

A Genetic Study of the Veddas and the Sinhalese

by

S. B. ELLEPOLA*

Pathologist, General Hospital, Badulla

and

EUGENE R. WIKRAMANAYAKE

Department of Anatomy, University of Peradeniya

SUMMARY. Sixteen polymorphic and 6 monomorphic gene loci have been studied in Veddas from Kandeganwila, Millana and Dimbulagala and in Sinhalese from Badulla, in Sri Lanka.

The genetic identity between the two populations was calculated from frequencies of the phenotypes at each locus, using the formula of Nei. The genetic distance between the two population groups, calculated from these genetic identities, also using Nei's formulae, is approximately 50,000 years. This corresponds to the period in which *Homo sapiens balangodensis* is postulated to have been in existence in Sri Lanka.

It is postulated that the differences in some of the allele frequencies between the two populations date from the late paleolithic and mesolithic periods of Sri Lanka.

INTRODUCTION

There are a number of communities in the world today who live in rather primitive conditions and obtain food by means that were prevalent in the early phase of human development, i. e. hunting, food - gathering, nomadic pastoralism and rudimentary agriculture. Since it is believed that the pressure of evolutionary forces working in conjunction with the changes in life - style have affected the genetic endowments of modern man, genetic studies concerning these primitive people have become a matter of much scientific interest.

Primitive communities of the type mentioned above are found in several parts of the globe: in the Arctic, Asia and Africa, South America and in Australia. Because these communities today seem to face an imminent threat of extinction as a consequence of contact with more advanced societies, studies concerning them have assumed an urgency and an importance quite out of proportion to the relatively small number of people involved³².

Present address : Department of Medicine, University of Peradeniya.

The Veddas of Sri Lanka belong to this category and are being gradually assimilated into the more advanced Sinhala society, a process that will be accelerated with the relocation of the Veddas in System "C" of the Mahaweli Development Scheme. Therefore there is an urgent need to investigate them before their disappearance as an ethnic group.

Most of the Veddas are found in the South Eastern plains of Sri Lanka in the area referred to as Bintenna. The largest groups are those who live in Dambana and Kandeganwila. The Veddas of Dambana are physically close to the Sinhalese and have intermarried with the Sinhalese, while those living in Kandeganwila are more isolated and therefore have less intermarriage with the Sinhalese. These two villages lie on one side of the Mahaweli Ganga. The total number of Veddas in Kandeganwila in 1983 was 400 (Pathirana, personal communication). However, at the time of the study (1972, 1973), there was a total population of 275 at Kandeganwila. The Veddas of Kandeganwila lived as close to nature as possible and practised a very basic kind of agriculture called chena cultivation, essentially a slash - and - burn type of agricultural method. Maize, chilli and a few vegetables are grown on these agricultural plots. The flesh of certain animals like deer, wild boar, ant-eater and langur Monkey was their regular source of animal protein. The meat that is left over is preserved by sun drying. Wild honey also formed an important constituent of their diet.

A second group of Veddas studied lived in the district of Polonnaruwa in the villages of Dimbulagala and Millana and called themselves the Thamankaduwa Veddas. They were studied in 1976 and their total population was one hundred and twenty.

This is a report of a study of 16 polymorphic and 6 monomorphic genetic loci in the Veddas and the Sinhalese. The data has been used to determine the phylogenetic relationship between the two groups.

The alleles studied at each locus and the number of Sinhalese and Veddas investigated for each locus are indicated in Table 1.

A GENETIC STUDY OF THE VEDDAS AND THE SINHALESE

TABLE 1. The alleles studied at each locus and the total number of Veddas and Sinhalese investigated for each locus.

Locus	Alleles	Number investigated Vedda	Sinhalese
ABO	A	54	268
	B		
	O		
Rhesus	D	53	268
	d		
Haptoglobin	Hp ¹	60	146
	Hp ²		
Transferrin	Tf ^C	60	146
	Tf ^D		
HbE	A	54	146
	E		
Acid phosphatase	P ^a	54	146
	P ^b		
Adenosine deaminase	1	54	124
	2		
Adenylate kinase	1	74	148
	2		
Esterase D	1	54	116
	2		
Glutamate oxalo acetate transaminase	1	54	111
	2		
Glutamate pyruvate transaminase	1	54	111
	2		
Isocitrate dehydrogenase	1	54	111
	6		
Phosphogluconate dehydrogenase	A	45	142
	C		
Phosphoglycerate kinase	1	34	74
	2		
Phosphoglucomutase	1	47	146
	2		
Uridine monophosphate kinase	1	34	74
	2		

Monomorphic enzymes		
System	Number investigated	
	Vedda	Sinhalese
2,3-Diphospho glycerate mutase	18	60
Enolase	18	60
Guanyl kinase	18	60
Nucleoside phosphorylase	18	60
Monophospho glycerate mutase	18	60
Phosphoglyceric acid dehydrogenase	18	60

MATERIALS AND METHODS

Selection of samples:

To obviate the criticism that has been made on some of the earlier studies on the Veddas, namely, the lack of attention to the genealogical background in the selection of the Vedda population, the Vedda population of Dambane and Kandeganwila were visited by the study team on six occasions in 1971, 1972, 1973 and 1974 during which they were treated for medical problems such as conjunctivitis, scabies, impetigo, yaws, upper respiratory infections, hay fever and asthma. These visits could be made only during the dry season, from February to July, as the jeep tracks were impassable during the North - East monsoon and the inter - monsoon rains.

By 1973 and 1974 the Veddas of Kandeganwila were willing to co-operate with the team. They were identified by their household numbers, clan names (waruge), individual names, age and sex. The replies given by them were checked with the Vedda chief and the Sinhala school master who taught in the village school at Kandeganwila and a pedigree was drawn for each household. Only those from Vedda matings within their ethnic group during the last three generations were included in this study. Although the samples were from related individuals, for purposes of allele frequency calculations they were treated as being derived from unrelated persons. Allele frequency data collected from closely related individuals are not subject to bias except when collected from a few large families.²⁹

The Sinhala population studied were school children, ages 17 to 19, in the General Certificate of Education (Advanced Level) class at Badulla Maha Vidyalaya. Badulla, being 70 miles from Kandeganwila, was considered sufficiently distant from the plains of Bintenne where the Veddas live. These children were considered to be representative of the Sinhala population of Badulla.

Collection of blood samples

Blood samples were collected by venepuncture using disposable sterile syringes, with heparin as an anticoagulant, and stored in cool containers (thermos flasks containing ice) till they were transported to the pathology laboratory at General Hospital, Badulla. A portion of the blood was retained in the syringes for blood grouping and the balance centrifuged (3000 G, 10 min). Plasma was stored at -20°C for the detection of haptoglobins and transferrins. The red cells were washed three times in normal saline ($0.9 \text{ g NaCl dl}^{-1}$) and lysed according to the method of Dacie and Lewis.⁶ The clear haemoglobin solution was pipetted off into bijou bottles and stored at -20°C .

A total of 70 blood samples was collected from the Veddas of Kandeganwila and 150 samples from the Sinhalese of Badulla in December 1973 and January 1974. The red cells were studied for blood groups and the serum for haptoglobins and transferrins, at Badulla. Haemolysates were taken by one of us (S. B. E.) to the Department of Immunogenetics, King and County Central Blood Bank, Seattle, Washington, USA for red cell enzyme studies.

In 1976, 25 blood samples were collected from the Veddas of Millana and Dimbulagala, who had also been screened genealogically. The red cell enzyme studies on these samples were carried out at the Department of Scientific Services, Singapore, and at Badulla.

The methods used for red cell antigen detection were those of Dacie and Lewis.⁶ Haptoglobins and transferrins were studied by the methods described by Giblett.¹² The separation of the iso-enzymes of red cells were done by starch gel electrophoresis at 4°C for 17 h by the techniques of Harris and Hopkins.¹³

Genetic Similarity and Genetic Distance

The genetic similarity (I_{jk}) per locus was calculated using formula of Nei (1978)²⁰

$$I_{jk} = \frac{\overline{\sum_i q_{ij} q_{ik}}}{(\sum_i q_{ij}^2)(\sum_i q_{ik}^2)^{1/2}}$$

where q_{ij} and q_{ik} are the frequencies of the i^{th} allele at a locus in taxa j and taxa k respectively, and the averages were over all gene loci examined. The genetic distance (D) was calculated by the application of formula of Nei²⁰ :

$$D = -\text{Lg } I$$

and the corrected genetic distance was calculated by the formula of Nei :

$$D_v = \frac{1 - I}{I}$$

The phylogenetic divergence time in years (t) is given by the formula :
 $t = 5 \times 10^6 D$ and the corrected phylogenetic divergence time (t_v) is given by the formula :

$$t_v = 5 \times 10^6 D_v$$

The genetic identity I , of each locus, calculated from the allele frequencies at that locus is indicated below the table giving the results for that locus.

RESULTS

I. Red cell surface antigens

1.1 ABO blood groups

Table 2 shows the distribution of the phenotypes at the ABO locus in the Vedda and the Sinhalese. There is a significant difference in the allele frequencies of A and B ($p < 0.05$) but not of O. The genetic identity between the Vedda and the Sinhalese in respect of A is 0.9899 and of B is 0.9890.

TABLE 2. The distribution of the ABO phenotypes and the allele frequencies among the Veddas and the Sinhalese

Ethnic group	n	Phenotype						Allele frequency				
		A	Freq	B	Freq	AB	Freq	O	Freq	A	B	O
Veddas	54	7	.1296	23	.4259	2	.0370	22	.4074	0.0871	0.2672	0.6383
Sinhalese	268	37	.1387	95	.3523	17	.0637	119	.4413	0.1094	0.2383	0.6523
		I(A) 0.9899		I(B) 0.9890		I(O) 0.9985						

1.2 The Rhesus blood groups.

The distribution of the phenotypes at the Rhesus locus is given in Table 3. There is no significant difference in the allele frequencies of D and d between the Vedda and the Sinhalese. The genetic identity between the two groups is 0.9975

TABLE 3. The distribution of the phenotypes at the Rhesus locus between the Veddas and the Sinhalese

Ethnic group	n	Phenotype				Allele frequency	
		D	Freq	d	Freq	D	d
Veddas	53	51	.9615	2	.03844	0.8039	0.1961
Sinhalese	268	260	.9697	8	.0302	0.8261	0.1739

$$I = 0.9975$$

2. Serum proteins

2.1 Haptoglobins

Table 4 indicates the distribution of the phenotypes for haptoglobins among the populations studied. The phenotypes 1, 2-1 and 2 were seen in the Veddas and the Sinhalese. There is a significant difference in the phenotype frequencies of Hp¹, Hp 2-1, Hp², between the two groups ($p < 0.05$). The genetic identity between the Veddas and Sinhalese is 0.9599.

TABLE 4. The distribution of haptoglobin phenotypes and allele frequencies between the Veddas and the Sinhalese

Ethnic group	n	Phenotype						Allele frequency	
		1-1	Freq	2-1	Freq	2	Freq	Hp ¹	Hp ²
Veddas	60	8	.133	26	.866	26	.866	0.3500	0.6500
Sinhalese	146	2	.136	47	.3216	97	.6643	0.1746	0.8254
$P < 0.05$								$I = 0.9599$	

2.2 Transferrins

Table 5 indicates the distribution of the phenotype of transferrins among the Veddas and the Sinhalese. The phenotypes CC, CD and DD were seen in the Veddas. The phenotype DD was not seen in the Sinhalese. There is a significant difference ($p < 0.05$) in the phenotype frequencies between the two groups. The genetic identity between the Vedda and the Sinhalese is 0.9980.

TABLE 5. The distribution of the transferrin phenotypes and allele frequencies between the Veddas and the Sinhalese.

Ethnic group	n	Phenotype			Allele frequency	
		CC	CD	DD	TfC	TfD
Veddas	60	53	6	1	0.9333	0.666
Sinhalese	146	144	2	0	0.9932	0.0068
$P < 0.05$					$I = 0.9980$	

3. Haemoglobin variants

The only haemoglobin variant that was detected was haemoglobin E. The distribution of phenotypes AA and AE among the Veddas is shown in Table 6. There is a significant difference ($p < 0.05$) in the allele frequency of E between the Veddas and the Sinhalese. The genetic identity between them is 0.9990. Homozygous E was not found in the Vedda or in the Sinhalese in this study.

4.3 Adenylate kinase (AK)

Table 9 shows the distribution of the phenotypes 1, 2-1 and 2 and the allele frequencies of AK¹ and AK². There is no significant difference in the phenotype frequencies between the Veddass and the Sinhalese. The genetic identity is 0.9989.

TABLE 9. The distribution of the phenotypes and allele frequencies of adenylate kinase between the Veddass and the Sinhalese

Ethnic group	n	Phenotype				Allele frequency	
		1-1	Freq	2-1	Freq	AK ¹	AK ²
Veddass	74	60	.8108	14	.1891	0.9054	0.0946
Sinhalese	148	132	.8918	16	.1081	0.9459	0.0540

I = 0.9989

4.4 Esterase D (ESD)

Table 10 shows the distribution of the phenotypes 1, 2-1, and 2 and the allele frequency of Esterase 1 and 2. There is no significant difference in the incidence of the phenotypes between the Vedda and the Sinhalese. Rare variants of csterase D were not seen. I = 0.9642.

TABLE 10. The distribution of the phenotypes and allele frequencies of esterase D between the Veddass and the Sinhalese

Ethnic group	n	Phenotype						Allele frequency	
		1-1	Freq	2-1	Freq	2-2	Freq	1	2
Veddass	54	15	.2777	27	.5000	12	.2222	.05277	0.4722
Sinhalese	116	50	.4310	45	.3879	16	.1379	0.6681	0.3319

I = 0.9642

4.5 Glucose - 6 - phosphate dehydrogenase (Gd)

Table 11 shows the distribution of the phenotype B and the slow variant of B. The phenotype A was not detected in the Veddass or in the Sinhalese.

TABLE 11. The distribution of the phenotypes B and the variant of B of glucose-6-phosphate dehydrogenase and allele frequency of phenotype B between the Veddass and the Sinhalese

Ethnic group	n	Phenotype					
		A	Freq	B	Freq	Variant of B (slow)	Freq
Veddass	51	0	0.0	45	0.8823	6	0.1176
Sinhalese	122	0	0.0	116	0.9508	6	0.0491

I - 1

4.6 *Glutamate oxalo acetate transaminase (GOT)*

also known as Aspartate aminotransferase

Table 12 shows the distribution of the phenotypes 1, 2-1 and 2 as well as the allele frequencies of GOT₁ and GOT₂. There is no significant difference in the phenotype frequency between the Veddas and the Sinhalese. The allele GOT₂ was not found in the Veddas or in the Sinhalese. The genetic identity was equal to 0.9998.

TABLE 12. The distribution of the phenotypes and allele frequencies of GOT₁ and GOT₂

Ethnic group	n	Phenotype						Allele frequency	
		1	Freq	2-1	Freq	2	Freq	1	2
Veddas	54	54	1	0	0	0	0	1	0
Sinhalese	111	109	.9819	2	.0180	0	0	.9819	.0180

I = 0.9998

4.7 *Glutamate pyruvate transaminase (GPT)*

also known as Alanine aminotransferase

Table 13 shows the distribution of the phenotypes 1, 2-1 and 2 and the allele frequencies of GPT₁ and GPT₂. There is a significant difference ($p < 0.05$) in the phenotype frequencies between the Veddas and the Sinhalese. The genetic identity is 0.8974.

TABLE 13. The distribution of the phenotypes and allele frequencies of GPT₁ and GPT₂

Ethnic group	n	Phenotype						Allele frequency	
		1	Freq	2-1	Freq	2	Freq	1	2
Veddas	54	27	.5000	18	.3333	9	.1666	0.6666	0.3334
Sinhalese	111	25	.2252	46	.4144	40	.3603	0.4324	0.5676

I = 0.8974 P < 0.05

4.8 *Isocitrate dehydrogenase (ICD)*

Table 14 shows the distribution of the phenotype 1 and 2-1. No cases of the phenotype 2-1 was seen in the Veddas while only a single Sinhalese was found who had this phenotype. There is no significant difference in the allele frequencies between the Veddas and the Sinhalese. The genetic identity is 1.

TABLE 14. The distribution of the phenotypes and allele frequencies of isocitrate dehydrogenase

Ethnic group	n	Phenotype						Allele frequency	
		1-1	Freq	2-1	Freq	2-2	Freq	1	2
Veddias	54	54	.1	0	0	0	0	1.000	0.000
Sinhalese	111	110	.9909	1	.0090	0	0	0.9914	0.0010

I = 1

4.9 Phosphogluconate dehydrogenase (PGD)

Table 15 shows the distribution of the phenotype AA and AC as well as the allele frequency of PGD^A and PGD^C in the Veddias and the Sinhalese. The difference in the phenotype frequencies of PGD^A and PGD^C between the Veddias and the Sinhalese is not significant.

The genetic identity is 0.9991.

TABLE 15. The distribution of the phenotypes and the allele frequencies of the PGD locus among the Veddias and the Sinhalese

Ethnic group	n	Phenotype				Allele frequency	
		AA	Freq	AC	Freq	PGD ^A	PGD ^C
Veddias	45	40	.8888	5	.1111	0.94444	0.0556
Sinhalese	142	136	.959	6	.0405	0.9840	0.0160

I = 0.9991

4.10 Phosphoglucomutase (PGM)

Table 16 shows the distribution of phenotypes 1, and 2-1 as well as the allele frequencies of PGM₁¹ and PGM₁²⁻¹ in the two groups. The phenotype 2 was not found in the Veddias or the Sinhalese. There was no significant difference in the phenotype frequencies of PGM₁ between the Veddias and the Sinhalese. The genetic identity is 0.9946.

TABLE 16. The distribution of the phenotypes and allele frequencies of PGM among the Veddias and the Sinhalese

Ethnic group	n	Phenotype						Allele frequency	
		1-1	Freq	2-1	Freq	2-2	Freq	PGM ₁ ¹	PGM ₁ ¹⁻²
Veddias	47	26	.5531	21	.4468	0	0	0.7761	0.2239
Sinhalese	148	70	.4729	78	.5270	0	0	0.7360	0.2640

I = 0.9946

4.11 *Phosphoglycerate kinase (PGK)*

Table 17 shows the distribution of the phenotype 1, 2-1 and 2. The phenotype 1 was present in both the Veddas and Sinhalese but not the other phenotypes. The genetic distance is 1.

TABLE 17. The distribution of the phenotypes and allele frequencies of the PGK locus among the Veddas and the Sinhalese

Ethnic group	n	Phenotype						Allele frequency	
		1	Freq	2-1	Freq	2	Freq	Pgk ¹	Pgk ²
Veddas	34	1	1	0	0	0	0	1.0	0.0
Sinhalese	74	1	1	0	0	0	0	1.0	0.0

I = 1

4.12 *Phosphohexose isomerase (PHI)*

Table 18 shows the distribution of the phenotype 1 and 2-1. The phenotype 2-1 was not found in the Veddas but was seen in one Sinhalese. There is no significant difference in the allele frequency between the Veddas and the Sinhalese and the genetic identity is 1.

TABLE 18. The distribution of the phenotypes and allele frequencies of phosphohexose isomerase among the Vedda and the Sinhalese

Ethnic group	n	Phenotype						Allele frequency	
		1	Freq	2-1	Freq	2	Freq	1	2
Veddas	54	54	1	0	0	0	0	1.0	0.0
Sinhalese	111	110	.9999	1	.0090	0	0	0.9954	0.046

I = 1

4.13 *Uridine monophosphate kinase (UMPK)*

Table 19 shows the distribution of the phenotypes 1, 2-1, 2. The phenotype 1 was present in both the Veddas and the Sinhalese but not the other phenotypes. The genetic distance is 1.

TABLE 19. The distribution of phenotypes and allele frequency of UMPK among the Veddas and the Sinhalese

Ethnic group	n	Phenotype						Allele frequency	
		1	Freq	2-1	Freq	2	Freq	1	2
Veddas	34	34	1	0	0	0	0	1.0	0.0
Sinhalese	74	74	1	0	0	0	0	1.0	0.0

1 = 1.0

4.14 Monomorphic red cell enzymes

The following enzymes were also studied in the Veddas and the Sinhalese, and confirmed to be monomorphic. Blood samples from Veddas ($n = 18$) and from the Sinhalese ($n = 60$) were tested at the Department of Immunogenetics, Seattle, USA.

2,3 Diphospho - glycerate mutase

Enolase

Guanylate kinase

Nucleoside phosphorylase

Monophospho glycerate mutase

Phosphoglyceric acid dehydrogenase

DISCUSSION

1. Red cell surface antigens

1.1 ABO blood groups

The allele frequency of group B in the Veddas was higher than in the Sinhalese (Table 2) as has also been demonstrated in 1963.³¹ There was a significant difference between the Veddas and the Sinhalese in respect of the allele frequency of Group A as well, but not in respect of Group O.

1.2 The Rhesus blood group

The Veddas and the Sinhalese have a very high allele frequency for group D and a low frequency for group d. There was no significant difference in the allele frequencies of the Veddas and the Sinhalese (Table 3).

2. Serum proteins

2.1 Haptoglobins (Hp)

All three phenotypes 1, 2-1, 2 were seen in the Veddas and the Sinhalese (Table 4). The difference in the allele frequencies of Hp¹ and Hp² is significant ($p < 0.05$). No case of anhaptoalbuminaemia (Hp⁰) was seen in either of the two groups although its presence in the Sinhalese has been reported.²⁴ Hp⁰ is however not considered to be inherited,³ but to be a common feature in patients with increased red cell destruction (as in haemolytic anaemia). Further, the genetically determined phenotypes Hp₁ and Hp₂ are often masked by haemolysis due to the utilization of all free haptoglobin available to combine with free haemoglobin. Therefore in areas where there is haemolytic anaemia due to malaria or due to the use of anti-malarial drugs, anhaptoalbuminaemia might be present.

The allele frequency in this series of Hp¹ and Hp² in the Sinhalese was similar to the values reported by Kirk and Lai¹⁵ and by Papiha.²⁴

2.2 Transferrins

There was a significant difference in the allele frequency of Tf alleles between the Veddas and the Sinhalese (Table 5). The presence of Tf^C and Tf^D have been reported in the Veddas.¹⁵ Tf^D found in the Veddas is considered to be the Chinese variant, as Tf^D is found in the mongoloid populations, the allele having gained entry to Sri Lanka along with traders from Indonesia.¹⁵

3. Haemoglobin variants

There was a significant difference in the allele frequency of haemoglobin E between the Sinhalese and the Veddas (Table 6).

Haemoglobin E is the commonest type of abnormal haemoglobin reported in Sri Lanka.^{4, 10, 11, 18, 24} The incidence of HbE in Sri Lanka among all communities is very low. According to Blackwell *et al.*⁴ it is 0.0021. The allele frequency of haemoglobin E in the present study of Sinhalese is 0.0068 and is similar to that found by Papiha.²⁴

Blackwell's study was confined to people living in an area where the incidence of malaria is low, namely, the Western Province. On the other hand, the present study and that of Papiha²⁴ involved persons living in Badulla and Anuradhapura, districts in which the incidence of malaria is high. It is therefore possible that the allele for Hb E is high in these areas because Hb E may give some protection against malaria.¹⁷

Haemoglobin E is present in the Bengalis but has not been detected in the other states on the Eastern side of India.³ This is one of the reasons for considering the Sinhalese to be of Bengali origin.¹⁹

4. Red cell enzymes

4.1 Acid phosphatase (AcP)

There is a significant difference ($p < 0.05$) in the allele frequency of p^a and p_b between the Veddas and the Sinhalese (Table 7). The main phenotype of the Vedita was the heterozygote BA while in the Sinhalese it was the homozygous phenotype BB. The allele p^c was not detected in this study but has been reported to be present in the Sinhalese with an allele frequency of 0.13.²⁶ The allele p^c is considered to be a Caucasian character.¹²

4.2 Adenosine deaminase (ADA)

The allele frequency of ADA¹ tends to be high while that of ADA² is low throughout the world.¹⁴ There was no significant difference in the allele frequencies between the Veddas and the Sinhalese. The allele frequencies of ADA¹ and of ADA² of the Sinhalese in this series is similar to those of Roberts *et al.*,²⁵ who reported values of 0.857 and 0.143 respectively (Table 8).

4.3 Adenylate kinase (AK)

The allele frequencies of AK¹ and AK² among the Sinhalese are 0.95 and 0.50, respectively (Table 9), as against values of 0.91 and 0.083 obtained by Roberts *et al.*²⁶ High values for AK was found in the Bengalis and other Indians and it was suggested that there may be a focus of high frequency for AK¹ in India which could be due to natural selection.¹ This is another reason for considering the Sinhalese to be of N-Indian origin.

There is no significant difference in the allele frequencies of AK¹ and AK² between the Veddas and the Sinhalese.

4.4 Esterase D (ESD)

Esterase D is a more recently described polymorphic enzyme system with two common alleles ESD¹ and ESD². Most of the world's population have a high allele frequency for ESD¹.

There is no significant difference in the ESD allele frequencies between the Veddas and the Sinhalese and no variants have been found in either population in this study (Table 10).

4.5 *Glucose - 6 - phosphate dehydrogenase (G6PD)*

The electrophoretically fast moving G6PD isoenzyme type A was not seen in the Veddas or in the Sinhalese. They both have the isoenzyme type B and a slow variant of B (Table 11). Further studies would be required to identify this variant. A slow variant of B has also been recognized in Bengal².

4.6 *Glutamate oxaloacetate transaminase(GOT)*

There was no significant difference in the allele frequency of GOT¹ among Veddas and the Sinhalese (Table 12). The allele GOT², though present, is very rare in the Sinhalese. It is absent in the Vedda.

4.7 *Glutamate pyruvate transaminase (GPT)*

There is a significant difference in the allele frequency of GPT¹ and GPT² between the Veddas and the Sinhalese.

4.8 *Isocitrate dehydrogenase (ICD)*

Both the Veddas and the Sinhalese showed a similar allele frequency for the phenotype 1 - 1 (Table 14). None of the Veddas and only one Sinhalese possessed the phenotype 2 - 1.

4.9 *Peptidase*

Low levels of Peptidase A were noted in both the Veddas and the Sinhalese. A reduced level of peptidase activity is considered to be due to a silent allele. However, peptidase A activity is also dependent on the time taken in the laboratory to make the erythrocyte haemolysates. Therefore it is not possible to draw conclusions from the reduced levels which were noticed in this study.

4.10 *Phosphogluconate dehydrogenase (PGD)*

The allele frequencies of PGD^A and PGD^C in the Veddas are not significantly different from that of the Sinhalese (Table 15).

The allele frequencies of PGD^A and PGD^C in the Sinhalese are similar to the values of 0.0984 and 0.016 obtained in an earlier study.²⁵

4.11 *Phosphoglycerate kinase (Pgk)*

Phosphoglycerate kinase is a polymorphic enzyme which has a rare variant sporadically distributed in different geographic areas of the world. The Veddas and the Sinhalese are monomorphic for the allele Pgk¹ (Table 17).

4.12 *Phosphohexose isomerase (PHI)*

The allele PHI¹ has the highest allele frequency in the world. The allele frequencies in the Vedda and the Sinhalese are very similar (Table 18).

4.13 *Phosphoglucomutase (PGM)*

There is no significant difference in the allele frequencies of the Veddass and the Sinhalese (Table 16).

The allele frequencies for PGM¹ and PGM² in the Sinhalese are similar to the values of 0.75 and 0.26 respectively obtained by earlier studies.²⁶ The Veddass and the Sinhalese are monomorphic for the PGM² locus 2.

4.14 *Uridine monophosphate kinase (UMPCK)*

Only UMPCK¹ could be detected in the Veddass and the Sinhalese (Table 19). UMPCK² has been detected in the Europeans and the Japanese.²² It is labile and has a tendency to disappear from aged haemolysates.

5. A comparison of allele frequency of the Veddass and the Sinhalese

There is no significant difference in the allele frequencies between the Veddass and the Sinhalese in respect of 14 alleles, namely, Blood group O, Rhesus D and d, Haemoglobin A, ADA, AK, Esterase D, GOT, ICD, PGD, PGK, PGM, PHI and UMPCK as well as the monomorphic enzymes.

There was a significant difference in the phenotype frequency in respect of seven alleles, viz. blood groups A and B, haptoglobins, transferrins, haemoglobin E, ACP, GPT.

Table 20 summarises the allele frequency and the genetic identity between the Veddass and the Sinhalese in respect of the polymorphic loci and Table 21 lists the monomorphic loci of the Veddass and the Sinhalese which were also studied.

TABLE 20. The allele frequency and genetic identity of the Veddas and the Sinhalese in respect of nineteen loci

Number	Locus	Allele	Allele Frequency of Veddas	Allele frequency of Sinhalese	Genetic Identity
1	ABO	A	0.0871	0.1094	0.9899
		B	0.2672	0.2383	0.9890
		O	0.6383	0.6523	0.9985
2	Rh	D	0.8039	0.8261	0.9975
		d	0.1961	0.1739	
3	Hp	1	0.3500	0.1746	0.9599
		2	0.6500	0.8254	
4	Tf	C	0.9333	0.9932	0.9980
		D	0.0666	0.0068	
5	Hb F	A	0.9508	0.9931	0.9990
		E	0.0492	0.0068	
		Pa	0.4167	0.2770	
6	ACP	Pb	0.5873	0.7229	0.9688
		1	0.9443	0.8870	
7	ADA	2	0.0556	0.1130	0.9978
		1	0.90554	0.9459	
8	AK	2	0.0946	0.0540	0.9989
		1	0.5277	0.6681	
9	Est. D	2	0.4722	0.3319	0.9642
		B	0.8823	0.9508	
10	G 6PD	Slow Variant of B	0.1176	0.0491	1
		1	1.0	0.9819	
11	GOT	2	0.0	0.0180	0.9998
		1	0.6666	0.4324	
12	GPT	2	0.3334	0.5676	0.8974
		1	1.000	0.9914	
13	ICD	2	0.000	0.0010	1.0
		A	0.9444	0.9840	
14	6PGD	C	0.0566	0.0160	0.9991
		1	1.0	1.0	
15	PGK	2	0.0	0.0	1.0
		1	0.7761	0.7360	
16	PGM ₁	2	0.2239	0.2640	0.9946
		1	1.0	0.9954	
17	PHI	2	0.0	0.0046	1.0
		1	1.0	1.0	
18	UMPK	2	0.0	0.0	1.0

TABLE 21. The monomorphic loci of the Veddas and Sinhalese in respect of six red cell enzymes and the genetic identity

	No. of Veddas	No. of Sinhalese	Genetic Identity
1. 2:3 Diphosphoglycerate mutase	18	60	1
2. Enolase	18	60	1
3. Guanyl kinase	18	60	1
4. Monophosphoglycerate mutase	18	60	1
5. Nucleoside phosphorylase	18	60	1
6. Phosphoglyceric acid dehydrogenase	18	60	1
No. of alleles studied	= 36		
Mean genetic identity (mean genetic similarity)	=		0.9901967

Using Nei's formula²⁰ the mean genetic identity between the Veddas and the Sinhalese is found to be 0.9901967. The genetic distance is 9.851668×10^{-3} . The phylogenetic divergence time (tv) is 49,258 (years).

There is a suggestion that the above formula over-estimates the minimum divergence time by a factor of four.²² If this be correct, the actual separation time between the Vedda and the Sinhalese will be reduced to approximately 12,000 years. It is therefore interesting to compare this figure with results of morphological studies on the Veddas.

6. Morphological studies on the Veddas

Most authorities agree that the Veddas have existed in Sri Lanka longer than the Sinhalese. Parker²³ identifies the Yakas, mentioned in the Mahavamsa, as the progenitors of the Veddas. Sarasin and Sarasin,²⁷ Pole²⁵ and the Seligmans²⁸ demonstrated the presence of prehistoric remains in Sri Lanka. The Sarasins²⁷ were of the view that the lithic remains were representative of the direct ancestors of the Veddas, and Wayland³⁰ assigned the artefacts found in Sri Lanka to a cultural phase falling between the palcolithic and the mesolithic periods.

The first scientific evidence of the presence of mesolithic man in Sri Lanka was the discovery of a frontal bone at Ravanella in 1946, followed by the excavation of 12 skeletons at Bellan Bandi Pellessa in 1956 and 1961.^{7,8} Deraniyagala named this man *Homo sapiens balangodensis* or "Balangoda Man".⁷

Homo sapiens balangodensis had features which Deraniyagala considered to be Proto-Australoid as they bore a strong physical resemblance to the Aborigines of Australia and the Dani of New Guinea. He used stone implements of the paleolithic and mesolithic periods.

Deraniyagala⁸ postulated that *Homo sapiens balangodensis* was ancestral to the Veddas, and classified the Veddas into Australoid and Negroid types.

Kennedy¹⁴ made a detailed comparison of the remains of the "Balangoda Man" with the Vedda skeletal remains found in the museums of Europe, India and Sri Lanka, using morphometric, radiographic and chromatographic techniques. He found osteological features in the Veddas which occurred in a relatively high frequency in the skeletons of the "Balangoda Man" and concluded that "it would be difficult to explain the presence of these unique features in the Vedda without recognising the biological affinity of the Veddas with the late Stone Age people of Sri Lanka."

It is therefore possible that the differences in allele frequencies between the Veddas and the Sinhalese have been derived through the years from the "Balangoda Man" who lived during the late paleolithic and mesolithic periods of Sri Lanka, which is now estimated to be about 30,000 years (K.A.R. Kennedy, personal communication).

ACKNOWLEDGEMENTS

We wish to thank Prof. T. W. Wikramanayake and Dr. R. Sri Pathmanathan for their assistance as members of the team that investigated the Veddas, and Prof. H. Giblett and J. Anderson of the Department of Immunogenetics, King County Central Blood Bank, Seattle, for introducing one of us to the techniques of enzyme electrophoresis and permitting the use of the facilities in their laboratories.

We also wish to thank T. B. Meedeniya, V. Somasundaram, S. Sivasubramaniam of General Hospital, Badulla, and the technical staff at the Department of Scientific Services, Singapore, for their technical assistance.

The data published in this paper formed part of a thesis submitted by one of us (S. B. Ellepola) to the Postgraduate Institute of Medicine of the University of Colombo for the award of the degree of Doctor of Medicine (Pathology) in 1986.

REFERENCES

1. ANANDAKRISHNAN, R. (1972). Further studies on the distribution of serum proteins and enzyme groups in S. India. *Humangenetik* 15, 172-276.
2. AZEVEDO, E., KIRKMAN, H. N., MORROW A. C. and MOTULSKY, A. (1968). Variants of red cell glucose six phosphate dehydrogenase among Asiatic Indians. *Annals of Human Genetics* 31, 373-378.
3. BARNICOT, N. A., KRIMBAS, D., CONNELL, M. C., BRAVEN, G. H. (1955). A genetic survey of Sphokia, Crete. *Human Biology*, 37, (3) 274-298.

4. BLACEWILL, R. O., SILVA, W. A. S., WARNASURIYA, N., SOYSA, P. E., NAGARATNAM, N., ABBYRATNA, D. D., OREGAN, N. D. and WONG, F. (1980). Structural identification of haemoglobin E in ethnic groups from Sri Lanka - Personal communication.
5. CHATTERJEE, J. P. (1966). Haemoglobinopathies, Glucose six phosphate dehydrogenase deficiency and allied problems in the Indian subcontinent. *Bulletin of the World Health Organization* 35, 837-856.
6. DACEY, J. V. and LEWIS, S. M. (1968). *Practical Haematology*. London: Churchill-Livingstone.
7. DERANIYAGALA, P. E. P. (1958). Open air habitation site of *Homo sapiens balangodensis*. *Spolia Zeylanica* 30, (1), 87-110.
8. DERANIYAGALA, P. E. P. (1963). The Hybridization of the Veddas with Sinhalese. *Spolia Zeylanica* 30, (1), 111-145.
9. DERANIYAGALA, S. U. (1980). Pre-historic research in Sri Lanka. P. E. P. Deraniyagala Commemoration Volume 152-207. Colombo: Lake House Investment Ltd.
10. DE SILVA, C. C., JONKIS, J. H. P. and WICKRAMASINGHE, R. I. (1959). *Abnormal Haemoglobins*, p. 340. Oxford: Blackwell Scientific Publications.
11. ELLIPOOLA, S. B., BEAVEN, G. H., GUNASEKERA, L. S. and ARUNANANDAN, P. (1980). Haemoglobin E-Trait, Haemoglobin E-Thalassemia in Sri Lanka. *Ceylon Medical Journal* 25, 29-34.
12. GOWLES, C. G. (1969). *Genetic Markers in Human Blood*, pp. 64-127. 426-537. Oxford: Blackwell Scientific Publications.
13. HARRIS, H. and HOPKINS, D. A. (1976). *Handbook of Enzyme Electrophoresis in Human Genetics* Chapters 1,2,3,4. Amsterdam: Elsevier-North Holland.
14. KENNEDY, K. A. R. (1972). The concept of the Vedda phenotype pattern. A critical analysis of research on the osteological collection of a remnant population. *Spolia Zeylanica*, 32, (1), 25-60.
15. KIRK, R. L. and LAI, L. Y. L. (1961). The distribution of haptoglobin and transferrin groups of South East Asia. *Acta Genetica* 11, 97-105.
16. KIRK, R. L., LAI, Y., VOS, G. H., WICKRAMASINGHE, R. L. and PERERA, D. J. B. (1962). Blood and serum groups of selected populations in South India and Ceylon. *American Journal of Physical Anthropology* 20, (4), 458-397.
17. MOTULSKY, A. G. (1964). Hereditary red cell traits and malaria. *American Journal of Tropical Medicine and Hygiene* 13, 147.
18. NAGARATNAM, N., WICKRAMASINGHE, R. L., JAYAWICKRAMA, U. S. and MAHESAN, V. S. (1958). Haemoglobin E syndromes in a Ceylonese Family. *British Medical Journal* 866-868.
19. NAGARATNAM, N., SILVA, M. and ATAPATTU, A. (1975). Personal communication.
20. NEI, M. (1978). The theory of genetic distance and evolution of human races. *Japan Journal of Human Genetics* 23, 241-369.
21. OMOTO, K. and BLAKE, N. M. (1972). Distribution of genetic variants of erythrocyte phosphoglycerate kinase (PGK) and phosphohexos: isomerase (PHI) among some population groups in South East Asia and Oceania. *Annals of Human Genetics*, London, 36, 61-67.
22. OMOTO, K. (1980). Genetic variants of red cell enzymes as potential anthropological markers. *Haemoglobin* 4, 755-769.
23. PARKER, HENRY (1909). *Ancient Ceylon* 3, 3-112. London: Luzak and Co.
24. PAPIHA, S. S. (1973). Haptoglobins and abnormal haemoglobin types in Sinhalese and Punjabis. *Human Heredity* 23, 147-153.
25. POLP, J. (1907). A few remarks on Prehistoric Stones in Ceylon. *Journal of the Royal Asiatic Society (Ceylon)* 19(58), 272-281.
26. ROBERTS, P. F., PAPIHA, S. S. and ABBYRATNA, K. P. (1972). Red cell enzyme polymorphism in Ceylon Sinhalese. *American Journal of Human Genetics* 24, 181-188.
27. SARASIN, P. and P. SARASIN (1907). Outline of 2 years scientific researches in Ceylon. *Journal of the Ceylon Branch of Royal Asiatic Society* 9, 32, 289-305.
28. SELIGMANN, C. G., and SELIGMANN, B. Z. (1969). *The Veddas*. Anthropological Publications. Netherlands: Oosterhout. N. S.
29. SMITH, C. A. B., CHERRIENT, R. and SINISCALO, M. (1955). The estimates of gene frequencies in a random mating population. *Annals of Human Genetics*. London. 20, 97-115.
30. WAVLAND, E. J. (1914). Paleolithic remains at Kosgalla Estate, Ratnapura District. *Journal of the Royal Asiatic Society (Ceylon)* 23, 117-119.
31. WICKRAMASINGHE, R. L., IRIN, E. W., MOURANT, A. E. and LERMAN, H. (1963). Blood groups and haemoglobins of the Veddas of Ceylon. *Journal of the Royal Anthropological Institute* 93, 1, 117-125.
32. WHO (1958). Technical Report Series 387. Research on Human Population Genetics. Geneva: WHO.