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Genetic structure and microgenetic differentiation among populations of Terai belt of Bihar, India

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ABSTRACT

Distribution of ABO blood groups, Rh, PTC taste ability and colour blinness was investigated among twelve endogamous populations of Terai belt (Indo-Nepal border) of Bihar, who belonged to different castes and ethnic groups with different migrational history. The phenotypic and gene frequencies of four common loci show wide differences between the populations. In general, group B is dominant over group A, but in four populations (Badhiya, Kulhayya, Mushar and Tharu) group O was prevalent. Rh- negative frequency was highest among Bania and lowest in Tharu. Frequency of non taster was highest among Tharu and lowest in Oraon. The incidence of red-green blindness was lowest in Santal and highest in Mushar. The pooled heterozygosity was highest (0.545) for ABO locus and lowest for colour blindness (0.055).The average heterozygosity value varies in between 0.228 among the Tharu and 0.420 among the Bania. The studied populations share low Gst value i.e. 0.020, which is almost negligible. The dendrogram depicts bifurcation of the population groups into two lines: one line leading to Tharu alone and the other line leads to Santal and other populations. The other line bifurcates again into Muslim and a bunch of populations. This bunch of populations includes Mushar, Rajbanshi, Chamar, Brahmin, Oraon, Munda, Dhobi, Bania, Kulhayya. Muslims share more or less proximal ancestor. From the genetic distance analysis there is some evidence of close genetic relationship among the population groups belonging to same region, irrespective of their caste, religion, linguistic or any other affinities.

Key words: Genetic diversity, Genetic distance, Heterozygosity, Gene flow and Terai belt.

INTRODUCTION

Humans are product of biology as well as culture and always strive for better life. People do not hesitate to leave their motherland for foreign land which assures them better standard of living. Thus people migrate from one zone to another for better life and food opportunity. But during the course of migration as well as adaptation to the new zone, they have to face a lot of difficulties. What help the migrating populations to resist with these diseases and to adapt in the new zone? It is their genetic structure may be considered to make them able to copes the burden of migration as well as to adapt them in the new zone. So, study of genetic structure of populations is of great importance in knowing their adaptation as well as ethnic relationships.

Genetic variability is the common feature of many organisms. The modern human populations carry in their genes, the encoded history of remote past and earlier migration history. Human populations differ genetically in varying proportions of the alleles of various sets. Human biological variations are related to the ethnic and ecological

background of the population. The genetic similarities within the population show the common origin or the admixture of gene pool. The existence of genetic variation in man is caused by many factors along with selection, migration, gene flow and genetic drift. Populations of the same ethnic origin living in different geographical regions appear to show variation in biological characters among them. A population is characterized by a set of gene frequencies. Hence, the gene frequency data are essential prerequisite for studying the genetics of any population.

The Indian population is structured into 40,000 endogamous groups, of which 37,000 groups belong to the Hindu caste system [1]. Hindu population constitutes the largest community and the second largest is the Muslim population. The Indian population also includes Hindu, Muslims, Christians, Sikhs, Jains, Buddhists and Parsis. Throughout the ages many population groups have migrated toward India along north eastern and north western routes [2]. A look at the ethnic history of India reveals that Indians belong to two different categories: the Dravidians (aborigines) and the Aryans or Sanskrit speaking groups (with mixed groups known as the Musalmans [2]. The caste system in India has its origin in the verna system, with its language, state, and religious base; hence caste differentiation can be studied from these three points of views [3]. The caste system reflects the Indian occupationally and religiously defined hierarchies.

North Bihar in East India has been populated since the ancient times (5000B.C.) and it was centre of two great historical dynasties of the Maurya and Gupta rulers in India. During the recent time there have been several waves of migration from the neighbouring states into Terai belt of Bihar. The present population of the state has a diverse mixture of old stock endogamous populations belonging to a common origin and recent heterogeneous populations of different neighbouring states. From the genetic point of view, the Terai belt of Bihar represents a mosaic pattern of gradients of homozygosity and heterozygosity in the same region, which could be explained primarily due to ethanohistorical reason, rather than geographical proximity. As majority of population of this zone are migrant of some other places and nothing is known about their genetic makeup as well as ethnic relationship, so the present work was undertaken.

MATERIALS AND METHODS

Population and sample collection

Terai belt of Bihar is an agricultural zone. Economically it is a backward zone. It was full of jungles and was popularly known as the ‘Paradise of Hunters’. Once upon a time, it was called as ‘Kala Pani’ due to its bad climatic condition. But after earthquake of 1934, there was a drastic change in its climatic condition and migration of people from different corners of the country started. Slowly urbanization and industrialization begins. In broad sense, there is a drastic change in environmental conditions. All the studied populations have migrated from Bangaladesh, Nepal as well as from the neighbouring state of India.

Blood samples were collected from 3331 unrelated male individuals from 12 endogamous populations: Brahmin, Bania, Mushar, Dhobi, Chamar, Rajbanshi, Santal, Oraon, Munda, Tharu, Badhiya and Kulahayya Muslims living in and around different villages in Terai belt of Bihar. Only four Mendelian traits were considered for the present study due to lack of proper laboratory facilities and chemicals. These were ABO blood groups, Rh system, PTC taste ability and Colour blindness.

The sample varies from 175 males in Dhobi to maximum 509 males in Badhiya Muslim. The 12 populations belong to four different (caste) groups:

A) Forward caste – Brahmin
B) Backward caste – Bania
C) Scheduled castes – Mushar, Chamar, Dhobi and Rajbanshi
D) Scheduled tribes – Santal, Oraon, Munda and Tharu
E) Muslims – Badhiya and Kulhayya

Laboratory Analysis

i) The standard methodology was followed for the detection of the ABO blood groups by slide agglutination method using anti A and anti B. Rh blood group was detected by slide agglutination method using anti D.

ii) Phenyl thiocarbamide (PTC) taste ability was studied using method of Harris and Kalmus, 1949a [4] and colour blindness was detected by using Ishihara, 1959 colour plates [5].

Statistical Analysis

Phenotypes were recorded for each trait. The allele frequencies were calculated according to Mourant *et al*., 1976 [6]. Heterozygosity for a given locus was calculated using the genotype frequencies (for heterozygous genotype). The level of Heterozygosity was calculated using the formula:

(i) $H=1-\Sigma Xi^2$

Gene diversity was calculated using Nei 's, 1973 [7] methods of gene diversity analysis in sub divided populations [7].

(ii) HT =HS + DST

Genetic distance (D) was determined using Nei's 1972 [8] formula. The normalized identity of gene between the X and Y with respect to all loci is defined as follows Nei, 1972 :

(iv) $I=Ia/\sqrt{I^{aIb}}$

(v) D= -1n I

The dendrogram was drawn as per UPGMA clustering method.

RESULTS

Phenotypic frequency

In the ABO blood group the most frequent blood was B (33.44%) followed by O (31.95%) and A (25.63%). Blood group AB was least frequent (8.98%). The frequency of this marker in present studied population was found to be **B>O>A>AB** (Table1). Blood group A manifested highest frequency in Brahmin and Oraon while in Bania, Chamar, Santal, Munda, Dhobi and Rajbanshii blood group B was dominant. In Badhiya, Kulhayya (Muslim), Mushar and Tharu blood group O was found highest. The highest frequency of Rh- was found in Bania (15.8%) and minimum in Tharu (0%). In other populations the frequency of Rh- ranges from 0.57% to 4.93% (Table – 1). The frequency of PTC non taster was highest in Tharu (58.9%) while in other populations it ranges from 28.7 to 47.7%. The incidence of colourblindness ranged from 1.7 to 15.8% being highest in Mushar (5.8%) and lowest in Santal (0.6%). .

Allele frequency

The distribution of allele frequencies among the studied populations are given in the Table – 2. Oraon has the highest frequency of A (0.263) while Munda has the highest frequency of B with 0.345. Both Chamar and Kulhayya Muslim have the least A with each 0.14. In case of B allele, Tharu has the least frequency with 0.147. The O allele varies from 0.442 among the Mundas to 0.666 among the Tharu. All populations showed trend of $I^O>I^B>I^A$. The Rh D allele was maximum in Tharu and minimum in Bania (Table – 2). Chamar manifested highest frequency of allele T (taster) while Tharu manifested its lowest frequency. The frequency of non taster gene was more than 50% in all studied populations (except Chamar and Rajbanshi). The allele frequency of colour blindness was maximum in Mushar and minimum in Santal

Heterozygosity

The Table – 3 provides the heterozygosity and co-efficient of genetic differentiation (Gst) among the studied populations. The pooled heterozygosity was highest (0.545) for ABO locus and lowest for colour blindness (0.055).The average heterozygosity value varies in between 0.228 among the Tharu and 0.420 among the Bania. In overall, the average heterozygosity lies below 0.5 which indicates the studied populations are more or less in equilibrium and homogenous. (The studied populations share low Gst value i.e. 0.020, which is almost negligible. The low Gst value among the studied populations can be accounted either on the basis of sharing a common geographical location or a common genetic substratum where the studied populations might share the ancestral population.

Genetic distance

The Table – 4 provides the Nei's genetic distance matrix among the studied populations. Neighbour Joining tree (NJ tree) based on Nei's distance is given in dendrogram depicts bifurcation of the population groups into two lines: one line leading to Tharu alone and the other line leads to Santal and other populations. The other line bifurcates again into Muslim and a bunch of populations. This bunch of populations includes Mushar, Rajbanshi, Chamar, Brahmin, Oraon, Munda, Dhobi, Bania, Kulhayya Muslim (share more or less proximal ancestor).

Table – 1: Frequency of ABO blood groups, Rh, PTC non taster and red-green blindness in studied populations of Terai belt of Bihar

Population	Number	A	B	AB	O	Rh^-	PTC non taster	Red-green blind
Badhiya	509	111 (21.8%)	170 (33.5%)	35 (6.8%)	193 (38%)	14 (2.7%)	178 (34.98%)	14 (2.7%)
Bania	190	36 (18.9%)	70 (36.8%)	28 (14.7%)	56 (29.5%)	30 (15.8%)	58 (30.5%)	9 (4.7%)
Chamar	368	80 (21.7%)	150 (40.8%)	15 (4.1%)	123 (33.4%)	11 (2.9%)	69 (18.7%)	8 (2.2%)
Kulhayya	304	50 (16.4%)	82 (27%)	30 (9.8%)	142 (46.7%)	15 (4.9%)	98 (32.2%)	11 (3.6%)
Mushar	325	103 (31,7%)	81 (25.0%)	35 (10.7%)	106 (32.6%)	8 (2.46%)	82 (25.2%)	19 (5.8%)
Tharu	190	50 (26.5%)	37 (19.1%)	15 (8.0%)	88 (46.4%)	0 (0.0%)	112 (58.9%)	6 (2.63%)
Santal	350	96 (27.4%)	141 (40.3%)	45 (13%)	68 (19.3%)	2 (0.6%)	118 (33.8%)	6 (1.7%)
Munda	202	52 (25.7%)	90 (44.5%)	24 (11.9%)	36 (17.9%)	9 (4.4%)	68 (33.7%)	11 (5.4%)
Oraon	202	70 (34.6%)	60 (29.7%)	22 (10.9%)	50 (24.8%)	12 (5.9%)	55 (27.2%)	9 (4.4%)
Dhobi	175	55 (31.4%)	69 (39.4%)	15 (8.6%)	36 (20..6%)	10 (5.7%)	83 (47.4%)	10 (5.7%)
Brahmin	208	72 (34.6%)	54 (26%)	15 (7.2%)	67 (32.2%)	15 (7.2%)	60 (28.8%)	4 (1.9%)
Rajbanshi	308	79 (25.6%)	110 (37.7%)	20 (6.5%)	99 (32.2%)	14 (4.5%)	62 (20.1%)	6 (1.9%)

Table – 2: Allelic frequencies of different genetic markers in twelve population groups of Terai belt of Bihar

Population	ABO			Rh		PTC		Color blindness	
	A	B	O	D	D	t	T	C	C
B. Muslim	0.156	0.227	0.617	0.166	0.834	0.591	0.409	0.028	0.972
Bania	0.183	0.299	0.518	0.397	0.603	0.553	0.447	0.047	0.953
Chamar	0.14	0.261	0.599	0.173	0.827	0.433	0.567	0.022	0.978
K. Muslim	0.14	0.202	0.658	0.222	0.778	0.568	0.432	0.036	0.964
Mushar	0.24	0.197	0.563	0.157	0.843	0.502	0.498	0.058	0.942
Tharu	0.187	0.147	0.666	0	1	0.768	0.232	0.026	0.974
Santal	0.229	0.318	0.453	0.0756	0.924	0.581	0.419	0.017	0.983
Munda	0.213	0.345	0.442	0.211	0.789	0.536	0.464	0.054	0.946
Oraon	0.263	0.231	0.506	0.244	0.756	0.522	0.478	0.045	0.955
Dhobi	0.23	0.285	0.485	0.239	0.761	0.689	0.311	0.057	043
Brahmin	0.239	0.184	0.577	0.269	0.731	0.537	0.463	0.019	0.981
Rajbanshi	0.178	0.242	0.58	0.213	0.787	0.449	0.551	0.019	0.981

Table – 3. Average Heterozygosity and Gst in twelve populations of Terai belt of Bihar

Locus	**ABO**	**Rh**	**PTC**	**Color Blindness**	**Average**	**SE**
B Muslim	0.545	0.277	0.484	0.055	0.340	0.111
Bania	0.612	0.481	0.497	0.090	0.420	0.114
Chamar	0.555	0.287	0.492	0.043	0.344	0.116
K Muslim	0.508	0.347	0.492	0.070	0.354	0.102
Mushar	0.588	0.266	0.502	0.110	0.366	0.109
Tharu	0.503	0.000	0.358	0.051	0.228	0.121
Santal	0.643	0.141	0.488	0.034	0.326	0.143
Munda	0.643	0.335	0.500	0.103	0.395	0.116
Oraon	0.625	0.371	0.502	0.086	0.396	0.115
Dhobi	0.634	0.366	0.431	0.108	0.385	0.109
Brahmin	0.579	0.395	0.500	0.037	0.378	0.120
Rajbanshi	0.575	0.336	0.496	0.037	0.361	0.119
Gst	0.009	0.049	0.025	-0.001	0.020	0.017

Table – 4: Estimates of Nei's measure of genetic distance (D) among twelve population groups of Terai belt of Bihar

	Bania	Chamar	K. Muslim	Mushar	Tharu	Santal	Munda	Oraon	Dhobi	Brahmin	Rajbanshi
B. Muslim	0.010	0.003	0.001	0.003	0.028	0.006	0.005	0.004	0.005	0.004	0.003
Bania		0.011	0.007	0.012	0.067	0.022	0.006	0.005	0.007	0.006	0.008
Chamar			0.004	0.004	0.041	0.009	0.006	0.005	0.013	0.006	0.001
K. Muslim				0.004	0.036	0.011	0.006	0.004	0.006	0.003	0.003
Mushar					0.032	0.007	0.004	0.002	0.007	0.004	0.003
Tharu						0.022	0.044	0.045	0.039	0.045	0.044
Santal							0.007	0.010	0.010	0.012	0.009
Munda								0.002	0.004	0.006	0.005
Oraon									0.004	0.002	0.003
Dhobi										0.006	0.010
Brahmin											0.003

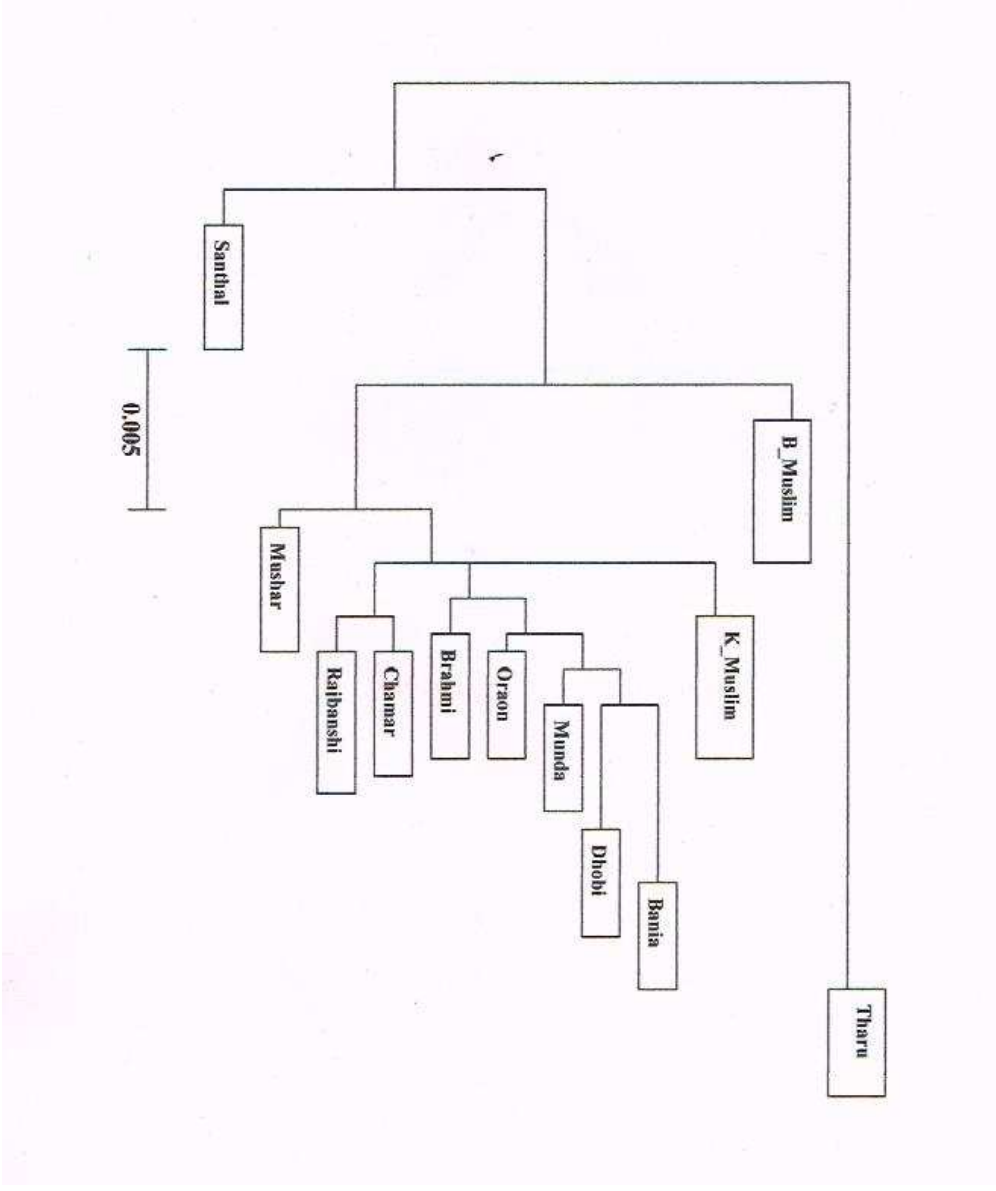

Fig. 1. Dendrogram showing the relationship between twelve populations of Terai belt of Bihar. (Based on Nei's maximum Genetic distance measure)

DISCUSSION

In Gangetic plain, generally blood group B is more frequent than A [9], for example, among Bania, Rajput and Yadav of Uttar Pradesh [10] and among Chamars of Punjab [11]), Jats of Haryana [12] and among Brahmin, Bania, Rajput, Kayastha, Sudra and Muslim of Madhya Pradesh [13] as well as populations from Kashmir [14]. In Bihar higher incidence of group B and O among Dusadh of Bhagalpur region [15], Bania, Bengali, Bhumihar, Dusadh,

Kayastha, Kurmi, Mushar, Rajput, Yadav, Muslim, Tharu, Dhobi, Kulhayya, Santal and Munda of Koshi zone [16,17, 18] and among Oraon of Ranchi and Purnia division [19, 20, 21, 22].

The most widely studied ABO blood groups show that in general the allele frequencies of the total population of the world found to be O = 62.3, A = 21.5 and B = 16.2 [23]. The European populations have more than 25 of A allele (varies from 25 to 35) and B allele frequency below 10. Among the population groups of Southwest Asian countries (Saudi Arabia, Jordan, Kuwait, Yemen, Israel, Lebanon, Syria, Iraq, Iran and Afghanistan) the frequencies of alleles A and B are about 23 and 15, respectively except in Afghanistan where the allele B is higher than allele A. [6, 24]. In India, the distribution of allele B frequency is higher (23.3) as compared to allele A (18.6), whereas the frequency of allele O is 58.1. In the present study the frequency of allele B is higher (24.48) as compared to A (19.99) and allele O is 55.53 (Table – 1). Normally, the distribution of ABO blood group varies from one population to another. In many other studies, blood group O has been found to be the most common blood group. In the Caucasians in the United States, the distribution is group O, 47%, group A, 41%, group B, 9% and group AB, 3% [25]. Among Western Europeans about 42% are group A, 9% group B, 3% group AB and the remaining 46% group O. For blacks in United States, the distribution is group O, 46%, group A, 27%, group B, 2%, and group AB, 7% [25]. Similarly, in Pakistan, blood group O is the most common 35%, blood group A is 24%, blood group B is 33% and blood group AB is 8%. In Lagos Nigeria, blood group O is 55.3%, blood group A, 25.3%, blood group B, 16.7% and blood group AB, 2.7% [26].

The Rh distribution also varies within any group of population. High incidence of Rh- have been found in other studies among Dusadh (6.2%), Chamar (4.8%), Mushar (4.7%) of Bhagalpur region of Bihar [15]. and among Chamar of Punjab (4%) by Sidhu [11]. Pandey *et al*. 2003 have reported incidence of 8.57% among Julhaha of Koshi zone of Bihar [17]. The recessive allele (d) ranges from as high as 40% to its virtual absence in Chinese Australian aborigines, Negrito etc. Exceptionally high incidence of Rh negatives yielding frequency of recessive allele (d) in the range of 50 to 60% have been reported in Basque (Europe) and Berbers of Moracco [6].

The variation in frequency of the Rh negative gene (i.e., Rh d) is 15-30% in the majority of the Indian population compared to 35-45% in Europeans and 0-10% in Asian population [9]. For the Rh system the overall frequencies for D and d are 80.71% and 19.29% respectively [27], which corresponds to 1% and 73.1% in the present study.

The average frequency of t allele among Indian populations is 53.4% (varies from 8.8% among scheduled caste of Andhra Pradesh to 89.2% in Munda of Ranchi, Bihar) while in European populations it varies from 25 to 57% which is little higher but similar to that of South west Asian [28]. For the Ansari from Bihar, the frequency of the *t* allele is 71% [29]. In the present study the average frequency of non taster was 56%. The frequency of colour blindness is lowest in hunting and gathering societies, with highest frequency in industrial societies [30]. The defect shows quite a bit of variation in different populations. The frequency of colour blind males among Indian populations on an average is 3.6% which varies from complete absence to 23%. Shah *et al*, 2013 have reported Muslims belonging to caste Sheikh, Pathan, Syed and Moghul shows overall prevalence rate of 5.34 %, 4.58%, 6.89% and 3.70% respectively [31]. The incidence of colour blindness in Chamars and Muslims of Bhagalpur, Bihar has been found to be 2.2% and 2.7% respectively [15]. Pandey *et al*., 2000 [16] have reported 7.3% among Mallah of Purnia district of Bihar while in present study the average frequency of color blindness was 3.58% which is within the Indian range.

The study in Bihar and other neighbouring regions both in North [33] and Eastern and Southern regions [33, 34, 35, 36, 37, 38, 39, 40] and many others suggest that both the phenotypes and gene frequencies of ABO, Rh, PTC and colour blindness in Terai belt of Bihar is in agreement with other populations in Northern, Southern and Eastern regions.

The variability observed in the distribution of various genetical parameters (such as ABO and Rh blood groups, PTC taste ability and colour blindness) in the present population groups of Terai belt of Bihar suggest that the genetic composition of the investigated groups is more or less homozygous. Much of the genetic similarity among the present populations primarily drives in their ethnic background, environmental condition and geographical isolation. However, differences in certain genetical parameters may be attributed to marriage pattern which involves the flow of genes.

The dendrogram (Fig. – 1) based on genetic distance depicts bifurcation of the population groups into two lines:
i) One line leading to Tharu alone and the other line leads to Santal and other populations.
ii) The other line bifurcates again into Muslim and a bunch of populations.
iii) The bunch of populations includes Mushar, Rajbanshi, Chamar, Brahmin, Oraon, Munda, Dhobi, Bania, Kulhayya. Muslim share more or less proximal ancestor.

iv) Badhiya and Kulhayya Muslims as well as Chamar and Rajbanshi manifested the lowest genetic distance while Bania and Tharu showed highest genetic distance.

Though the study is based on only four loci, the results manifested a clear possibility of identifying population structure variables that influence the genetic diversity at the regional level. In case of Terai belt of Bihar, the diversity is related to ethano-historical migration of the populations.

It has been reported that caste group is invariably clustered with the scheduled caste and the community, while the scheduled tribe is distinct from all [41]. The scheduled castes may have in them substantial contribution of gene flow from the higher castes in past generations [42] .Such instances of clustering of forward castes with backward castes, scheduled castes as well as with scheduled tribes have been reported in Koshi zone of Bihar [16, 17, 18].

CONCLUSION

In the present investigation the patterns of gene diversity between populations, the genetic distances, and the relation of heterozygosity between population have been studied. The extent of genetic divergence (G_{ST}) varies considerably from locus to locus. Gene diversity is the most important measure of genetic variability of a population and can be related to the number of codons different per locus [43.] Bania manifested highest heterozygosity (0.420) while lowest heterozygosity was found in Tharu. Pooled heterozygosity was highest for ABO and lowest for Color blindness. The pooled G_{ST} (0.020)) gives an estimate of the degree of genetic differentiation present among different populations.

The genetic distance between Badiya and Kulhayya as well as in Chamar and Rajbansji was the lowest (0.001)) and that between the Bania and Tharu was the highest (0.067). The dendrogram based on genetic distances clearly shows that the Tharu and Santal differentiated from other population groups earlier. The study reveals that these populations are at an early stage of genetic differentiation.

Though the study is based on only four loci, the results manifest a clear possibility of identifying population structure variables that influence the genetic diversity at the regional level. In case of Terai belt of Bihar, the diversity is related to ethano-historical migration of the populations. The results shed some light on the genetic composition as well as fitness of the populations in the new ecological zone.

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