Chapter 6

Summary and Conclusion
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6.1. Introduction

Anthropology is a multidisciplinary approach to study the biological and socio-cultural attributes of human societies/populations of all kinds. In the earlier days, anthropologists were mainly concerned with documenting socio-cultural aspects of tribal communities but now various aspects of all types of human ethnic groups are a subject matter of interest. Biological or Physical Anthropology is the branch of Anthropology that is concerned with documenting and understanding biological attributes of human populations; and their interaction with culture. One of the prime areas of interest for physical anthropologists has been to understand origin of man within the framework of human evolution. In this respect, science of population genetics becomes an integral component of biological anthropology as it aids in understanding human evolution and pursuing the goal of understanding human origin vis-a-vis the divergence of human groups. It provides the foundation for evolutionary biology and assumes the existence of mechanisms for heredity and variation and inquires into the ways in which the genetic makeup of the population is altered or is held in equilibrium by the multiple influences of selection, migration and breeding structure. As an extension, study of genetic polymorphisms becomes immensely important in seeking answers to queries on evolutionary significance of diversity patterns. Evaluating role of evolutionary forces such as selection, mutation, gene flow, drift and others in shaping the diversity patterns of population groups becomes vital. Population genetics can thus broadly be defined as study of gene and genotype frequencies in a population, and predicting the way they would change or would be maintained over a period of time under the differential effects of various micro-evolutionary forces. The unit of examination in population genetic studies is Mendelian population. The field has seen a lot of development in terms of use of different types of markers to understand intra and inter-population variations, dynamics and relationships. Population genetics is also concerned with applying models of gene frequency change involving different factors in the context of Mendelian genetics to examine evolution in a quantitative manner. With specific
reference to evolution at molecular level, the concept of neutral or nearly neutral mutations along with genetic drift has been consistent with many observations of genetic variations.

By virtue of the fact that India is a ‘hotbed’ of immense cultural, ethnic, linguistic, demographic and genetic diversity, it provides ample opportunities to study a whole range of gene differentiation patterns between groups and action of different evolutionary forces. The inhabitants of the subcontinent have received variable gene flow (both in terms of source and degree) at different points in time; and more or less strictly adhere to the practice of endogamy. These factors have played an important role in defining the structure of these population groups. This combination further strengthens the scope of such research endeavours. The population groups are stratified at multiple levels into different categories- into tribal groups and non tribal groups; religious groups; linguistic groups and several others. This has created a milieu of immense cultural and genetic diversity which makes the subcontinent and its populations very appealing for evolutionary diversity studies. Several views have been proposed to interpret the existence of various ethnic and linguistic elements in the subcontinent and their complex interactions. One such view is regarding the entry of Indo-European linguistic element and its retreating effect on the previously widespread natives belonging to the Dravidian linguistic fold.

The two study populations, Dhodia and Dubla, residing in southern part of Gujarat are culturally tribal groups but linguistically they are affiliated to Indo European linguistic family. This is an interesting combination considering the fact that Indian tribal groups are considered to be the native inhabitants of the area by virtue of higher heterozygosity levels observed in these groups; and are believed to be affiliated to one of the three older linguistic families- Austro Asiatic, Dravidian and Tibeto Burman. But these tribal groups of Gujarat, along with some other tribal groups of north and north-western India belong to Indo European linguistic fold, a late immigrant in the subcontinent. These tribal groups are considered to be probably of proto-Australoid racial affinity, belonging to the earliest group of settlers in India who must have interacted with the people of various racial affinities passing through their habitat. This would have resulted in change in their racial constitution to some degree (Fuchs, 1964).
Also, despite of the fact that the tribal groups of western and north-western India constitute 29.31% of the total tribal population of India (Census of India, 2001), most of the studies conducted to explore the genetic and linguistic structure and affinities of different population groups of India have largely ignored these population groups. Most of these studies have stated that linguistic diversity concurs with genetic differences among populations.

It was thus aimed to genetically examine these population groups to explore whether they have greater genetic similarities with the immigrant groups that brought Indo European languages to the subcontinent; or these native groups only adopted/ were forced to adopt the new language while retaining their original genetic composition which is expected to be similar to other native groups of the mainland. This will aid in understanding the dynamics of genetic versus linguistic affinities. Also, the present study will lead to generation of comparable baseline data that can be used in the future for genome diversity analysis of populations of Indian origin. The population groups under study are also known to contain sickle cell gene and thus presented an opportunity to study this polymorphism as well.

6.2. Objectives of the Study

The study thus aimed to examine the extent of genetic heterogeneity and assess the genetic structure of the two tribal population groups, Dhodia and Dubla, in light of their ethno-historical and linguistic affiliations. The main objectives of the study were:

- To study the genetic heterogeneity among the Dhodia and Dubla tribes of southern Gujarat.
- To study pattern of gene differentiation among the Dhodia and Dubla tribes of southern Gujarat.
- To study genetic and linguistic affinities of the tribes under study.
- To find out the prevalence of HbS gene in the two populations understudy.
- To find out the most common haplotype(s) associated with the sickle cell mutation.

Keeping in view the above objectives, following molecular markers were selected and analysed in the study populations.
• **Alu Insertion Deletion** (*Alu* InDel) markers
  ✓ PV92, FXIIIB, D1, APO, ACE, CD4, PLAT

• **Restriction Fragment Length Polymorphisms** (RFLPs)
  ✓ **Unlinked**: ESR (*Pvu*II), NAT (*Kpn*I), PSCR (*Taq*I), T2 (*Msp*I), LPL (*Pvu*II), ADH2 (*Rsa*I) and ALAD (*Rsa*I)
  ✓ **Linked**: These permit haplotype reconstruction and have been examined for two gene systems:

Apart from analysing these molecular markers, sickle cell status of the collected samples was also determined. The data obtained was subjected to various statistical measures to derive comprehensible information.

### 6.3. Area and People

#### 6.3.1. Area

Study area, Valsad, is one of the 26 districts of the Gujarat state and is situated at the southernmost tip of Gujarat, near Gulf of Khambhat in the Arabian Sea. It is bound by Navsari district to the north, Dang district to the east and the State of Maharashtra to the south. The Arabian Sea lies west of the district. It is spread over an area of 2,939 sq km and has the population density of 561 persons per sq km. It has a total population size of 17,03,068, constituting 2.82% of total population of Gujarat (Census of India, 2011). It is subdivided into five talukas, namely Valsad, Pardi, Dharmapur, Kaprada and Umergaon.

#### 6.3.2. People

Study groups, Dhodia and Dubla, are two of the 29 notified Scheduled Tribes (STs) of Gujarat of which Dhodia and Dubla respectively constitute 7.90% and 8.00% of the total ST population of the state. According to Census of India (2001), population sizes of the two populations Dhodia and Dubla are 5,89,108 and 5,96,865 respectively. Dhodia are mainly distributed in Surat and Valsad districts of Gujarat and are also
found in Bharuch, Vadodra, Ahmedabad and Dang districts. Surat, Valsad, Bharuch and Vadodra districts also show high concentration of the Dubla population. In physical appearance the two groups do not differ much other than that the Dubla are darker in complexion with broader face and medium to broad nose as compared to the Dhodia.

There are several theories regarding origin of the two tribes. It is believed that the term Dhodia has been derived from Dhulia, a place in Maharashtra which is considered as the centre of origin of Dhodia tribe. Another legend traces their origin to Dholuka-Dhandhuka, a place near the south bank of Narmada. Term Dubla has been variably interpreted to mean ‘Durbala’ or weak. Dubla have been classed among the Kaliparaj i.e. dark race, a common term used for the aboriginal tribes of Gujarat. Both the tribes are known to claim descent from Rajputs. By most accounts the two study groups are considered to be the native inhabitants of Gujarat. Both the tribes are known to claim descent from Rajputs.

Both the population groups speak Gujarati language which belongs to the Bhili group of Indo-European family. Dhodia are bilingual. They also speak Dhodi dialect which is also grouped under Indo-European linguistic family but they don’t have a script. Dubla on the other hand have no dialect of their own. Dhodia society is divided into exogamous kuls. One cannot marry in one’s father’s kul (Pagdi kul) but can marry in one’s mother’s kul (Kachli kul). Dubla on the other hand show a lot of ambiguity with respect to the mating pattern. About 20 endogamous sub divisions are reported in literature but in reality there is a lot of uncertainty regarding this. Consanguineous marriages are prohibited in both the groups.

Dhodia enjoy good social status in the local social hierarchy and are placed above Dubla. Both groups are primarily agriculturists or agricultural labourers but are involved in other subsistence activities as well. They have access to education and health care facilities, drinking water, bank loans etc. and are also politically aware. Under the influence of factors such as increased education, means of communication and people moving out of their homelands for occupation in the cities, the study groups have experienced changes in their way of living over the years. This change has been more prominent in Dhodia as compared to Dubla, probably because of their better economic conditions and education level.
6.4. Materials and Methods

In order to minimise the bias because of flaws in research methodology, a well planned out research design was attempted at.

6.4.1. Selection of the Field Area and the Populations for the Study

Interest in the present study was prompted by the evident scarcity of genetic information on the tribal populations residing in western most region of the country. The interest was further heightened by the fact that most of the ‘indigenous tribal’ populations living in this part have affiliations with Indo-European family of languages, a late linguistic immigrant in the subcontinent. Due to these reasons, Valsad district lying in the ‘tribal belt’ of Gujarat was selected from the southern part of the state to carry out the study. The tribal populations Dhodia and Dubla selected for the study are numerically dominant tribal groups residing in the area living in close geographic and economic contact with each other but still maintaining their endogamous gene pools. Also, these groups are known to have a high frequency of sickle cell allele and thus provided an opportunity to study the gene.

6.4.2. Collection of the Data

At the start of study, clearance was taken from Ethical Review Committee of the Department of Anthropology to carry out the work. The study was initiated by conducting a pilot survey to get an overview of distribution of the study populations and to check the feasibility of carrying out research in the area. Various officials were approached to intimate them about the study objectives and to seek permissions. Valsad Raktdan Kendra, an organisation working in the health sector in Valsad district was also approached. They were of great help in rapport establishment in the area and with the population groups and in sample collection later on. After the initial survey, subsequent field works were carried out to collect blood samples and detailed ethnographic information. After explaining the purpose of the study and the procedure involved, written consent was taken from the study subjects and 5 ml of intravenous blood sample was collected in EDTA vaccutainers by a trained medical practitioner using sterilised disposable syringes. Sampling was done from unrelated subjects. Non-random sampling was also performed to collect known sickle cell trait or disease
samples from Dhodia population but this was not possible in case of the Dubla population due to non-availability of information. In total, samples were collected from 120 and 100 individuals from Dhodia and Dubla populations respectively. But DNA could later be isolated from 116 samples from Dhodia population and 97 samples from Dubla population.

6.4.3. Laboratory Techniques

Collected samples were subjected to two kinds of analyses-biochemical and molecular. Biochemical analysis was performed at Valsad Raktdan Kendra to detect sickling status of the collected blood samples. Sickling status was first detected using Dithionite Tube Turbidity test (Nalbandian et al., 1971) and then confirmed using Cellulose Acetate Membrane Electrophoresis. Following blood sample collection, DNA was isolated from them using salting out method (Miller et al., 1988). In the early phase of the study collected samples were transported to Biochemical and Molecular Anthropology Laboratory, Department of Anthropology, University of Delhi for DNA extraction but in later fieldworks DNA isolation was done in the field in a makeshift laboratory established at Valsad Raktdan Kendra. 25 markers analysed in the present study were then standardised in the laboratory in the department of Anthropology. They can broadly be classified into Alu Insertion Deletion Polymorphisms and unlinked and linked Restriction Fragment Length Polymorphisms. DNA was amplified for specific markers using Polymerase Chain Reaction (PCR) technique. Gel electrophoresis was then carried out to confirm the correctness of amplified product. In case of restriction fragment length polymorphisms, amplified product was subjected to restriction digestion and subsequently agarose gel electrophoresis and then the results were documented. In case of Alu insertion deletion polymorphisms, restriction digestion was not involved.

Protocols used for typing of different markers were as follows: Alu InDels (Majumder et al., 1999b); Unlinked Restriction Fragment Length Polymorphisms (Jorde et al., 1995); linked restriction fragment length polymorphisms at DRD2 locus (Kidd et al., 1998) and linked restriction fragment length polymorphisms of β-globin gene cluster (Sutton et al., 1989; Trabuchet et al., 1991; Majumder et al., 1999a; Lee et al., 2002).
6.4.4. Genetic Structure Analysis

Data generated using the above stated techniques was subjected to various statistical measures to derive comprehensible values from the raw data to aid in understanding of population structure of the populations under study and to help in their comparisons with available data from other population groups. The two study population groups were compared with each other and other population groups of India and Eurasia. A comprehensive search of literature was done to narrow down on the population groups to be used in the analysis based on their ethno-linguistic affinities. The two study groups were compared with six other Indo European speaking tribal groups of Gujarat for 20 autosomal loci; with nine other Indo European speaking groups of Gujarat for DRD2 gene system; and with other tribal and non tribal groups of India of different linguistic affiliations and Indo European speaking groups of Eurasia for different sets of markers.

Allele frequencies for the typed markers were computed using maximum likelihood scoring method, following an updated version of Reed and Schull (1968). Estimated allele frequencies were then subjected to chi-square goodness of fit test to determine whether the phenotype (genotype) frequencies were in accordance with Hardy-Weinberg proportions. Bonferroni’s correction was applied to correct for multiple comparisons in case of unlinked markers. Genetic distances among the population groups were determined using Nei’s standard genetic distance (1972) and their standard errors were computed using Nei and Roychoudhury’s 1974 method. To determine the significance of genetic distances among the different populations, the gene frequency data was compared pairwise using the chi-square statistic (Nei and Roychoudhury, 1974). Extent of genetic variability in the population groups was measured using heterozygosity and gene diversity estimates (Nei, 1973). To determine population structure, regression analysis of heterozygosity on genetic distance was carried out with the method described by Harpending and Ward (1982). The significance of regression equation was assessed by the method of Snedecor and Cochran (1967). Haplotype frequencies in case of the linked loci were calculated by maximum likelihood method using an Expectation Maximisation (EM) algorithm in the software ARLEQUIN, version 3.1 (Excoffier et al., 2005). Standard deviations of the haplotype frequencies were estimated by a parametric bootstrap procedure.
(Excoffier and Slatkin, 1995). Linkage Disequilibrium (LD) between pair of linked loci was tested for genotypic data using a likelihood-ratio test (Slatkin and Excoffier, 1996) in the ARLEQUIN software. Clustering of different population groups on the basis of analysed markers was determined using Principal Coordinate Analysis (Gower, 1966). Analysis of Molecular Variance (AMOVA) was done to analyse population differentiation among different categories of populations in terms of component of variance (Excoffier et al., 1992).

6.5. Salient Findings

Allele frequency estimates for the 25 analysed markers revealed that both the study groups, Dhodia and Dubla, are polymorphic with respect to these markers. Both the populations were found to show similar frequency distribution patterns. They were observed to differ significantly at four loci, FXIIIB, ALAD, TaqI ‘A’ and HinfI 5′β. The allele frequency estimates were used for the goodness-of-fit chi-square test to determine whether the phenotype and genotype frequencies depart from Hardy-Weinberg proportions. Out of all the loci analysed, two showed significant deviation from Hardy-Weinberg equilibrium in both groups. But, the overall pattern of phenotype (genotype) distribution was found to be in accordance with the expected proportions. Both the population groups were found to show high levels of heterozygosity for the 25 markers analysed; 41.46% and 40.79% in Dhodia and Dubla respectively. The extent of gene differentiation ($G_{ST}$) between the two study groups was found to be 0.59% on the basis of 25 autosomal loci. Haplotype analysis at DRD2 locus revealed haplotype sharing between the two population groups. Dhodia was found to have a set of 6 haplotypes occurring with frequencies ≥2.00% whereas in Dubla all the 8 haplotypes occurred with frequencies ≥2.00%. Ancestral haplotype, B2D2A1 was found to be present in both populations but with higher frequency in Dubla (5.60%). The overall pattern of haplotype distribution was similar in both groups with same set of three haplotypes contributing predominantly to the haplotypic pool. All three DRD2 sites were found to be in significant linkage disequilibrium in Dhodia but LD was not found to be significant between Taq I ‘D’ and Taq I ‘A’ sites in Dubla. With respect to haplotype analysis at β-globin gene cluster, Dhodia and Dubla were again found to exhibit same set of three predominant haplotypes. The two
populations were observed to share most of the haplotypes in all the three combinations but some variation in the haplotypic distribution pattern was also observed. Dhodia was observed to have more number of haplotypes with frequencies ≥2%. Slight discrepancy was observed with respect to linkage disequilibrium pattern between the two groups with more restriction sites showing significant LD in Dubla. Hb*S was found to occur with almost double the frequency in Dhodia (14.65%) than in Dubla (7.73%). Differences were observed in terms of allele frequency distribution pattern at restriction sites of β-globin gene cluster among the HbAA and HbAS/SS samples with higher frequencies of alleles for presence of restriction sites observed at most loci in HbAA samples of both study groups, other than at HindIII Aγ, HinfI 5′β and BamHI loci where the trend was opposite. Analysis of haplotypes associated with Hb*S could be done only for Dhodia population because of lack of sufficient samples from Dubla population. Arab Indian haplotype was found to be the most frequently associated haplotype in the study samples, occurring with a frequency of 78.97%. Five atypical haplotypes occurring with appreciable frequencies (2.00-4.99%) were also observed. These were mainly 5′ variant forms of other four major haplotypes known to be associated with the HB*S i.e. Senegal, Benin, Bantu and Cameroon. The processes such as probable admixture, gene conversion or chance mutation at the linked restriction sites studied can be considered as an explanation for existence of the atypical haplotypes (Niranjan et al., 1999). But recombination of the frequent haplotypes associated with HbA and HbS alleles seems to be the more plausible explanation (Majumder et al., 1999). In case of Dhodia, differences were observed in linkage disequilibrium patterns between the HbAA and HbSS samples, with significant LD observed between restriction sites 5′ and 3′ to the β-globin gene only in case of HbSS samples which has been taken as an indicator of young age of βS mutation (Magana et al., 2005).

When the two study groups were compared with six other Indo European speaking tribal population groups of Gujarat for 20 autosomal loci, they were observed to show similar frequency distribution patterns as the other tribal groups. Polymorphisms at the studied loci observed for the eight tribal groups of Gujarat were found to be more or less in line with the findings from other studies on Indian populations especially south Indian populations (Majumder et al., 1999b; Mujherjee et al., 2000; Veeraju et
al., 2001; Vishwanathan et al., 2004; Saraswathy et al., 2008) and also from global populations (Batzer et al., 1994, 1996; Stoneking et al., 1997; Kidd et al., 1998).

When frequencies of alleles for presence of restriction sites at the \textit{DRD2} locus were compared among the 11 Indo European speaking tribal groups of Gujarat, it was observed that the distribution pattern was similar in all the groups, including Dhodia and Dubla. Frequency distribution of ancestral alleles B2, D2 and A1 was found to be similar to Dravidian speaking tribal populations of South India (Vishwanathan et al., 2003; Bhaskar et al., 2008; Prabhakaran et al., 2008; Saraswathy et al., 2009c), but not to African and European populations (Kidd et al., 1998) and Indo-European speaking north Indian population groups (Saraswathy et al., 2010).

Heterozygosity estimates from the two datasets (8 tribal groups and 20 autosomal loci; and 11 tribal groups and 3 \textit{DRD2} sites) revealed moderate to high average heterozygosity levels across the tribal population groups of Gujarat, with the two study populations consistently showing high levels. When compared with studies carried out on similar set of markers it was seen that the heterozygosity levels are similar to those observed in south Indian populations (Majumder et al., 1999b; Mujherjee et al., 2000; Veeraju et al., 2001; Vishwanathan et al., 2004; Saraswathy et al., 2008) and higher than in other non-African populations (Stoneking et al., 1997; Novick et al., 1998). Genetic distances among the paired populations based on analysis of 20 autosomal loci did not show significant differentiation as determined by the pair-wise chi-square statistics.

When the gene diversity analysis was done using 20 autosomal loci for the 8 tribal groups of Gujarat, gene differentiation between the populations was found to be 2.20%. Gene diversity analysis done using three \textit{DRD2} sites in 11 population groups of Gujarat revealed that the inter population differences accounted for only 2.00% of the observed heterozygosity. Most of the observed heterozygosity was attributable to diversity within the population groups. The values of extent of genetic differentiation observed from the two analyses are smaller than the observations from other studies done using molecular markers (Mujherjee et al., 2000; Veeraju et al., 2001; Watkins et al., 2001; Vishwanathan et al., 2004; Saraswathy et al., 2008). It is noteworthy that the sizes of most of the populations under study are fairly large and therefore, the genetic drift might not have played major role in the process of genetic differentiation.
The regression of heterozygosity on distances from centroid in both the analyses (8 tribal groups and 20 autosomal loci; and 11 tribal groups and 3 DRD2 sites) found the regression to be consistent with linearity indicating that the groups have received similar proportion of gene flow. All of these groups were found to be placed near theoretical regression ($r_{ii}$) line, indicating that there is a little possibility of assimilation of these groups into migrant groups (in this case Indo-European speaking immigrants entering the subcontinent).

Haplotype analysis at DRD2 system revealed extensive sharing of haplotypes among the tribal populations of Gujarat. Distribution pattern with respect to individual haplotypes was found to be largely in line with the findings from other south Indian population groups. Range of frequency of ancestral haplotype B2D2A1 was observed to be 1.90%-15.90%. This was comparable to the findings from other south Indian population groups (Vishwanathan et al., 2003; Prabhakaran et al., 2008; Aggarwal et al., 2010) but in contrast to the findings among European populations where the frequency of ancestral haplotype varies mostly between 0 and 4% (Kidd et al., 1998) and Indo-European speaking north Indian populations (Saraswathy et al., 2010).

Haplotype analysis at β-globin gene cluster among the normal individuals (i.e. individuals with genotype HbAA) did not reveal much information regarding relationships of the study populations with other population groups largely because of paucity of comparable data and patchy distribution of haplotypes observed both in India and other world populations. A study by Kshatriya et al. (2011) found the same set of three haplotypes to be the predominant contributor to the haplotypic pool of tribal groups of Gujarat on the basis of analysis of three sites, as seen in the study groups Dhodia and Dubla.

Finding of predominance of Arab Indian haplotype (based on the analysis of eight restriction sites) associated with Hb*S allele in the study group Dhodia is in line with the findings from other studies carried out in different population groups of India. (Labie et al., 1989; Majumder et al., 1999a; Niranjan et al., 1999; Uma Mahesh et al., 2011). These studies have also reported prevalence of one or the other atypical haplotypes. Five atypical haplotypes, variants of all the five major haplotypes-Senegal, Bantu, Benin, Cameroon and Arab-Indian, were found in the present study.
It is probably for the first time that atypical forms of all the major haplotypes have been reported in one population group from India.

Analysis of molecular variance (AMOVA) taking into account different populations groups of India and Eurasia and categorised on the basis of ethnic and linguistic affinities revealed an interesting pattern. The two study populations were categorised with other Indo-European speaking tribal groups. Maximum variance was observed between Dravidian speaking groups of India and Indo-European speaking groups of Eurasia (8.70%). Indo-European speaking tribes of Gujarat showed minimum between group variance with Dravidian-speaking groups of India (1.44%), followed by higher variance with other Indo-European speaking groups of India (2.83%) and maximum with Indo-European speaking groups of Eurasia (6.50%). This is indicative largely of genetic similarities of the Indo-European speaking tribes of Gujarat in general, and the two study populations in particular, with Dravidian speaking groups of southern India; and also of genetic contribution of Eurasians in the gene pool of these groups.

Principal Coordinate Analysis was done to see how the different population groups cluster on the basis of comparable data available on six Alu loci and it was seen that the two study populations tend to lie between the Dravidian speaking groups and Indo-European speaking groups of Eurasia, but, largely within the domain of the Dravidian groups indicating their affinities with Dravidian speaking population groups.

6.6. Conclusion

Ethno-historical records suggest that the study populations, Dhodia and Dubla, are the natives of Gujarat and / or adjoining areas. Nothing conclusive is known about their origins. Both population groups live in close geographic and economic contact with each other but still maintain their endogamous gene pools. These socio-culturally tribal groups are affiliated to Indo-European linguistic family which is an anomaly of sorts. It was thus aimed to study the dynamics of linguistic and genetic affiliations in these groups.

The two groups were found to have high prevalence of Hb*S allele, ranging between 7.73% in Dubla to 14.65% in Dhodia. Haplotype analysis at eight restriction sites of β-globin gene cluster in Dhodia revealed predominance of Arab Indian haplotype and
also presence of five atypical haplotypes associated with the mutation. It is probably for the first time that variant forms of all the five major haplotypes associated with Hb*S are being reported from one ethnic group from India.

Similarity in allele frequency distribution pattern at the 25 autosomal loci, very low value of coefficient of genetic differentiation ($G_{ST}=0.0059$), extensive sharing of haplotypes at both $DRD2$ locus and $\beta$-globin gene cluster and predominance of same haplotypes in both the groups are suggestive of the genetic uniformity between the two groups. At the same time, some variation was observed between the two groups with respect to number of haplotypes occurring with appreciable frequencies ($\geq 2.00\%$) and linkage disequilibrium patterns. This is understandable considering that the two populations maintain their distinct gene pools. High heterozygosity levels hint towards greater antiquity and greater genetic diversity.

Furthermore similar observations of uniformity in allele frequency distribution patterns at 20 autosomal loci including restriction sites of $DRD2$ locus, low $G_{ST}$ values for the different analyses, haplotype sharing between the different tribal groups of Gujarat reveal the underlying genetic homogeneity of these groups, which is consistent with underlying social and cultural homogeneity among them and is in accordance with historical past of these groups. Also, analysis of regression of heterozygosity on genetic distance among these groups was consistent with the linearity indicating that the groups have received similar proportion of gene flow. Thus, it can be said that the tribal population groups of Gujarat are neither overtly admixed nor are they completely isolated. Moreover, all of these groups are placed near theoretical regression ($r_{ii}$) line, indicating that there is little possibility of assimilation of these groups into migrant groups (in this case Indo-European speaking immigrants entering the subcontinent). Clearly, these groups, including the study populations, with high genetic diversity have experienced a slow process of genetic differentiation.

The two study groups, along with other tribal population groups of Gujarat were found to cluster with Dravidian speaking groups of south India in Principal Coordinate Analysis. Also they showed least among group variance with Dravidian speaking groups in AMOVA analysis. This, along with similarities in allele frequency
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(such as those of DRD2 ancestral alleles) and haplotype frequency distribution patterns and high heterozygosity levels point towards their similarities with Dravidian speaking groups of south India.

Influence of Indo-European speaking populations on the tribal groups of Gujarat was also observed in terms of their intermediate position between Dravidian speaking groups and Indo European speaking Eurasians as seen in PCO analysis and also in high but intermediate value of among group variance observed for comparison between Gujarat populations and Indo-European speaking Eurasians (6.50%). These findings, thus, pinpoint to their diverse genetic makeup and Eurasian contribution to gene pool of these groups as well.

Hence, the autosomal DNA marker analysis in the study groups with respect to pattern of allele frequencies, heterozygosity levels, haplotype frequencies, AMOVA and Principal Coordinate Analysis point to their closer genomic proximity with Dravidian-speaking south Indian groups and the acquisition of Indo-European language appears to be a recent phenomenon with the study populations still retaining pre-Indo-European genetic component and this is in agreement. The original composition of proto-Australoid genes in these tribes might have been diluted to some extent through the differential contributions from various incoming groups from north and north-western corridor of India but their proximity with the Dravidian-speaking tribal populations of south India suggests that genetic affinities may not necessarily be dependent on linguistic similarities.