Summary and Conclusion

Chapter V__________
India, which is known for its rich heritage and culture, offers a wide scope for genetic research on account of its population diversity. Ethnic populations of India are culturally, morphologically, linguistically and genetically very diverse (Majumder, 1998). Multiple waves of migration into India during prehistoric and historic times and the subsequent cultural differentiation resulting in strict rules governing mating practices are two of the major causes of genomic diversity observed among contemporary ethnic groups of India. The present diversity of man is result of the development of genetically distinctly population within a single species or sub-species through the process of microevolution (Hirszfeld, and Hirszfeld, 1919). The way in which genes are organized into genotypes in human populations is called genetic structure of population (Harrison and Boyce, 1972). Due to interbreeding with neighboring populations some group may lose their identity and this change in gene frequencies of population thus result into genetic micro-differentiation (Roychoudhary, 1976).

The central Indian State of Chhattisgarh (C.G.) is inhabited by a large number of ethnic groups. It is considered to be the home of many tribal populations (consisting of 32.83% tribal population) therefore, it offers a unique opportunity of study of human genomic diversity research, particularly in the Bastar Division that consists of 70% tribal population. Different tribal groups living side by side for hundreds or even thousands of years try to retain their separate entities by practicing endogamy consequently retaining their genetic entities within the groups. Thus, the ethnic tribal populations harboring the Bastar are considered a hotspot for human genome diversity research (Mitra, 2003). Despite this, so far little work has been done to tap the vast repertoire of unexplored wealth of information. Therefore, it is of immense interest to study patterns of genetic affinities among endogamous groups inhabiting in Bastar region.

Many investigators have studied the genetic structure of sub-divided human populations by the use of specific markers. In the past the vast majority of genetic studies were based on classical genetic markers viz. blood group, serum protein, and red-cell enzyme polymorphisms. The levels of polymorphism at the loci that code for expressed proteins and enzyme are generally low because mutations at these loci are commonly deleterious and, therefore, are often strongly selected against. In recent times, polymorphic DNA are widely used to study the genomic diversity of Indian populations because these are most selectively neutral, more ubiquitous and have higher heterozygosities than polymorphic protein and enzyme markers. Past studies, using DNA markers on Indian ethnic groups, have employed mitochondrial DNA markers (Mountain et al., 1995; Bamshed et al., 1996; Roychoudhury et al., 2000); Y chromosomal DNA polymorphisms (Thangaraj et al., 1999; Bhattacharya et al., 1999).
1. To quantify genomic diversity in three tribes of Bastar, Chhattisgarh namely Abujhmaria, Bison-horn Maria and Muria.

2. To estimate genomic diversity and affinities among the tribes of Bastar, Chhattisgarh.

3. To see congruence of genomic and ethno-linguistic affinities among tribes of Bastar, Chhattisgarh.

Subjects and Methods

Located in Central India, Chhattisgarh state was born, through carving out of about 33 percent area of eastern part of Madhya Pradesh, on November 1, 2000, to become the 26th state of the Indian Union. Chhattisgarh extends southeast of Madhya Pradesh from 17°43'S to 24°5'S latitude and from 80° 5'E to 80° 20'E longitude, with an area of 1,35,191 sq.kms. The region has a population of 2,07,95,956 according to Census 2001. As its name implies, the region is covered by thirty-six forts and is
often referred to as “bowl of rice”. Uttar Pradesh in north, Jharkhand in the northeast, Orissa in the southeast, Andhra Pradesh in the south, Maharashtra in the southwest bound it and Madhya Pradesh in the northwest (Tiwari, 2004). The state comprises three Commissioners’ Division including sixteen districts. The Bastar division situated at the extreme southeast corner of Chhattisgarh. It lies between latitudes 17°46’ to 20°34’ N and longitudes 80°15’ to 82°12’ E with an area of about 39171 sq. kms. In the year 1999, the Bastar has been divided into three districts namely Bastar, Kanker, and Dantewada.

The present study was conducted on three sub-tribes of Gond from Bastar, Chhattisgarh namely- Abujhmaria, Bison-horn Maria and Muria. Gonds are geographically the most widespread and numerically very large tribe not only in C.G. but India too. They are said to be have migrated from the southern region of India and some anthropologists consider them as pre-Dravidian (Venkatachar, 1935).

The Abujhmaria tribe is one of the dwindling tribes of Chhattisgarh, with a population of only 19,401 in C.G. They inhabit in remote forest of Abujhmarh region of Bastar, suffering from geographical isolation of extreme degree. It is an identified primitive tribe of India and facing the problem of depopulation or slow population growth rate, which draw alarming attention from all corners not only to protect their cultural heritage but also to protect their biological heritage or protect them from extinction. Grigson (1938) called the Abujhmaria as Hill Maria and considered it to be most backward tribe between Godavari and Ganga. Elwin (1938) considered them to be a lowly section of the Gond tribe.

Bison-horn Maria tribe is another sub-group of Gond tribe and they call themselves as Maria or Dandami Maria. Their culture and living ways is almost similar to Abujhmaria. They reside in the south of Indravati River, Jagdalpur, Dantewada, and Bijapur Tehsils. Their characteristic dance is marriage dance with a splendid Bison-horn head dress (Grigson, 1938).

The Murias are a numerically large (~ 100,000) sub-tribe of the Gond, inhabits only in Bastar district, and as Elwin (1947) has stated, are the only civilized group among the other Gond sub-tribes of the same region. They are highly endogamous but do not practice close interbreeding. Like the Marias, they are lumped together with the Gonds and therefore, their exact number is not known.

Three tribal groups viz. Abujhmaria, Bison-horn Maria and Muria have been sampled from their primary regions of habitat. Blood samples (5-10 ml) were collected from 163 healthy unrelated individuals with individual informed consent. All sampled individuals were unrelated at least to the first cousin level. Collection of blood samples
was carried out from several villages of Bastar Division, Chhattisgarh (The location of sampling is given in Chapter III, Table 3.3 and Figure 3.9). Blood samples were transported in ice to the Human Genomics Lab of School of Studies in Anthropology, Pt Ravishankar Shukla University, Raipur for further analysis.

DNA Markers Studied

A total of 163 unrelated individuals from three tribes, viz. Abujhmaria (ABM), Bison-horn Maria (BHR) and Muria (MUR) were genotyped for 15 biallelic polymorphic loci.

Out of 15 DNA markers, eight are insertion/deletion polymorphic loci: Alu ACE (chromosome: 17q23); Alu PV92 (chromosome: 16); Alu CD4 (chromosome 12p12); Alu PLAT (chromosome: 8); Alu D1 (chromosome: 3); Alu APO (chromosome: 11); Alu FXIIIIB (chromosome: 6p25-24) and one mitochondrial DNA (mt-NUC) insertion/deletion locus on chromosome 11. Along with these seven autosomal unlinked restriction site polymorphism (RSPs), that are ESR/PvuII (chromosome: 6q25.1); NAT/Kpnl (chromosome: 8p22); LPL/PvuII (chromosome: 8p22); ALB/HaeIII (chromosome: 4q11-13); PSCR/TagI (chromosome: 21q11.2); CYP1A/MspI (chromosome: 15q22-q24); T2/MspI (chromosome: 13q14-q24). Their brief descriptions such as primer sequence, PCR protocol, Band size etc. are given in Chapter III).

These are the “core set” of DNA markers approved by the Department of Biotechnology (DBT), Government of India to study Human Genomic Diversity (HGD) initiative in the context of Indian populations in order to generate database.

Laboratory Analysis

High-molecular-weight DNA was extracted by the salting out procedure of Miller et al. (1988). PCR reactions were carried out in a Perkin Elmer 2400 Thermal Cycler. Each DNA sample was analyzed for polymorphisms at 15 biallelic loci, of which eight were insertion/deletion polymorphisms (IDPs) and remaining seven were RFLPs. Primers and protocols used for screening of the IDPs were as given in Majumder et al. (1999) and Tishkoff et al. (1995) and those for RSPs were as given in Jorde et al. (1995), Majumder et al. (1999) and from Kidd (personal communication). For the mtNUC locus, the reaction mixture for amplification comprised 0.25μl Taq DNA polymerase, 0.25μl of each primer, 0.5μl genomic DNA, in a total volume of 10μl. PCR cycling temperature protocol was: 30 cycles X (94° C for 15s, 63° C for 30s, 72° C for 1min). For the Alu insertion loci, the reaction mixture comprised 0.25μl Taq DNA polymerase, 0.3μl of each primer, 0.5μl genomic DNA, in a total volume of 10μl. Each sample was subjected to the following amplification conditions: 1min at 94° C
denaturation), annealing at 58°C (ACT), 50°C (APO), 66°C (D1), 56°C (FXIIIB), 54°C (PV92) and 60°C PLAT for 1 min, and 1 min at 72°C (extension) for 30 cycles. For Alu CD4 locus, the reaction mixture comprised 0.25 μl Taq DNA polymerase, 0.3 μl of each primer, 0.5 μl genomic DNA, in a total volume of 10 μl. PCR cycling temperature protocol was: 30 cycles X (94°C for 1 min, 58°C for 1.30 min, 72°C for 1.30 min). Likewise for RFLPs each sample was subjected to the following condition: 1 min at 94°C (denaturation), annealing at 63°C (ISR), 56°C (NAT), 60°C (PSCR), 56°C (CYP1A), 65°C (T2), 62°C (LP1) and 63°C (ALB) for 1 min, and 1 min at 72°C (extension) for 30 cycles. For all other loci the cycling protocol was the same except for the annealing temperature. Amplified PCR products were digested with appropriate restriction enzymes (where necessary) and run on agarose gel, stained with ethidium bromide and visualized under UV light.

Gene counting method was used for estimating allele frequencies and their standard errors. Using these allele frequencies heterozygosities at individual loci were estimated. Average heterozygosity, gene diversity and genetic distance were computed using DISPAN software. This software also constructs the phylogenetic tree (Dendrogram). To assess the relative amount of gene flow experienced by each population, a regression model originally proposed by Harpending and Ward model (1982) was used.

**The analysis of the data reveal following few facts:**

The frequency of human specific deletion allele (-) at the CD4 locus varies from about 95% in ABM to about 98% in MUR. The frequency of insertion allele (+) at the mtNUC locus, which is also human specific and that the event reported to have occurred before the divergence of human populations from Africa, varies about 33% in ABM to about 38% in MUR. Polymorphic Alu insertion and RFLP loci showed high levels of polymorphisms in the populations under study, as also found previously for other groups from India (Majumder et al., 1999; Mukherjee et al., 2000; Veerraju et al., 2001; Vishwanathan et al., 2003) and elsewhere (Batzer et al., 1994, 1996; Stoneking et al., 1997). Similarly, the RSPs loci also showed high levels of polymorphisms in the studied populations, which is in good agreement with earlier reports on global populations (Jorde et al., 1995; Kidd et al., 1998).

χ² tests for goodness of fit to Hardy-Weinberg Equilibrium (HWE) were significant in six (13.33%) out of forty-five cases for goodness of fit. All the values that were significant are probably due to a deficiency in the observed heterozygosities, where, if they were normal statistical fluctuations, or similar number of departures from the expected number of heterozygotes for excess and deficiency would be expected. Another explanation is that the tribal groups reflect a true heterozygote deficiency, possibly because of inbreeding. Gonds in general practice endogamy. Therefore, it may be
concluded that the deficiency in heterozygotes is real, possibly due to the reflection of a variable degree of inbreeding within the populations.

Excess frequency of heterozygous genotypes than expected for biallelic loci was observed for three loci (Alu ACE, Alu PV92, Alu PLAT), one locus in ABM and BHR and two loci in MUR. Homozygous genotypes for the deletion allele at the Alu CD4 locus are absent in all populations.

Genetic structure of three tribes of Bastar was studied using measures of genetic heterozygosity, genetic differentiation & genetic distances. Estimates of heterozygosity revealed that Alu CD4 was least variable while AluPV92, ALB, CYP1A and T2 loci were most variable in all the populations studied. Thus, the autosomal biallelic DNA markers in the study groups exhibit high levels of genetic diversity. In the present study, the estimated levels of heterozygosities were consistently high in all the populations under study. The heterozygosity levels were similar to those of other Indian populations studied using nuclear DNA markers (Majumder et al., 1999; Mukherjee et al., 2000; Veerraju et al., 2001 and Vishwanathan et al., 2003). Average heterozygosity (H) overall the studied loci was 0.4321. Interestingly, the average heterozygosity levels were higher than in other global populations studied (Europe & America), with the exception of African populations (Stoneking et al., 1997; Novick et al., 1998). Evolutionary theory predicts that an older, "source" populations will typically have greater genetic diversity than a population derived more recently from it (Mayr, 1970). ABM also shows a higher diversity than BHR and MUR implying their greater antiquity.

The total genomic diversity (H_t) varied between 0.36 (Alu FXIIIB) to 0.50 (Alu PV92) with the exception of the locus Alu CD4 at which the value is 0.0528. The overall genomic diversity is 0.4321. The genomic diversity attributable to between populations (G_{st}) is 0.0091 (0.91%). Despite some variability, majority of genetic diversity exists within (H_s, 0.4282), rather than between populations. One interpretation of this result is that gene flow between the populations has been sufficiently large to maintain genetic similarity, which could be consistent with the populations separated relatively recently, with little time for divergence to occur (which would support the replacement hypothesis) (Jorde et al., 1998).

The extent of genetic differentiation G_{st}, based on fifteen autosomal polymorphic loci for the three tribal populations of Bastar, is about 1% (0.91%), which is lower than that observed in other parts of India (Majumder et al., 1999; Mukherjee et al., 2000; Veerraju et al., 2001; Vishwanathan et al., 2004). Watkins et al. (2001) reported a G_{st} value of 2.4% for twelve Indian populations using Alu insertion polymorphisms, which is quite higher than the G_{st} value estimated in the present study. Thus a substantial inter-population variability was observed in the populations under study.
The genetic distances ranged from 0.0035 between (MUR & BHR and MUR & ABM) to 0.0041 (between BHR & ABM). The neighbor joining phylogenetic tree indicates that MUR and BHR closer and ABM is distinct from them. From genetic distance point of view, the MUR and BHR are nearer to each other as also the MUR and ABM. Our result of DNA markers suggests that the populations under study share a common ancestry. According to Grigson (1938), the BHR are the only descendants of the ABM trekked through the hills and forests from Abujhmarh hills towards plateaus and plains to their south. Considering the observations of anthropometric survey, further strengthens the above inference (Rakshit, 1974). However, both Grigson and Elwin are of the opinion that the Muria were originally Hill Maria and having migrated to low land area (foot of Abujhmarh hills), in course of time they formed a separate population. Saba (1993) also supported this view so far as the data on marriage, clan organization and anthropometric measurements are concerned. He also gave evidence of marital relation between MUR and ABM. This indicated that geographic proximity; ethno history & biosocial and cultural affiliation are important determinants of genetic affinity. Thus analysis based on genetic distances among populations corroborated with the ethno-historical findings.

Pairwise comparisons of gene frequencies between the populations under study showed statistical significant difference in eleven (24.4%) comparisons. 40% of these significant comparisons are between ABM and BHR. Chi square values show homogeneity between MUR and BHR. The analysis of anthropometric variables attempted by Rakshit (1974) also suggests relative homogeneity between BHR and MUR.

An attempt to seek an interpretation of the observed genetic variability, the available such DNA markers data in literature on some other groups from India and abroad were also compiled and compared with the present findings. Neighbor-Joining (NJ) Tree depicting relationship with the global populations, using Alu InDel markers, is in consistent with that reported by Majumder et al. (1999), which states that the Indian populations are genetically in between the Caucasoid and Mongolid.

The analysis based on Harpending and Ward model revealed that the ABM & MUR have experienced higher gene flow than predicted in terms of having lower than predicted heterozygosity while the BHR has experienced less gene flow than predicted. This observation may be explained by their population history. Ethno-history of Abujhmaia tribe reveals that these people were once the highly cultured Chhindak Nagas of Karnataka, who were ruling at Chakrakota (the modern Barsur). In a terrible war waged around 1112 AD between king Jajjalla Deva Kallachuri & Someswara-I, the king of Chhindak Nagas, the latter was vanquished and many of his army and retinue fled into the forests of Abujhmaria for good. The wild situation killed all but 50 or 60
The progeny of these are the present Marias of Abujhmaria (Hubey, 2000). A likely explanation is that a gene flow occurred prior to the subdivision of these populations into largely endogamous units, long before the region has been restricted for outsiders. However, scanning through anthropological literature reveals that there is some evidence favoring inflow of genes from different external sources pertaining to the study populations. Jain (1961) reported that the first person who entered in the Abujhmaria for study was Captain C.L.R. Glasfurd in 1906. Grigson (1938) was the first who presented a detailed description of the area and people. Roy (1938), Elwin (1945), Noronha (1950-52) made extensive tour in the area and made these people known to outsiders. Drift effect could have accentuated the process of genetic differentiation and has altered ancestral gene frequencies. Several lines of evidence suggest that genetic drift/gene flow has been the major evolutionary force to shape genetic variation in populations. This, by and large, is in agreement with anthropological findings. Since this analysis does not permit timing of the period during which the gene flow may have occurred between these populations, we unable to offer a clear interpretation of this finding. This represents important feature of populations under study, which has to be taken into account in any attempt to reconstruct the history of this populations.

The results of the present study also confirm earlier findings based on classical genetic markers (Majumder, 1998) that (i) genetic affinities among Indian populations do not correlate well with socio-cultural rankings, geographically closer populations are also genetically closer, and (ii) Indian populations are genetically between Caucasoids & Mongoloids.

Our findings are in agreement with Rakshit (1974) that “in general we may say that the Maria (MUR) and Hill Maria (ABM) and Bison-horn Maria (BHR) are nearer to each other vis-à-vis”. The findings suggest that these tribes are though distinct, are to be considered as an ethnic continuum in terms of genes, language, culture and geography. It is in conformity that ABM, BHR and MUR are sub-tribes of Gond. The core of this continuum is Abujhmaria. Analysis of further data on more biallelic loci, autosomal linked loci and then studying variation at micro-satellite loci, on the multi-biallelic locus backgrounds, in these populations will contribute to a deeper understanding on the population history.

The findings of the present study is very important in terms of wealth of information that has come out with regard to their socio-cultural characteristics and various customary practices and would form the cornerstone for epidemiological research. This is the first genetic study of any population of Chhattisgarh using DNA markers. It is modestly hoped that this study would help to the other researchers, with findings usable for comparison. In future, this may provide invaluable information in tackling numerous genetically control human disorders.