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Genomic structure of Zeme and Kuki tribes of Manipur, North-East India

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ABSTRACT

Introduction: Zeme and Kuki tribes are the hill inhabitants of Manipur who belong to TibetoBurman speaking groups. Aim: The present study attempt to assess the extent of heterogeneity of the genetic structure of Zeme and Kuki tribes of Manipur. Methods: Five Alu InDel markers were screened on 188 individuals of two tribal groups of Manipur. Results and Conclusion: All the five loci are found to be polymorphic. Genotype frequencies for all the loci are in reasonable agreement with their respective Hardy-Weinberg proportions except in PV92 among the Kuki tribe. Average heterozygosity levels are high and degree of differentiation is low (Gst-1.7%), revealing that genetic drift is unlikely to play a significant role in the process of genetic differentiation. Genetic distance analysis indicates that Zeme and Kabui, Kuki and Meitei are close to each other. However, the affinities of Kukis with Meitei need further investigation. The genetic structure by and large revealed that the population groups of Manipur are neither overtly admixed nor isolated. Furthermore, the composition of genes in the population groups of Manipur are comparable to each other and that differential heterozygosity might have led to variable influx of genes into these groups, but with similar sources.

Keywords: *Genetic affinities, population structure, gene flow, heterozygosity.*

1. INTRODUCTION

India is a country having twenty nine states and six Union territories and one national capital Region-Delhi. It is the second most populous country in the world with 1.21 billion people

(Census of India 2011) and is composed of a large number of populations sub-divided by caste, tribe, religion, region and language. Atleast 50-60 thousand essentially endogamous groups exist in the country (Gadgil and Malhotra 1983; Joshi et al. 1993). Thus, it exhibits enormous genetic, cultural and linguistic diversity which can in part be attributed to its position at the tri-junction of Africa, and the northern Eurasian and oriental regions, giving rise to a great variety of environmental conditions and associated biodiversity (Gadgil et al. 1997). North-east India is dominated by Tibeto-Burman speaking Mongoloid groups (Grierson, 1903, 1909) except the Khasi and its sub-tribes who are Mongoloids but speak dialects of the Austrio-Asiatic linguistic family. As a whole, Tibeto-Burman speakers constitute < 2% of the India's total population (Malhotra and Valusu, 1993). They are assumed to have arrived at different periods from eastern, southern, and central Asian regions (Rapson, 1955; Dani, 1960; Parrat, 2005). Indian tribes are considered as the autochthones of India. But, this situation does not hold true in the context of Manipur because they are considered as late arrivals (Meitei et al., 2010). The Manipur state comprises mainly of two well defined regions i,e, valley and hill, and 29.3 percent of the total population of the State belongs to Schedule Tribes (Sanajaoba 1995). The present study is based on two populations who inhabit the hills of Manipur namely Zeme and Kuki. Both the population belong to Tibeto-Burman speaking linguistic family. It may be mentioned here that few studies (Saraswathy et al. 2009 and Meitei et al. 2010)have been carried out on molecular markers among the populations of Manipur.

Keeping this in view, an attempt has been made to study genomic diversity using five *Alu* InDel markers, with the available linguistic and genetic data. The objective of the study is to assess the extent of heterogeneity of the genetic structure of Zeme and Kuki tribes of Manipur.

2. METHODS

Five *Alu* insertion-deletion polymorphisms were analyzed among 188 individuals belonging to Zeme and Kuki tribal (Zeme=90, and Kuki=98) groups of Manipur speaking Tibeto-Burman languages. The details of the study populations are given in Table 1. 5ml of intravenous blood sample were collected from unrelated individuals by a trained medical practitioner after taking written consent from the subjects. The ethical clearance was obtained from Ethical Committee of the Department of Anthropology, University of Delhi.

Table 1Name of the presently studied population groups, linguistic group, ethnicity, sample sizes and area of sample collection

Sl No.	Population	Sample size	Linguistic group	Ethnic groups	Area of sample collection
1	Zeme	90	Tibeto-Burman, Naga-Bodo subgroup	Mongoloid, tribal group	Tamenglong District
2	Kuki	98	Tibeto-Burman, Kuki-Chin subgroup	Mongoloid, tribal group	Churchandpur, Chandel, Imphal West, and Senapati Districts

2.1. Laboratory analysis

DNA was extracted from each blood sample using salting out method (Miller et al., 1988).Each DNA sample was screened for *Alu* insertion-deletion autosomal markers. The loci studied were ACE (Tirret et al., 1992), APO (Karathanasis, 1985), D1 (Batzer et al., 1996), PV92 (Batzer et al., 1996) and PLAT (Ludwig et al., 1992). The primers and protocols used for typing of the insertion-deletion markers were as given by Stoneking et al., 1997, Majumder et al., 1999, and Watkins et al., 2001. Genotyping was done using polymerase chain reaction, followed by electrophoresis in 2% agarose gel at 100 V. They were viewed in a gel documentation system.

2.2. Statistical analysis

Allele frequencies for each population were obtained using the software POPGENE version 1.31 (Yeh and Yang, 1999). Hardy-Weinberg equilibrium was calculated using the test for chi-square goodness of fit. Average heterozygosities and standard genetic distance (DS) matrix were calculated following Nei (1973). Genomic diversity analysis was calculated using the software DISPAN (Ota, 1993). A regression analysis of heterozygosity on genetic distance (Harpending and Ward, 1982) was carried out to understand the population structure of the tribes under study. A dendrogram was constructed using the neighbour-joining method (Saitou and Nei., 1987) to identify affinities among the neighbouring populations of Manipur.

3. RESULTS

3.1. Allele frequency and Heterozygosity

Allele frequencies for all the five lociamong the Zeme and Kuki tribes of Manipur presented in the Table 2 are found to be polymorphic. When applying the chi-square goodness of fit test to determine whether the phenotype and genotype frequencies depart from Hardy-Weinberg proportions and using Bonferroni's correction, It is observed that the phenotype (genotype) frequencies for all the loci are in reasonable agreement with their respective Hardy-Weinberg expectations and do not show significant departure from expected frequencies except in PV92 among the Kuki tribe. The locus PV92 shows the highest variation between the two populations.

Heterozygosity values for most of the loci are noticeably high among the Kuki population whereas it is noticeably low (APO and PV92 which are below 30%) among the Zeme population. The average heterozygosity for Zeme and Kuki is 0.373 and 0.427 respectively (Table 3).

Table 2 Allele frequencies at 5 autosomal loci among the Zeme and Kuki tribes of Manipur.

Locus	**Zeme**			**Kuki**		
	P[a]	**P[b]**	**2n[c]**	**P[a]**	**P[b]**	**2n[c]**
ACE	0.471	0.150	170	0.566	0.052	196
APO	0.829	0.249	176	0.770	0.965	196
D1	0.217	0.089	152	0.212	0.064	184
PV92	0.835	0.392	152	0.599	0.000	192
PLAT	0.378	0.567	180	0.391	0.528	192

[a] frequency of '+' allele; [b] p-value; [c] number of chromosome tested

Table 3Heterozygosities at individual level and average heterozygosity based on 5 autosomal DNA loci among the Zeme and Kuki tribes of Manipur.

Locus	**Zeme**	**Kuki**
ACE	0.4983	0.4912
APO	0.2828	0.3538
D1	0.3399	0.3341
PV92	0.2749	0.4804
PLAT	0.4701	0.4761
All loci	0.3732	0.4271

3.2. Genomic diversity analysis

The analysis of genomic diversity of the loci among the Zeme and Kuki tribes of Manipur revealed a total average genomic diversity of 40.7% (Table 4). Most of the genomic diversity computed on the basis of 5 loci can be attributed to individual variations within the populations as only 1.7% of the total genetic diversity comes from variation between the populations (G_{ST}).

Table 4 Gene diversity analysis based on 5 autosomal loci among the Zeme and Kuki populations of Manipur

Locus	H_T	H_S	G_{ST}
ACE	0.499316	0.494803	0.009037
APO	0.320599	0.318859	0.005429
D1	0.336979	0.336967	0.000037
PV92	0.405822	0.377974	0.068621
PLAT	0.473320	0.473235	0.000179
All loci	0.407207	0.400368	0.016796

H_TTotal average heterozygosity, $H_S$individual variation within population, G_{ST} degree of genetic variation

3.3. Genetic distance and genetic affinity

Standard genetic distance analysis among the Manipur populations shows that Zeme and Kabui, and Kuki and Meitei are close to each other as shown in Table 5. Overall,Kom shows the maximum distance from the rest of the populations of Manipur. Furthermore, the dendrogram (Figure 1) generated from the genetic distance matrix also revealed that Kuki and Meitei, and Zeme and Kabui are close to each other, while Manipur Muslim and Kom are far apart from the rest of the studied populations.

Table 5 Standard Genetic Distance (DS) matrix among the two present studied tribes and other previously studied populations of Manipur, based on 5 autosomal loci.

	Kuki	**Zeme**	**Kabui**	**Meitei**	**Manipur Muslim**	**Kom**
Kuki	-					
Zeme	0.02219	-				
Kabui	0.03421	0.01377	-			
Meitei	0.01130	0.01493	0.02054	-		
Manipur Muslim	0.02692	0.07505	0.06468	0.02949	-	
Kom	0.07130	0.09251	0.11744	0.06009	0.05578	-

Data on Kabui, Meitei, Manipur Muslim and Kom were obtained from Meitei et al 2010

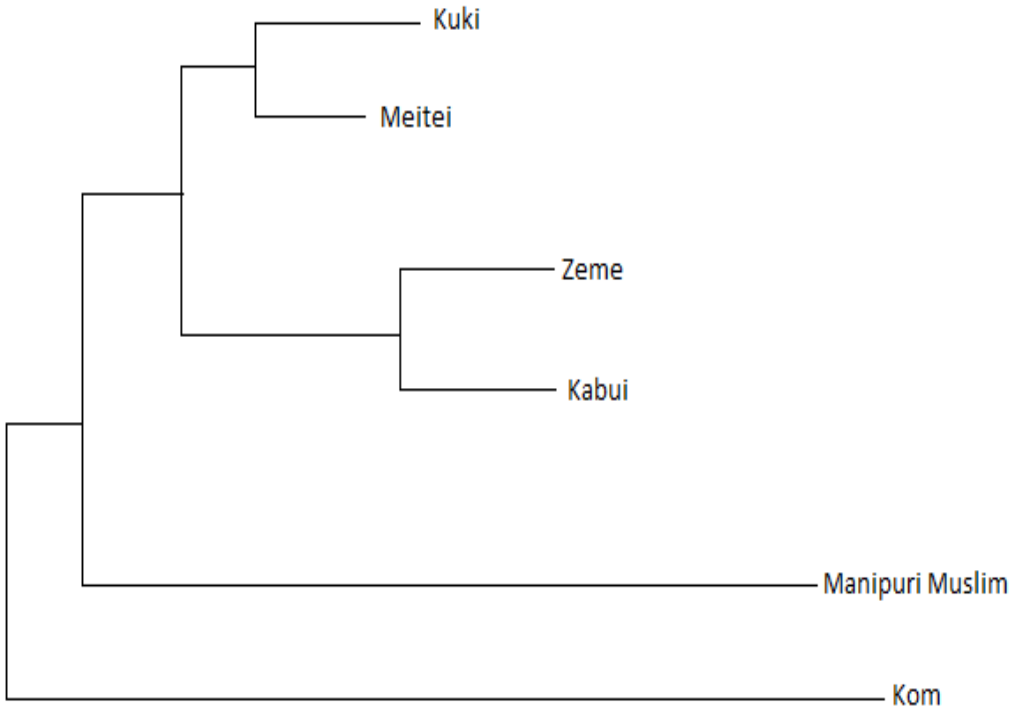

Fig. 1 Genomic affinity among the two present studied tribes and other previously studied populations of Manipur, based on 5 autosomal loci.

3.4. Population structure and gene flow

When the 6 population groups of Manipur (Zeme, Kuki and their neighbouring groups) are pooled, the average heterozygosity in the pooled population (42.43%) does not differ significantly from the regression coefficient of 42.49% in the populations (Table 6), signifying that the populations have received similar proportions of gene flow with the exception of Manipur Muslim and Kom. It appears that differential admixture among them might have resulted in their characteristic genetic structure. Nevertheless, the plot made between the observed heterozygosity and distance from the gene frequency centroid indicates that all the other groups are placed along with the theoretical regression line (Fig 2). By and large, the results imply that the population groups of Manipur are neither overtly admixed nor isolated.

Table 6 Average Heterozygosity (H_i) and Genetic Distances from the centroid (R_{ii}) among the two present studied tribes and also other previously studied populations of Manipur, based on 5 autosomal loci.

Population	**R_{ii} ± SE**	**H_i ± SE**
Zeme	0.037339 ± 0.028128	0.373216 ± 0.046890
Kuki	0.014885 ± 0.001964	0.427110 ± 0.034185
Kabui	0.053061 ± 0.038924	0.387698 ± 0.050620
Meitei	0.012705 ± 0.010939	0.393890 ± 0.063697
Manipur Muslim	0.060784 ± 0.037836	0.431236 ± 0.038950
Kom	0.137652 ± 0.048994	0.407318 ± 0.062983

Regression analysis: $H_i = b\ (1-r_{ii})$; H_i plotted against $1-r_{ii}$ through the origins has t=-1.9130, 4 df, p>0.05. Regression coefficient through origin (b) =0.4249 ± 0.031414. Average heterozygosity in pooled population H=0.424338 ± 0.040079.
(Data on Kabui, Meitei, Manipur Muslim and Kom were obtained from Meitei et al 2010)

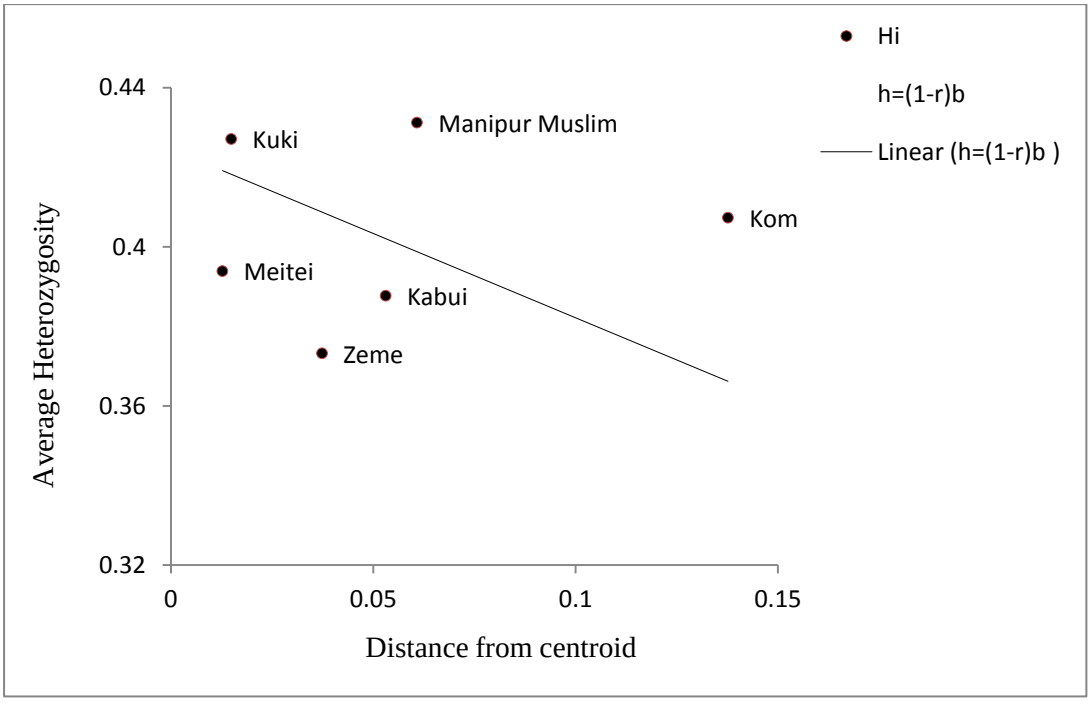

Fig. 2 Gene flow among thetwo present studied tribes and other previously studied populations of Manipur, based on 5 autosomal loci.

4. DISCUSSION

Polymorphisms at the studied loci are comparable to the findings from other studies of Manipur and also to those of the Chinese and Southeast Asian populations (Meitei et al., 2010). The average heterozygosityof Kuki, Muslim and Meitei at the 5 studied loci is higher as compared to other populations of Manipur. However, the genomic diversity in the present study computed on the basis of 5 loci can be attributed to individual variations within the populations as only 1.7% of the total genetic diversity comes from variation between the populations (G_{ST}). Genetic differentiation is found to be lower than that observed for the populations of Manipur but is comparable to the population of western India (Kshatriya et al., 2011). Kshatriya et al. (2011) also pointed out that genetic drift is unlikely to play a significant role in the process of genetic differentiation if the value is small and the population size is large.

The Dendrogramgenerated in Fig 1 and the standard genetic distance (Ds) matrix in Table 5 showing close affinities between Zeme and Kabui is in accordance with the common origin theory given by AZSU (2009). Furthermore, Meiteis are found to be clustering with Kukis (Fig 1).Kukis manifest preponderantly mongoloid ethnicity while Meiteis exhibit non-mongoloid ethnicity. Burling (2003) and Thurgood (2003) pointed out that Manipuri population group share some lexical similarities with the Kuki-Chin languages and Tangkhul due to prolonged contact between these languages. This prolonged contact may not only be in terms of language but also in terms of interexchange of genes. Meiteis are reported to be the autochthones of Manipur (Singh, 2005) and are the products of admixture between the then existing Proto-Australoid stock with the incoming Mongoloid stock (Das, 1960; Saraswathy et al 2009). However, the affinities of Kukis with Meiteis need further investigation by studying more molecular markers along with proper ethnographic account before arriving at valid inferences.

Harpending Ward analysis demonstrates that of all the six groups, Kabui, Meitei and Zemeare less heterogenous as compared to Kuki, Manipur Muslim and Kom. Gene differentiation is small in these groups. Thus, moderate to high diversity with low level of genetic differentiation indicates differential gene flow into the study populations and that these groups are receiving alleles from similar sources. Hence, autosomal DNA markers analysis of the population groups of Manipur point out their proximity with each other with

theexception of Manipur Muslims and Kom. These two groups are more heterogenous and with the low genetic differentiation, the possibility of comparatively greater gene influx in them in shaping their genetic structure cannot be rule out.

Finally, it will not be out of place to emphasize that the composition of genes in the population groups of Manipur are similar to each other and that differential heterozygosity might have led to variable influx of genes into these groups, but with similar sources.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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