Table 4.2 Enzymes/Proteins studied and their respective staining mixture used in the study.	76
Table 4.3 Loci and allele frequencies of ten populations of <i>Mus terricolor</i> chromosome type I. Alleles are designated as a, b, c, d or f, s for different loci and sample size, N is also given for each loci.	94
Table 4.4 Mean allele frequency for the Dooars and Terai populations and population mean(Total).	95
Table 4.5 Locus wise observed and expected heterozygosity of Terai and the Dooars populations of <i>M. terricolor</i> chromosome type I.	96-97
Table 4.6 Summary of genetic variation in the Dooars, and Terai populations of <i>M. terricolor</i> chromosome type I.	99
Table 4.7 F-statistics and gene flow for all loci among populations of the Dooars, Terai and Total populations.	100
Table 4.8 Mean Gene flow (Nm) among the populations of the Dooars, Terai and over all populations (Total).	101
Table 4.9 Nei's genetic identity (above diagonal) and genetic distance (below diagonal) values by pair wise comparison of ten populations of <i>M. terricolor</i> chromosome type I.	102

Table 4.10 Pair wise comparison of genetic identity and genetic distance values of GDH, NXL and BDN populations with a weighted mean of the Dooars population.

103

Table 5.1 Composition of reaction mixture of PCR.

117

Table 5.2 Nucleotide distances spanned by tRNA genes and D-loop regions in *M. m. domesticus* and *M. terricolor* (adopted from Pogożelski *et al.*, 2008).

124

Table 5.3 Pairwise differences of mtDNA sequences (CR I) of the *M. m. domesticus* strain C57BL/6J and ten populations of *M. terricolor*. For each pair of sequences the values above the matrix diagonal represents the total number of differences (to the left of the slash) and the number of length differences (to the right of the slash). The values below the diagonal are the number of transition (left of the slash) and transversion (right of the slash).

128

Table 5.4 Pairwise differences of the mtDNA sequences (CR II) of the *M. m. domesticus* strain C57BL/6J and eight populations of *M. terricolor*. For each pair of sequences the values above the matrix diagonal represents the total number of differences (to the left of the slash) and the number of length differences (to the right of the slash). The values below the diagonal are the number of transition (left of the slash) and transversion (right of the slash).

128

Table 5.5 Comparisons of different regions of mtDNA sequences from *M. terricolor* populations and *M. m. domesticus* strain C57BL/6J. Total span of the genes or regions has been given under the name of gene.

129

Table 5.6 Pattern of nucleotide substitution by Maximum Composite Likelihood Estimate of CR I and CR II region.

129

Table 5.7 Estimates of evolutionary divergence (below the diagonal) and percent identity (above the diagonal) between mtDNA sequences. Results are based on the pairwise analysis of overall sequences.

131

LIST OF FIGURES

- Figure 1.1** Two models of the origin and expansion of house mouse, *Mus musculus*. A. The centrifugal model B. The sequential or linear model. (adopted from “The mouse in Biomedical Research, Diseases. Second Edition”). 6
- Figure 1.2** The phylogenetic relationship of different subgenera of the genus *Mus*. Species belonging to the four subgenera are bracketed. The phylogeny adopted from (“The Mouse in Biomedical Research, Diseases. Second Edition”). 7
- Figure 1.3** Dorsal (a) and Ventral (b) view of *Mus terricolor*. 9
- Figure 3.1** Map showing the collection sites of *M. terricolor* and *M. booduga* in Terai and the Dooars regions of West Bengal (not to scale). 25
- Figure 3.2** C-banded karyotypes of three chromosomal types of *Mus terricolor* (adopted from the Ph.D. Thesis of Bahadur, 1995 with kind permission): a. MdI: *Mus terricolor* chromosomal type I. b. MdII : *Mus terricolor* chromosomal type II. c. MdIII : *Mus terricolor* chromosomal type III. Arrows (→) and arrow heads (▶) indicate C-bands in autosomes and sex chromosomes. 27
- Figure 3.3** C-banded karyotypes of *M. terricolor* type I: a) APD population b)MNG population. Arrows (→) and arrow heads (▶) indicate C-bands in autosomes and sex chromosomes. 31
- Figure 3.4** C-banded karyotypes of *M. terricolor* type I: a)RBD population b)KGM population. Arrows (→) and arrow heads (▶) indicate C-bands in autosomes and sex chromosomes. 32
- Figure 3.5** C-banded karyotypes of *M. terricolor* type I: a) MLB population b) CBH population. Arrows (→) and arrow heads (▶) indicate C-bands in autosomes and sex chromosomes. 34
- Figure 3.6** C-banded karyotypes of *M. terricolor* type I of NGK population. Arrows (→) and arrow heads (▶) indicate C-bands in autosomes and sex chromosomes. 36

- Figure 3.7** C-banded karyotypes of *M. terricolor* type I : a) GDH population b) BDN population. Arrows (→) and arrow heads (▶) indicate C-bands in autosomes and sex chromosomes. **38**
- Figure 3.8** C-banded karyotypes of *M. terricolor* type I of NXL population. Arrows (→) and arrow heads (▶) indicate C-bands in autosomes and sex chromosomes. **39**
- Figure 3.9** Segmental C-band on short arm of X chromosome in *M. terricolor* from NXL population. Arrow heads (▶) indicate C-band in sex chromosomes. **39**
- Figure 3.10** NOR banded karyotypes of *M. terricolor* type I: a) APD population: b) MNG population. **42**
- Figure 3.11** NOR banded karyotypes of *M. terricolor* type I: a) RBD population b)KGM population. **43**
- Figure 3.12** NOR banded karyotypes of *M. terricolor* type I: a) MLB population b) NGK population. **44**
- Figure 3.13** NOR banded karyotypes of *M. terricolor* type I from CBH population. **45**
- Figure 3.14** NOR banded karyotypes of *M. terricolor* type I: a) BDN population b) GDH population **47**
- Figure 3.15** NOR banded karyotypes of *M. terricolor* from NXL populations (a & b). **48**
- Figure 3.16** NOR banded karyotypes: a) *M. musculus* b) *M. booduga*. **49**
- Figure 4.1** Schematic diagram of isozyme patterns expected for monomeric, dimeric, trimeric and tetrameric enzymes (adopted from Harris and Hopkinson, 1976). **75**
- Figure 4.2** Representative electrophoregram of serum protein, Albumin-1 of *M. musculus* and *M. terricolor*. *M. musculus musculus* (lane 1) and *M. terricolor* populations of BDN (lanes 2-4), NXL (lanes 5-7), RBD (lanes 8-10), MLB (lanes 11-13). **84**

Figure 4.3 Representative electrophoregram showing banding pattern of serum proteins Prealbumin-B and Transferrin of *M. terricolor* populations from NGK (lanes 1-4), CBH (lanes 5,6), BDN (lanes 7,8), RBD (lanes 9-11) and GDH (lanes 12,13). **86**

Figure 4.4 Electrophoregram showing the banding patterns of serum Esterase -5. Note the band patterns of *M. terricolor* populations of BDN (Lanes 1-8), APD (lanes 9,10), RBD (lanes 11,12) and KGM (lane 13). **86**

Figure 4.5 LDH patterns in different populations of *M. terricolor* type I:

a) *M. terricolor* individuals from NXL (lanes 1,2), KGM (lanes 3-5), MLB (lanes 6-10), RBD (lanes 11,12).

b) *M. terricolor* individuals from NXL (lanes 1-4), MLB (lanes 5-7), MNG (lane 8), BDN (lanes 9-11) and NGK (lane 12). **88**

Figure 4.6 The band patterns of Mdh-1 in *Mus musculus* (lane 1-3) and different populations of *M. terricolor* from NXL (lane 4,5,9,12,13), NGK (lane 6,7), RBD (lane 8) and GDH (lane 10,11). **90**

Figure 4.7 Electrophoregram showing the band patterns of Mod-1 in different populations of *M. terricolor*. Lanes 1,2: GDH, lanes 3-5: NGK, lanes 6-9: MNG, lanes 10,11: RBD, lane 12: CBH and lane 13: BDN. **90**

Figure 4.8 Electrophoregram showing the band patterns of GOT. Lane 1: *Mus musculus*, and Lanes 2-13: different population of *M. terricolor* (lanes 2-4: NXL, lanes 5-7: MLB, lanes 8,9: GDH, lane 10,11: CBH, lane 12,13: RBD). **92**

Figure 4.9 Electrophoregram showing the band patterns of Isocitrate Dehydrogenase in different populations of *M. terricolor*. NXL: lane 1, MLB: lane 2, MNG: lane 3, BDN: lane 4, NGK: lane 5 and GDH: lane 6,7. **92**

Figure 4.10 Distribution pattern of genetic identities in the population of *M. terricolor* chromosome type I. **103**

Figure 4.11 Dendogram showing phylogenetic relationship among the populations of *M. terricolor* chromosome type I. **105**

Figure 5.1 Amplified PCR product on 2% agarose gel with size markers 100bp ladder(A) and 500 bp ladder(B). **119**

Figure 5.2 Graphical representation of sequence showing 4 peaks of four nucleotides. **121**

Figure 5.3 Sequence alignment of 10 mtDNA sample sequence from 10 populations of *M. terricolor* and one sequence of *M.m.domesticus* strain C57BL/6 from GenBank spanning from 15338 to 15577 nucleotide positions including proline tRNA gene, ETAS I, ETAS II and HVR I of control region. **125**

Figure 5.4 Sequence alignment of 8 mtDNA sample sequence from 8 populations of *M. terricolor* and one sequence of *M. m. domesticus* strain C57BL/6 from GenBank, spanning from 16132-00065. This segment includes phetRNA gene and part of HVR II region. **126**

Figure 5.5 Phylogenetic tree based on mtDNA sequences using UPGMA (a), Neighbour Joining (b) and Maximum Parsimony Analysis (c) methods. **132**