The Distribution of The Sickle Cell Gene in Liberia

FRANK B. LIVINGSTONE

Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan

WHEN ALLISON (1954) first advanced the hypothesis that the high frequencies of the sickle cell gene were to be explained by a balanced polymorphism in which the heterozygotes for the sickle cell gene possessed a relative immunity to falciparum malaria, he attempted to show that the tribal frequencies of the sickle cell trait in East Africa were all in agreement with this hypothesis. His own investigations among 35 tribes showed a remarkable correlation between the frequency of the sickle cell trait and the intensity of malaria. All tribes with relatively high frequencies of the sickle cell trait inhabited areas in which malaria was hyperendemic, while the tribes with relatively low frequencies inhabited areas in which malaria was either absent or not transmitted for several months of the year. Although at that time data on the frequency of the sickle cell trait in West Africa were very fragmentary, Allison (1954) indicated that, insofar as known, they were in agreement with his hypothesis. More recently Allison (1957) has shown that the presence of the gene responsible for hemoglobin C, which is an allele of the sickle cell gene, complicates the distribution of the sickle cell gene in West Africa. In populations with high frequencies of the hemoglobin C gene, there tend to be low frequencies of the sickle cell gene.

It will be the purpose of this communication to present data on the frequency of the sickle cell trait in Liberia, a region of West Africa previously unstudied in this respect. The finding of a relatively low frequency of the sickle cell trait here, in an intensely malarious region, indicates that factors other than malaria pressure must be invoked to explain the present-day distribution of the sickle cell gene in Liberia —the nature of these factors will be explored briefly.

MATERIALS AND METHODS

The following data on the distribution of the sickle cell trait in Liberia were collected during ten months of field work, extending from September 1955 to August 1956. Most of the investigation was carried out at the Liberian Institute of Tropical Medicine, which is located near the seacoast about 40 miles southeast of Monrovia, the capital of Liberia. It is adjacent to the Firestone Rubber Plantation, which employs about 20,000 natives, among whom most of the tribes of Liberia are represented. The majority of the subjects tested were these workers or their families, but trips were also made to other parts of Liberia in order to test tribes which were poorly represented at the Firestone Plantation.

On most of these surveys sickle cell preparations were made and examined by the author in the field, but also included are the results of a survey on which blood was collected in 10 cc. "venules" containing oxalate and air-shipped to the Child Research

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Center, Detroit, Michigan, where a variety of studies were performed on the blood. Some of the results of the latter survey are reported in Neel *et al.* (1956).

Approximately 300 of the bloods which were sent to the United States for testing were collected by Dr. Marvin D. Solomon in the vicinity of Monrovia; I am indebted to him for permission to include the findings on these bloods in this paper.

The technique used for the determination of sickling in the field was the rapid sodium metabisulfite method. Experimental results with the various techniques fully coincided with Allison's (1954) remarks and recommendations on this subject. The presence of sickle-type hemoglobin (hemoglobin S) in the bloods shipped to the Child Research Center was ascertained by paper electrophoresis and solubility determinations. These techniques are given in detail in Zuelzer, Neel, and Robinson (1956). In order to make the results of the two surveys comparable, those individuals in the paper electrophoresis survey who would have been positive for the sickle cell test were counted as positive without regard to whether they appeared to be homozygous or heterozygous for the sickle cell gene. Thus, the frequency of the sickle cell trait, as used in this paper, includes both heterozygotes and any living homozygotes for the sickle cell gene. However, since recent studies (Lehmann and Raper, 1956) indicate that homozygotes for the sickle cell gene very rarely survive the first few years of life, it is very unlikely that the results of the surveys described in this paper include more than a few homozygotes—if any at all.

Subjects for testing were obtained in several ways. The great majority was obtained either from surveys of Firestone rubber tappers done at the weekly rice issue, from outpatient clinics, or from surveys of schoolchildren. The outpatient clinics and schools were located both around Firestone and in the interior. Strictly speaking, none of these methods of selection provides a random sample of the population. Moreover, some known relatives, and most likely some unknown ones, are included. In an effort to control the number of relatives in the tribal estimates of the frequency of the sickle cell trait, many surveys of small villages in which the majority of the inhabitants were known to be related have been omitted. The number of relatives in the remaining samples is not considered to be large enough to have any appreciable effect on the estimates of the frequency of the sickle cell trait.

Although on many surveys additional information was obtained, tribe, sex, and age were recorded for all subjects. Knowledge of age is exceedingly variable in Liberia, with the majority of adult subjects having no reliable knowledge about their exact age in years. Schoolchildren, however, usually had some information, or in some cases the school authorities knew the approximate ages of their pupils. No special techniques were used for the determination of age, so that the age groups are only approximate with a great deal of overlap between them.

Most of the natives of Liberia speak some English, and when asked their tribe, would respond with one of the tribal names used in this paper. In cases where the tribes of the parents were different and only one name was given in response, it was invariably the tribe of the father. The subjects were not routinely questioned on the tribes of their mother and father; thus it is not known what percentage of the subjects are the result of inter-tribal matings. For the adult subjects, this is most likely a very small percentage. Subjects whose parents were known to belong to different

tribes were included in the tribe of the father. Gene flow is undoubtedly occurring across tribal boundaries in Liberia and these subjects would only be instances of this gene flow.

Except for the peoples who inhabit the southeastern hinterland, the tribes of Liberia are well known. For the areas other than southeastern Liberia the tribal list used in this paper agrees with that of Schwab (1947), except that Schwab includes two additional tribes. These two tribal names were never given to the author in response to questions concerning tribal affiliations. Among authors who have written about the tribes of Liberia, there is considerable disagreement as to the tribes of the interior of southeastern Liberia. This is due mostly to the author's different opinions as to what local group fits the definition of a tribe. In this paper the peoples of this area have been grouped into two large linguistic units, Krahn and Webbo. Although these two groupings, undoubtedly contain several breeding isolates, it is highly unlikely that these isolates differ considerably in the frequency of the sickle cell gene, since the gene is almost completely absent in these peoples.

RESULTS

Table 1 gives the frequency of the sickle cell trait in the various populations of Liberia. With the exception of the Americo-Liberian, Mandingo, and Fanti, the populations are all indigenous peoples whose ancestors were living in Liberia at the time of European contact. It should be noted, however, that European contact was very late in some areas, as late as the 20th century in the hinterland of southeastern Liberia. The Americo-Liberians are the descendants of slaves who were repatriated

TABLE 1. THE FREQUENCY OF THE SICKLE CELL TRAIT IN THE POPULATIONS OF LIBERIA

Population	Number examined	Number positive	Sickle cell trait (%)
Americo-Liberian	106	17	16.2
Mandingo	38	7	18.4
Fanti	60	20	33.3
Kissi	298	58	19.5
Mende	77	13	16.9
Gbandi	352	54	15.3
Vai	93	13	14.0
Kpelle	982	128	13.0
Loma	511	65	12.7
Gola	183	22	12.0
Belle	29	3	10.6
Bassa	811	58	7.2
Dei	53	2	3.8
Mano	709	15	2.1
Gio	428	9	2.1
Grebo	69	1	1.4
Kru	148	1	0.7
Krahn	154	1	0.7
Webbo	77	0	0.0
Total (Kissi-Webbo)	4974	443	8.9

from the United States and are mostly scattered along the coast in the larger towns. The Mandingos are rather recent arrivals from French West Africa. Most of them are engaged in commerce and their settlements are located in the larger commercial centers in Liberia, both on the coast and in the interior. The Fantis are fishermen from Ghana. In recent years they have extended their fishing activities to many other countries in West Africa. This sample was obtained from the small Fanti village at Marshall, Liberia.

Prior to combining the data into tribal frequencies, an attempt was made to determine whether sex, age, or method of sampling had any effect on the frequency of the sickle cell trait. Tables 2–4 give the tribal frequencies according to sex, age, and method of sampling, the chi-squares for the differences in frequency for each tribe, and a total chi-square for each table. The five tribes which are shown in tables 3 and 4 are the only ones for which there was a sufficiently large sample for all the age groups and all the methods of sampling to warrant subdivision. The only chi-square which is significant in these tables is that for the frequencies of the sickle cell

TABLE 2. THE FREQUENCY OF THE SICKLE CELL TRAIT IN THE TRIBES OF LIBERIA ACCORDING TO SEX

Tribe	Males			Females			Chi-square*
	No. Exam.	No. Pos.	Freq.	No. Exam.	No. Pos.	Freq.	
Bassa	617	47	.076	194	11	.057	.843
Kpelle	841	111	.132	141	17	.121	.124
Loma	439	58	.132	72	7	.097	.679
Gbandi	279	39	.140	73	15	.206	1.923
Kissi	250	50	.200	48	8	.167	.265
Mano	564	14	.026	145	1	.007	1.790
Gio	375	8	.021	53	1	.019	.014
Gola	128	15	.117	55	7	.127	.037
Vai	54	8	.148	39	5	.128	.075
Dei	40	1	.025	13	1	.075	.729
Mende	70	11	.157	7	2	.286	.750

Total chi-square = 7.229, 11 D.F., $.7 < P < .8$.
* With one degree of freedom P = .05 at $\chi^2 = 3.841$.

TABLE 3. THE FREQUENCY OF THE SICKLE CELL TRAIT IN FIVE LIBERIAN TRIBES ACCORDING TO AGE

Age Group	Bassa		Kpelle		Loma		Gbandi		Kissi	
	No. Exam.	Freq.	No. Exam.	Freq.	No. Exam.	Freq.	No. Exam.	Freq.	No. Exam.	Freq.
0–5	67	.045	35	.114	30	.100	39	.128	16	.000
6–10	122	.066	80	.138	45	.089	11	.182	16	.250
11–15	119	.059	77	.221	52	.135	42	.214	19	.316
16–	503	.080	790	.122	384	.133	260	.146	247	.194
Total	811	.072	982	.130	511	.127	352	.153	298	.195
Chi-square*	1.564		6.256		.924		1.581		5.962	

Total chi-square = 16.287, 15 D.F., $.3 < P < .5$
* With three degrees of freedom P = .05 at $\chi^2 = 7.815$

TABLE 4. THE FREQUENCY OF THE SICKLE CELL TRAIT IN FIVE LIBERIAN TRIBES ACCORDING TO THE METHOD OF SAMPLING

Survey	Tribe									
	Bassa		Kpelle		Loma		Gbandi		Kissi	
	No. Exam.	Freq.	No. Exam.	Freq.	No. Exam.	Freq.	No. Exam.	Freq.	No. Exam.	Freq.
Bloods examined in Detroit (mostly outpatients)	201	.085	214	.094	159	.151	133	.135	106	.198
Firestone Rubber tappers	152	.072	473	.125	168	.125	127	.165	133	.203
Tappers' dependents	44	.000	103	.117	45	.067	11	.182	20	.150
Schoolchildren	263	.072	189	.196	123	.114	22	.227	19	.158
Lib. Inst. Trop. Med. Clinic	150	.080	3	.000	8	.375	—	—	4	.250
Total	810	.073	982	.130	503	.129	293	.157	282	.195
Chi-square*	4.018		10.566		6.816		1.412†		0.572	

Total Chi-square = 23.384, 19 D.F., $.2 < P < .3$

* With four degrees of freedom $P = .05$ at $\chi^2 = 9.488$.

† Three degrees of freedom; $.8 > P > .7$.

trait for the Kpelle tribe according to the method of sampling. The relatively high frequency for schoolchildren has made this test significant. Since the schoolchildren frequencies for the other tribes do not seem to be consistently higher than the other frequencies, and since the total chi-square for table 4 is not significant, this is most likely a random deviation. Thus, the schoolchildren have been included in the total Kpelle sample, although there does seem to be some heterogeneity in the sample.

The primary reason for the test shown on table 4 was to determine whether outpatient surveys, involving subjects with some type of illness, would yield results very different in the frequency of the sickle cell trait from those obtained from surveys of complete villages or of workers. In table 4 it can be seen that the results of surveys of outpatients are similar to the results of surveys of tappers and are not consistently higher or lower. Thus, there is no indication from the test that the different methods of sampling would result in different estimates of the tribal frequencies.

Although these statistical tests indicate that the effects of sex, age, and method of sampling are not large enough to influence significantly the tribal frequencies of the sickle cell trait, the possibility still remains that some of the tribes are composed of several breeding isolates which differ significantly from one another in the frequency of the sickle cell trait. Figure 1 shows the distribution of the native tribes of Liberia and their observed frequencies of the sickle cell trait. For southeastern Liberia the frequencies of the sickle cell trait are all very close to zero, so that significant differences between breeding isolates could not exist here. In northwestern Liberia the tribal frequencies are very close to one another, so that differences in frequency within any one tribe would most likely be small. In central Liberia, however, there

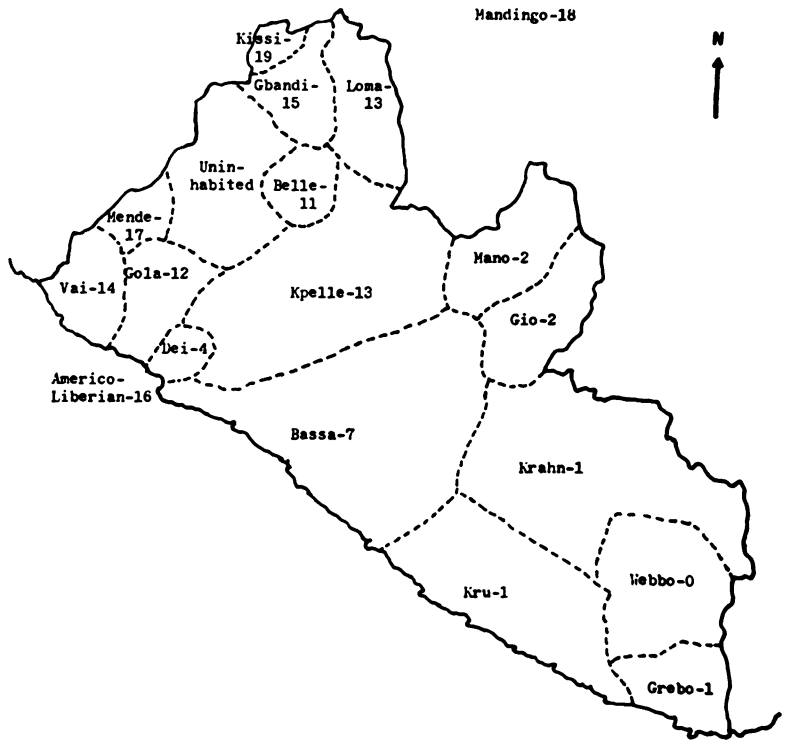

FIG. 1. Percentage incidence of the sickle cell trait in the tribes of Liberia.

are several tribes which border on other tribes with very different frequencies of the sickle cell trait. Some attempt was made to sample the sub-areas of these tribes, but the samples were all rather small. No significant differences were found, although there appeared to be some heterogeneity in the Bassa tribe. Further work on this aspect of the distribution is planned. However, even if differences in frequency exist within some of the tribes the general features of the distribution of the sickle cell gene which will now be discussed would not be changed.

DISCUSSION

From the map it can be seen that there is a northwest-southeast cline in the frequency of the sickle cell trait. With two exceptions the northwestern half of Liberia is occupied by tribes with frequencies ranging from 12 to 20 per cent. The tribes with the highest frequencies of the sickle cell trait are generally toward the interior on the Sierra Leone border, and there is a decrease in the frequency as one proceeds toward the center of Liberia. In the center of the country, the frequency decreases sharply from the Gola and Kpelle to the Dei and Bassa tribes and then from the Bassa to the Kru. In the interior it decreases sharply from the Kpelle to the Mano. In the southeast part of Liberia the frequency then decreases to almost zero per cent,

and in the tribe which is furthest to the southeast and the most isolated, no positives were detected.

Liberia is situated entirely within the West African Tropical Rain Forest, within which the epidemiological picture of malaria varies very little. Young and Johnson's (1949) malaria survey of Liberia showed high parasite rates throughout the country. In fact the highest overall parasite rates occur among the Mano, who have 2 per cent of the sickle cell trait. Thus, the differences in the frequency of the sickle cell trait do not seem to be explained by differences in the intensity of malaria in the areas concerned.

Elsewhere in West Africa, high frequencies of the gene responsible for hemoglobin C tend to be associated with low frequencies of the sickle cell gene and hence in some manner explain these low frequencies. However, the low frequencies of the sickle cell trait found in Liberia cannot be accounted for in this way since the hemoglobin C gene is either very rare or absent in the tribes of Liberia (Neel et al., 1956).

The fact that the frequency of the sickle cell trait in Liberia nowhere approaches the equilibrium value of 40 per cent postulated by Allison does not imply that selection by means of malaria is not an important factor maintaining the high frequencies of the sickle cell gene in Africa, or even in Liberia. It only means that the explanation of the distribution, at least in Liberia, must take into consideration other evolutionary factors. Since the cline in the frequency of the sickle cell trait is smooth and not erratic, and also since there is some correlation between the cline and cultural differences, gene flow, and not gene drift, would seem to be the major factor explaining this cline.

The tribes in the northwestern half of Liberia, who have frequencies ranging from 12 to 20 per cent, speak languages of the West Atlantic or Mande subfamilies of the Niger-Congo linguistic family (Greenberg, 1955). Within this group language affiliation does not seem to be correlated with the frequency of the sickle cell trait, the cline being gradual throughout regardless of language. The Kissi, who have the highest frequency in this area, and the Gola, who have the lowest, speak West Atlantic languages. The Mende, Gbandi, Loma, and Kpelle speak Southwest Mande-fu languages, while the Vai speak a Mande-tan language. The same is also true in Sierra Leone, where the Timne, who speak a West Atlantic language, have 29 per cent of the sickle cell trait (Allison, 1956), and the Mende, who speak a Mande language, have 30 per cent (Allison, 1956; Rose and Suliman, 1955). These frequencies are higher than any encountered in Liberia, and it is also interesting to note that the difference in frequency between the Mende in Liberia and the Mende in Sierra Leone is greater than the difference between the Timne and the Mende in Sierra Leone. Thus, the Mende tribe seems to contain several breeding isolates which differ significantly in the frequency of the sickle cell trait.

Although the cline in the frequency of the sickle cell trait in northwest Liberia and Sierra Leone is not correlated with language, the sharp decrease in the frequency which occurs in central Liberia is related to linguistic differences. The Dei, Belle, and Bassa, who have lower frequencies, are the northernmost speakers of Kru languages. Neither the Dei nor the Belle frequency is significantly different from that

of the Bassa, although with larger samples from these tribes they might very likely become so. Nevertheless, the decrease in the frequency of the sickle cell trait from the Gola and Kpelle on the north to the Dei and Bassa on the south is related to linguistic differences. Similarly, in the interior of central Liberia, the significant decrease which occurs on the Kpelle-Mano border is related to linguistic differences. Mano and Gio, which are Northeast Mande-fu languages, were previously thought to be closely related to Kpelle and other Mande languages whose speakers have high frequencies of the sickle cell trait, but Prost (1953) has recently shown that they are related to the Mande languages of the Ivory Coast and not to their Mande neighbors in Liberia.

Recent unpublished investigations by Binson, Neel, and Zuelzer in the Ivory Coast to the east of Liberia indicate a similar cline in the frequency of the sickle cell trait. Starting with the high frequencies of more than 20 per cent in Ghana, which is the east of the Ivory Coast, there is a gradual decrease in the frequency up to the central Ivory Coast where there is an abrupt decline on the border of the Akan peoples on the east and the Kru and Lagoon peoples on the west. The frequency then decreases to almost zero per cent among the Kru peoples of the western Ivory Coast. Thus, in eastern Liberia and the western Ivory Coast, there is a "pocket" of tribes with low frequencies of the sickle cell trait, who are surrounded by tribes with high frequencies. A similar "pocket" has also been found recently by Leite and Ré (1955) in Portuguese Guinea. Since the tribes with low frequencies in both these pockets are the more primitive and isolated tribes of West Africa, the sickle cell gene appears to have been introduced into the western part of West Africa and is still spreading to the more backward peoples.

The almost complete absence of the sickle cell trait among many of the Kru peoples is another surprising finding of this investigation. Although no anthropometric studies were done, the author would agree with most observers who have written about Liberia, that the Kru peoples are the most Negroid of the tribes of Liberia. Holas (1952, p. 339), on the basis of anthropometric measurements, has called the Krahn, who are one of the Kru peoples of the interior, the "true Negro". Johnston (1910) regards the Kru as the archtype of the race he calls the "Forest Negro", and Hooton (1947, p. 622) says "The purest type of African Negro, called by Sir Harry Johnston, the 'Forest Negro', is found along the coastal regions of West Africa . . . .". It thus seems that the sickle cell gene, although long considered a Negro characteristic, is absent in the "purest" representatives of the Negro race.

SUMMARY

Data are presented on the distribution of the sickle cell gene in Liberia. These data indicate a northwest-southeast cline in the frequency of the sickle cell trait. In the extreme northwest on the Sierra Leone border there is 20 per cent of the sickle cell trait, and the frequency decreases gradually to about 12 per cent in central Liberia. There is then an abrupt decline to 4 to 7 per cent among the coastal tribes and to 2 per cent among the tribes of the interior, and then a more gradual decrease to almost zero per cent in southeastern Liberia. The evidence seems to indicate that this cline is due to the recent advance of the sickle cell gene through Liberia.

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