Abstract

The Indian pygmy field mouse *Mus terricolor*, a chromosomal complex, is the indigenous *Mus* species of India with chromosome complement, 2n=40. It consists of three distinct karyotypic forms which are designated as *Mus terricolor* chromosome types I, II and III due to presence of variable number of heterochromatic short arms in homozygous condition. However, all the three chromosomal types invariably possess a large submetacentric X and large acrocentric Y chromosomes. In the light of karyotype divergence with respect to constitutive heterochromatin, only a limited work has been done in this species based on molecular techniques. Therefore, due to lack of substantial data the position of the Indian pygmy field mice is still in controversy in the phylogenetic relationship of the genus *Mus.* In the present study, a multidimensional investigation based on chromosomal, allozyme and mitochondrial DNA analyses have been carried out on ten populations of *M. terricolor* from Terai and the Dooars regions of West Bengal, India. The *M. terricolor* specimens were collected from Alipurduar (APD), Rahimabad (RBD), Kumargram (KGM), Cooch Behar (CBH), Maynaguri (MNG), Malbazar (MLB) and Nagrakata (NGK) in the Dooars and Naxalbari (NXL), Bidhan Nagar (BDN) and Garidhura (GDH) in Terai. The populations were designated with three letter abbreviation based on the place of collection shown in parentheses. A total of 1600 specimens were collected from ten populations and were chromosomally analysed to confirm the karyotype. Chromosomes in the karyotype have been grouped into A,B,C and D. Out of 1600 specimens, 12 were *Mus booduga* and rest of the specimens were found to be *M. terricolor* type I.

Cytogenetic Study

Heterochromatin and C-banding

Cytogenetic analyses using C and NOR-banding techniques showed intra and inter-population variation of C-positive heterochromatin and Ag-NORs. Centromere of autosomes, short arm and distal telomere of X-chromosomes and the entire Y were found to be C-banded. Variations have been recorded in the size of the C-band positive centromeric heterochromatin ranging from very large to minute and even absent in some cases. Very large blocks of centromeric C-bands were found in few D group chromosomes either in homozygous or in heterozygous condition in all

populations. Individuals of BDN, GDH, MLB, NGK and MNG had large blocks of centromeric heterochromatin in most of the autosomes, while NXL, RBD, APD, KGM and CBH populations have prominent large blocks of C-bands in few autosomes only. A few autosomes in RBD, MLB and NXL populations were found to have hardly detectable centromeric C-bands. In NXL autosome 16 was found to be C-band negative in homozygous condition.

Short arm of X-chromosomes revealed intense C-banding in the individuals of RBD, KGM, NGK, CBH and APD populations, whereas, it was faintly stained in individuals of MNG, MLB, NXL and BDN. X-chromosome in one female individual of GDH showed telomeric C- band in heterozygous condition. Interestingly, a few individuals of NXL and BDN showed a discrete localization of heterochromatin on the short arms of X-chromosomes showing segmental localization. The entire Y chromosome was found to be C-banded in all populations with variation in the banding intensity.

Besides variation in size of centromeric heterochromatin the results also suggest that *M. terricolor* has a trend of accumulation of heterochromatin in both autosomes and sex chromosomes which is a recently evolved trait in rodents and specifically in the genus *Mus*. Intra and inter population variation in size of C-positive heterochromatin suggests that heterochromatin play a significant role in genetic differentiation and karyotype evolution of these populations.

Nucleolar Organizing Regions (NORs) and Ag-NOR banding

M. terricolor possesses large number of Ag-NOR sites distributed in different chromosomes. The NOR bands were categorized as major, minor and diffused NORs according to the size of band and characteristic of silver deposition.

Major Ag-NORs were found to be present in centromeric or pericentromeric region on most of the autosomes in APD, RBD, in some individuals of NXL and MNG populations. Other populations showed major Ag-NORs on few autosomes only, while it was absent in GDH population. Excessively large and broad Ag-NOR band was found in some individual of RBD and in one individual of NGK in the autosome 9 in heterozygous condition.

The minor NOR bands were found to be present only on few pairs of autosomes of APD, KGM, NXL, BDN, and GDH populations, while NGK population consistently showed minor NORs in all autosomes including one individual with excessively large

NOR on autosome 9. Other populations showed minor NORs in most of their autosomes except MNG where minor NORs were not detected. Both X and Y chromosomes consistently showed minor NORs in all populations.

Diffused NORs were present in most of the autosome in all population except MNG, NGK and RBD.

Ag-NOR banding revealed polymorphism both at intra and inter-population level. The intra-population variation showed that the homologs of the pairs differed not only in the deposition of silver but also showed differences in position and number of Ag-NOR sites in the same individual. Though variations exist among populations in distribution of Ag-NORs, however, multichromosomal location of NORs was found to be a common feature in all population.

Genetic polymorphism in *Mus terricolor*

Genetic analyses were carried out on ten allozyme/ protein loci, i.e. Alb-1, Prealb-B, Est-5, Trf, LDH-A, LDH-B, Mdh-1, Mod-1, GOT-1 and Idh-1. A total of 30 alleles were delineated for ten loci studied, out of which 15 were found to be shared by all populations in different frequencies and the rest were fixed in one or other populations. The Terai populations showed uniformity in allele frequeny, with a high rate of fixation of specific alleles such as Trf^{b}, $Est\text{-}5^{b}$, $Ldh\text{-}b^{f}$, $Mdh\text{-}1^{a}$ and $GOT\text{-}1^{b}$. Genetic polymorphism was estimated based on percent polymorphic loci (P), heterozygosity (H) and effective number of alleles (A_E). All populations were highly polymorphic in terms of P ranging from 60 to 100% with slight differences of mean effective number of alleles (A_E) between Terai and the Dooars regions. Alb-1, Mdh-1, Mod-1 and Idh-1 showed higher observed heterozygosity (H_O) in most of the populations. The mean H_O have been found to be spread over a lowest value of 0.2950 ± 0.4020 to a highest value of 0.4917 ± 0.2732. Moreover, Terai populations showed higher mean H_O compared to the Dooars populations, however, H_O is less than expected in all population except APD. Genetic structure of population was also determined by estimating F_{ST}, F_{IT} and F_{IS} values. Mean F_{ST} for the Dooars, Terai and total population (Terai and Dooars together) were 0.1552, 0.0295 and 0.1246, respectively which indicates that at least 12% of the total variability of all populations is attributable to divergence between populations. A positive F_{IT} value in the Dooars populations at most of the loci indicated the dominancy of homozygotes, while Terai populations showed excess of heterozygote at least at four

loci i.e. Alb-1, Prealb-B, Mdh-1 and Idh-1. F_{IS}, a measure of random mating, was positive for most the loci of Terai and the Dooars populations indicating slight heterozygote deficiency.

Gene flow is another factor to measure genetic structure. The average gene flow among different populations of Terai, Dooars and all population (Terai and Dooars together) were estimated to be 8.2197, 1.3607 and 1.7563, respectively. The values revealed that the gene flow is operating but cannot be considered sufficient to homogenize all population. Therefore, variability exists in sufficient degree.

Allele frequencies were used to estimate the Nei's Genetic Identity (I) and Genetic Distance (D). *M. terricolor* MLB and NGK from Dooars and NXL and BDN of Terai showed 99% and 97.4% similarity (I), respectively. Out of 45 pair wise comparisons, 62% of total I-values were found to be ranging from 0.9 to 1.0, 24.4% were between I values 0.76 to 0.9 and 13% were between 0.61 to 0.75.

The genetic distance values ranged from a minimum, D=0.0139 between MLB and NGK to the maximum D=0.5023 between RBD and APD in the Dooars populations, while a minimum D=0.0266 was found between BDN and NXL populations from Terai which was slightly higher than the minimum genetic distance value for Dooars population (D=0.0139). The RBD population showed a lower D values 0.0916 and 0.0940 with two distantly situated populations NXL and BDN, respectively while KGM relatively closer population to RBD showed genetic distance value within the same range, 0.0975 as shown by distantly situated populations. The geographic distances and genetic distances do not show any correlation. Dendrogram based on genetic distance matrices showed three major groups of cluster. The populations MLB, NGK, MNG and CBH formed group I, the populations NXL, BDN and GDH of Terai were clustered in group II and RBD and KGM were in group III. APD appeared as an outgroup.

Moreover, a high level of heterozygosity indicating greater genetic polymorphism in the populations of *terricolor* may be due to different evolutionary factors acting separately or in combination.

Study of mitochondrial DNA

Control region and flanking tRNA genes of mtDNA were PCR amplified and sequenced for analysis. The total sequences were analysed in two parts i) The sequence spanning 15338-15577 (CR I) is the part of control region comprising

Hypervariable Region I (HVR I) with flanking Proline tRNA gene and the intermediate region and ii) The sequence spanning 16132-00066 (CR II) of the control region which contains the part of Hypervariable Region II (HVR II) and the Phenylalanine tRNA gene. The mtDNA sequences representing from all populations of *M. terricolor* were compared with the mtDNA sequence of *M. m. domesticus* (#AY172335) as reference. Comparisons were done on the basis of transition, transversion and insertion-deletion. HVR II was found to be more polymorphic than HVR I in terms of base substitution. Transversions were more frequent in interspecific comparison than interpopulation comparison of *M. terricolor*. In comparisons with other populations of *M. terricolor* the mtDNA sequence of MLB, NGK and GDH showed a higher rate of transversion type of base substitution, which reflects that these populations are more diverged than other populations. Overall nucleotide diversity (π) ranges from 0.011 to 0.566 among *terricolor* populations. A comparison between *M. m. domesticus* and NGK, MLB and GDH populations showed comparatively higher nucleotide diversity, π = 0.494, 0.467, 0.347, respectively. The level of inter population sequence (nucleotide) divergence between Terai and Dooars populations revealed that MLB-NGK and MLB-GDH are highly diverged showing π = 0.566 and 0.428, respectively.

Dendrograms were constructed based on mtDNA sequence data using UPGMA, Neighbour joining (NJ) and Maximum Parsimony (MP) methods. Out of the three phylogenetic trees, the tree obtained by UPGMA showed higher bootstrap value for maximum branches than NJ and MP dendrograms and was considered for analysis of the result. The dendrogram revealed that APD was clustered with CBH, a nearby population and the RBD with BDN, geographically distant populations with a high bootstrap value of 75%. NGK and MLB appeared as out groups. The clustering of populations based on mtDNA showed limited concordance between dendrograms and geographical distance. This discontinuity in the distribution of mtDNA may be explained in terms of ancestral polymorphism and gene flow.