POPULATION GENETIC STUDIES IN INDONESIA

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by

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A.S.M. SOFRO

CHAPTER 1

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ABSTRACT

Some three thousand blood samples from various Indonesian populations have been tested for electrophoretic variations of 21 red cell enzymes, three serum proteins and haemoglobin. In addition G6PD deficiency was also screened in the field and red cell morphology was also examined from unstained blood films to diagnose ovalocytosis.

Among 21 red cell enzymes tested, excluding G6PD, nine enzymes were polymorphic, five showed rare variants and six were monomorphic. In serum proteins, two proteins were polymorphic and one was monomorphic. For haemoglobin, in addition to two rare abnormal haemoglobins, Hb E was polymorphic. New variants were discovered in esterase D, carbonic anhydrase 2, 6-phosphogluconate dehydrogenase and transferrin.

A clinal pattern was observed in the distribution of $\underline{GLO}^1$, $\underline{GPT}^1$, $\underline{PGD}^C$ and $\underline{Hp}^1$ across the Indonesian archipelago. There is a trend of increasing $\underline{GPT}^1$, $\underline{PGD}^C$ and $\underline{Hp}^1$ gene frequencies and of decreasing $\underline{GLO}^1$ gene frequency towards the east. For systems with more specific alleles, the distribution of $\underline{PGM}_2^9$ and $\underline{Tf}^{D1}$ showed the western limit as far as Java and the distribution of $\underline{Tf}^{D}$Chi and $\underline{Hb}^E$ showed the eastern limit as far as Halmahera in the north and Timor in the south.

Variation in red cell enzymes, serum proteins and also Hb E were used to study the genetic relationship between populations. Employing data for gene frequency distributions in the populations, Nei's genetic distance measure was used to analyse the genetic relationship between Indonesian populations themselves and between Indonesian and neighbouring populations. A separate analysis was also made of the genetic relationship between the Balinese village

populations. The results showed that Indonesian populations may be grouped into a western and eastern Indonesian cluster. The western Indonesian cluster consists of all populations of the Greater Sunda Islands and the Lesser Sunda Islands from Sumbawa to the west, while the eastern Indonesian cluster is composed of populations of the Lesser Sunda Island beyond Sumbawa to the east and those from the Moluccas. When contrasted to the neighbouring populations, the Malays and Tagalog were grouped into the western Indonesian cluster, whereas the Fijians and Motu-speakers from Papua New Guinea were grouped into the eastern Indonesian cluster. The Asmat from Irian Jaya, Angurugu from north Australia and Kadazans from Sabah were distinctive from either cluster.

Analysis of the Balinese village populations showed that their genetic heterogeneity was compatible to some extent with geographical distance and/or topographical setting, but historical factors may also have played a part.

Examination of blood genetic traits associated with malaria indicated that G6PD deficiency and ovalocytosis were widely distributed in the archipelago, whilst the distribution of Hb E showed an eastern limit apparently associated with more recent population migration. Interaction between G6PD deficiency and ovalocytosis shown by the inverse proportion of the respective trait was to be observed beyond Java to the east.

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INTRODUCTION

1.1. The general background

The Indonesian archipelago (or the Nusantara archipelago) comprises a chain of islands between the Asian mainland in the northwest and the Australian continent in the southeast. Its important role in the history of population movement from the mainland of Asia toward the Pacific region has long been recognized.

Bellwood (1978) in his detailed review of population movement in the Pacific, points out that the complex racial history of Indonesia is due to the depth of the human occupation in the west of the country, and to a pattern of gene flow which has been superimposed on the earlier populations from Indian, Chinese, Arab and European sources during the last 2000 years. But he argues that this gene flow has not drastically altered the basic Indonesian pattern which may be considered as a cline running from Mongoloid populations in the west to Melanesian populations in the east. Bellwood believes that this cline may have attained almost its present form by 2000 years ago.

This present thesis will examine existing evidence from various disciplines which throws light on the nature of Indonesian populations. It will then present new evidence on the distribution of a wide range of blood genetic markers based on the examination of samples from populations in many parts of Indonesia and it will show the genetic relationship between these populations and their neighbouring populations in Asia on the one hand and on the other with populations in Australia, New Guinea and other parts of the Western Pacific.

1.2. The geographic setting

Administratively, at present Indonesia stretches from 92° to 141° east longitude and from 6° north to 11° south latitude, covering nearly 4 million square miles with its inland - seas included. Geographically

In the Lesser Sunda Islands, a large

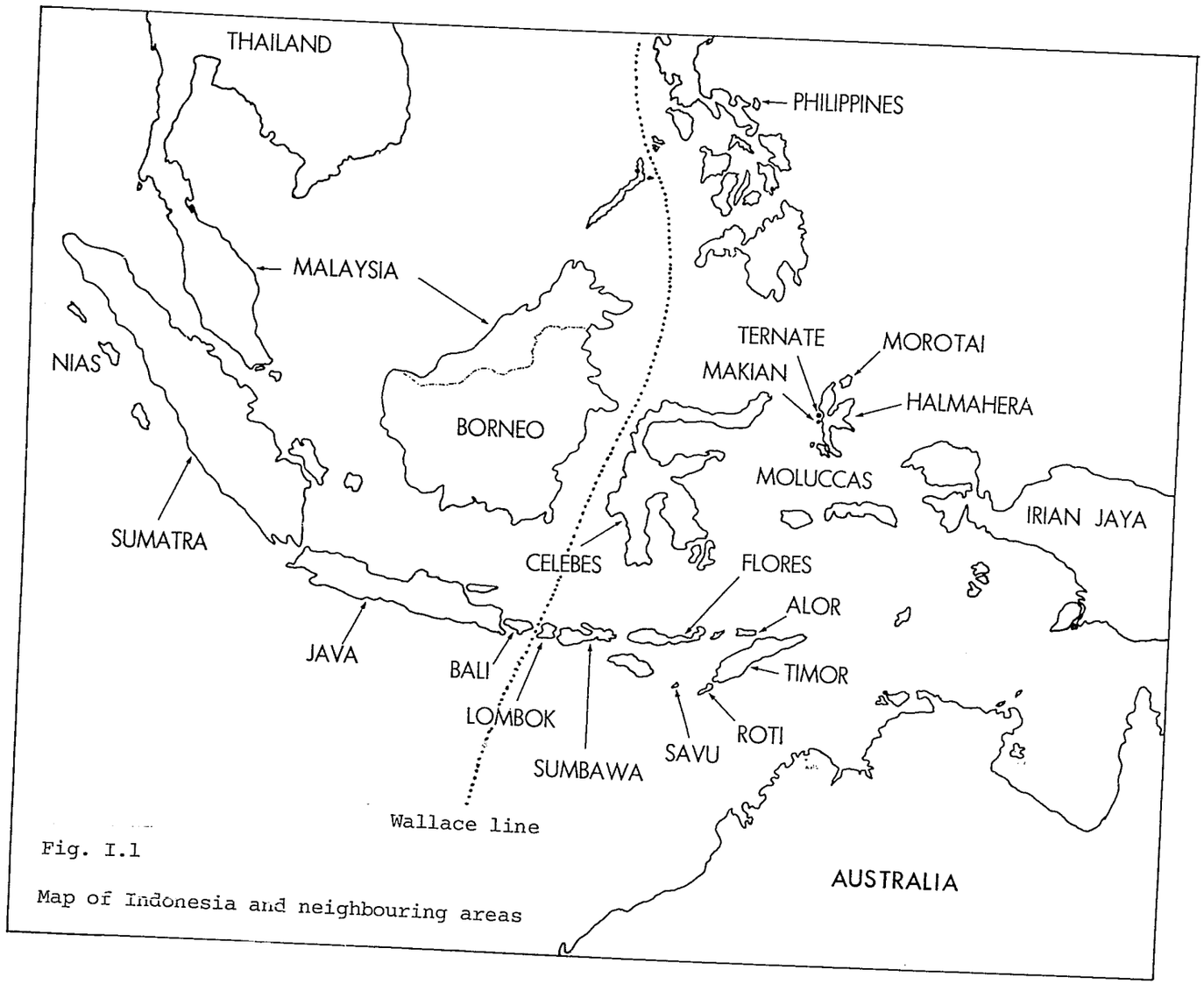


Fig. I.1

Map of Indonesia and neighbouring areas

the archipelago is commonly divided into four major groups. The first group is the Greater Sunda complex which includes Sumatera, Java, part of Borneo (Kalimantan) and Celebes (Sulawesi). The second group is the Lesser Sunda complex (Nusa Tenggara) which forms a chain of lesser islands stretching east of Java from Bali to Timor archipelago. The third group is the Moluccas (Maluku) which lies north of the Lesser Surda complex and east of Celebes. The last group is the western part of New Guinea (Irian Jaya) and its nearby islands. The country is divided biogeographically by Wallace's line into a western part closely related to the Asian continent and an eastern part related to Australia and New Guinea (Fig.I.1). This demarcation line has been of great importance in the early history of occupation by man of areas to the east of Wallace's line.

For much of the Pleistocene epoch, up to 10,000 years ago, when sea levels were much lower than during the Holocene, the western and northern portions of Indonesia were an extension of the Asian mainland, the Sunda shelf, while its eastern portion was the Sahul shelf which linked Australia and New Guinea into a single land mass, or Sahul land. The relatively short distance sea barriers at the time of maximum low sea level, made it possible for human populations to move from Asia into the Pacific through this region. Birdsell (1977) proposed two main routes of migration from Sunda land to Sahul land. The northern route was via Sulawesi - Sula - Seram - Misool - Sahul, and the southern route was via Lesser Sundas - Timor - Sahul. It is very possible that the migration must have been not just a single event, so that the region experienced occupation by different human types bringing with them different cultures.

One important consequence of the geographical transition at the end of the Pleistocene was that rising sea levels drowned vast areas of Sunda land and Sahul land, leaving the large number of islands which

today comprise the Indonesian archipelago. Here existing populations became relatively isolated, but were stimulated also to develop a seafaring technology to make possible inter-island communication and trade. It is plausible, therefore, to consider some parts of Indonesia as the cradle where the waves of later migrations started and then moved eastward to colonize the western Pacific beyond Melanesia, finally continuing out to become the Polynesians of the central Pacific (Brace and Hinton, 1981).

1.3. The population setting

The present population of Indonesia approximates 145 million (BPS, 1974), some 65% of which live in Java and its integral part, Madura. The remaining 35% is scattered in the other islands. According to Geertz (1963) the ethnic pattern in the population is very diverse, represented by more than 300 ethnic groups and over 250 languages or dialects. Reference will be made later to the major linguistic divisions. The multiplicity of ethnic groups is reduced by the "adat", or custom law system, into 19 local groups in the country (Koentjaraningrat, 1969). According to this grouping, the eastern Lesser Sunda area is represented by Timor. In this area the people are typologically very heterogenous; it is perhaps the most heterogenous area in the country. This situation brought about extensive studies by a number of physical anthropologists (e.g. Bijlmer, 1929; Keers, 1948; van Bork-Feltkamp, 1951; Glinka, 1978). Lebar (1972) has given detailed descriptions of the various ethnic groups in Indonesia. The present population also is diversified by religious affiliation. More than 90% of the population are Moslem, particularly those living in the Greater Sunda complex and other coastal areas. The remaining less than 10% are either Christians, Hindus or Buddhists. It is interesting that the population of Bali is almost exclusively Hindu.

Linguistically, the majority of the local languages spoken belong

to the major linguistic division called Austronesian (AN) which includes Malay, many languages in coastal areas of New Guinea and Polynesia . In Indonesia Austronesian speakers cover those living in the Greater Sundas, the Lesser Sundas and the Moluccas. The other major linguistic division is Non-Austronesian (NAN). Speakers of NAN are found in northern Halmahera, Alor and Timor (Howells, 1973; Lebar, 1972) and in all of West Irian except AN speakers in some coastal areas (Voorhoeve, 1975). Since AN languages have spread far out into the Pacific, linguistic affiliations are important also to the hypothesis of the migration of peoples from the Indonesian region eastwards into the Pacific world.

1.4. Physical diversity of the people

1.4.1. The Prehistoric period

Archaeological research during the past nearly 100 years has revealed that parts of Indonesia has been occupied by Hominids for a great length of time. Indeed, Java is among the relatively few places in the world where any substantial number of Hominid remains have been found. Jacob (1981a) classifies them into three taxa, Meganthropus, Pithecanthropus and Homo. Homo is defined by any continuously erect walking primate with a well developed neocortex and who has language, at least proto-language. Pithecanthropus is defined as primates with a smaller brain size; the erect posture is not continuous and is different from Homo, at least in the cervical lordosis and probably also in gait. Pithecanthropines have limited language capacity; at the most they possess pre-language. Meganthropus is distinct from early Pithecanthropines due to the differences in the masticatory apparatus and in the neurocranium.

The earliest stage in the prehistory of Java may start from the Lower Pleistocene with the Tji Djulang and Kali Glagah faunas without known Hominid finds (Heekeren, 1972). The oldest Hominid finds are

Pithecanthropus modjokertensis found in east Java, which is of Lower Pleistocene age, followed by *Meganthropus palaeojavanicus*. The latter were found at Sangiran in central Java, an important place providing the most fossils coming from the Lower and Middle Pleistocene and probably also from the Upper Pleistocene. The most recent find from this site is a natural pithecanthropine cranial endocast (S34) discovered in 1980 (Jacob and Kadar, 1981).

Jacob (1976b) recognizes three groups of Pithecanthropines in Indonesia. The first one is *Pithecanthropus modjokertensis* who lived around 1.9 million years ago. The second, which is the largest group, is *Pithecanthropus erectus* found in the Middle Pleistocene deposits of Sangiran, Trinil and Kedungbrubus: they lived from 900,000 to probably 600,000 years ago. The third group is *Pithecanthropus soloensis* found in the Middle Pleistocene deposits of Ngandong, Sambungmacan and Sangiran. They lived at the same time as *Pithecanthropus erectus* but survived up to probably 200,000 years ago.

Another classification also put forward is into the gracile pithecanthropines and the robust pithecanthropines (Jacob, 1976a). The first mentioned include the Trinil fossils and most of the fossils from the Kabuh formation of Sangiran. The robust one includes the fossils from the Puchangan formation of Sangiran, from the lower beds of the Kabuh formation of Sangiran and Sambungmacan and from Ngandong. *Pithecanthropus modjokertensis* and *Pithecanthropus soloensis* represent the early robust and the late robust respectively. It is suggested that *Pithecanthropus modjokertensis* evolved toward both *Pithecanthropus erectus* and *soloensis* (Jacob, 1976b), and *Pithecanthropus erectus* further evolved towards *Homo sapiens*. Therefore it is expected that future discoveries of Pithecanthropine remains from a later period would look more like *Homo sapiens*.

The late Upper Pleistocene period may be important not merely due

to the development of *Homo sapiens* in the region, but also because there had been many migrations from Indonesia to Melanesia and Australia. Jacob (1976a) assumed that some Pithecanthropus genes were absorbed by these early *sapiens* migrants.

The earliest *Homo sapiens* remains found in Java are two fossils, the Wadjak skulls. Jacob (1967) was the last to study the fossils. According to him Wadjak Man showed a mixture of Australoid and Mongoloid characteristics. He also suggested that this population represented the ancestral type of both the Proto-Malays and the Austromelanesians, but were not the direct ancestor of the living Australoids. Unfortunately the antiquity of Wadjak is not clearly understood though it is not more than the antiquity of the Niah skulls in northern Borneo or of Tabon skeletal remains in the Philippines (Jacob, 1976a).

Heekeren (1972) postulated that the physical evolution of man in Africa and Asia moved along closely parallel lines, although their cultural development was quite distinct. By looking at their tool industries he concluded that, in Pleistocene times, the Far East was more or less a technically retarded marginal area as compared to Europe, the Middle East and Africa.

During the post-glacial period, with the general rise in sea level and after landbridges became inundated, the further colonization of Indonesia took place by sea and by inland waterways by series of successive waves of races of *Homo sapiens: Homo erectus* was no longer present (Heekeren, 1972). In the Mesolithic and Neolithic periods various human remains have been discovered. Glinka (1981) provides a list of the discoveries including the sites and their racial affinities as given by Jacob (1967). In the Mesolithic period Australoid and Melanesoid elements were widely spread beyond the Indonesian archipelago. Mongoloid influences were seen only in the north and northeast of the territory. It was estimated that by the Mesolithic period the

Mongoloid element was represented by only 18% of the total. In the Neolithic period the Mongoloid element seems to have increased greatly to more than 50%. It is most likely that the Mongoloids expanded across the sea and replaced and/or interbred with the former Australoids and Melanesians.

1.4.2. Contemporary populations

As noted above the present populations of Indonesia represent a great diversity of cultures. According to Lebar (1972) this is the end result of the diversity of historical influences together with isolation on often remote islands or in interior mountain valleys. This cultural diversity may reflect itself also in genetic diversity, detectable in blood genetic markers or in anthroposcopic and anthropometric characteristics.

In the older physical anthropological literature the so-called 'Deutero-Malays' (represented by the peoples of Java, Sumatera and Borneo) are Mongoloids of medium stature. It is assumed frequently that they were preceded in the same areas by a migration of 'Proto-Malays' who are now represented by the Bontoc and Ifugao in northern Luzon, the Punans in Kalimantan, the Toala and Toraja of Sulawesi, the Kubu and Batak in Sumatera, the Tenggarese in eastern Java and some of the people of Nias, western Sumba and western Flores (Bellwood, 1978). These 'Proto-Malays' it is claimed have a higher proportion of Australoid traits, such as darker skin and curlier hair.

It has been suggested by Keers (1948) that major elements of a Negrito phenotype survive in western Timor, western Sumba, western Flores and southern Sulawesi. These groups together with the 'Proto-Malays' may well represent survivals of an earlier gene flow from the Asian mainland into the island world of Indonesia which, at that time, was already populated by peoples of Australoid type. This problem will be addressed in the concluding chapter of this thesis. What is difficult to assess, however, is whether the migration of Mongoloid peoples

took place in two waves, represented by the 'Proto-' and 'Deutero-Malays', or whether the infusion of Mongoloid genes was a continuous process over a long period of time.

1.4.2.1. Anthropometric and anthroposcopic traits

In the complex of Indonesian populations, physical anthropological studies have been carried out by a number of investigators (e.g. Bijlmer, 1929; Keers, 1948; van Bork-Feltkamp, 1951). Recently Glinka (1981) reviewed in detail the available physical anthropological data for Indonesian populations. Below is given briefly Glinka's summary.

Body stature:

Average body stature does not vary much and exceeds only sometimes the limits of "sub medium" (160-163 cm). Some tribes in the interior of Kalimantan and Sumatera have a predominantly low stature. In general the stature is a little higher in the Sunda islands such as Java, Bali, Flores and Timor.

Cephalic index:

The average of the cephalic index varies much more than body stature. In the eastern Indonesian islands (Flores, Alor, Timor) the populations are rather dolichocephalic, perhaps due to Melanesian influences, and these are to be seen in other characters as well. The other regions of Indonesia tend to be more brachycephalic, but there are many local differences.

Facial index:

Most of the averages point to low-broad faces, particularly in Kalimantan and, in a lower degree, in eastern Indonesia from Lombok to Timor. Higher averages, i.e. more high-narrow faces are found in the islands of western Indonesia.

Nasal index:

The highest averages of the nasal index, i.e. broad noses, are found in the interior tribes of the Malayan peninsula and of Kalimantan.

The Indonesian island chain from Nias to Timor shows in general lower averages, i.e. narrower noses.

Hair form and skin colour:

Wavy to curly hair predominates in the eastern part of Indonesia. Straight hair becomes more frequent in Celebes and west of Sumbawa. The distribution of skin colour is very similar to that of hair form. In general it is darker in the east and becomes fairer towards the west and north.

1.4.2.2. Blood groups

The distribution of the allele frequencies of blood groups in Indonesian populations have been compiled by Mourant *et al.* (1976). Among the earliest studies was one carried out by Simmons and Graydon (1951). More recently the blood group distribution in the Javanese has been reported by Jacob and Doerjadibroto (1981). Comparison has also been made with other available data from some Indonesian and neighbouring populations.

In the ABO system, in general, the incidence is O>B>A>AB except for some peoples from west Java who have the gene frequency of blood group A higher than that of B.

In the MN system, the gene frequency for gene M is higher than that of N. However, a reverse situation occurs in the Dayak. It seems that this system exhibits clines between the Mongoloid and Australoid areas. There is a trend for gene N to increase towards east and south.

In the Rh system the most frequent allele combination is CDe. Three other combinations; cDe, cDE and the rarest, CDE, are also present. Rh negative (cde) is absent in Indonesia though Simmons and Graydon (1951) reported the occurrence of Cde and cdE in Java.

A detailed study of population relationships in the South East Asian area, including Indonesia, based on available blood genetic marker data has been made by Lie-Injo (1976). Lie-Injo points out that the

high frequencies of blood group B in the ABO system and of R_1 (CDe) in the Rh system found in South East Asia generally is characteristic of Mongoloid populations, as also is absence of blood group A_2 and r (cde) and low values for N and S in the MNS system. She points out that, although this pattern is characteristic of the Senoi and Aboriginal Malays in the Malay peninsula, there are marked differences in gene frequencies in different settlements of the same groups and further that the Diego blood group, another Mongoloid trait was not found in a series of Senoi studied by Chin (1964). Referring to the Negritos, Lie-Injo states that they have low B frequencies and a high R_o frequency, although Lehmann and Ikin (1954) found a very high A_1 frequency but no R_o among the Onges in the Andaman islands. However, the population size of many of the Negrito groups is very small (the Onges number only about 100 at present) so that genetic drift could introduce large changes in frequencies of many alleles or even lead to their complete disappearance.

1.4.2.3. Serum protein and red cell enzyme groups

Lie-Injo (1976) also has summarized data for haemoglobins, serum proteins and red cell enzyme systems in the South East Asian-Indonesian region although, at that time, the only information available for Indonesia was based on the work of McDermid *et al.* (1973) and Lie-Injo *et al.* (1974). Using data for the occurrence of two abnormal haemoglobins, Hb A2 and Hb CoSp (Hb Constant Spring) described by Lie-Injo *et al.* (1971b), she concludes that Batak and Aboriginal Malays in the Malay peninsula are genetically related. Hb E, on the other hand, has low frequencies in the Batak, as also in the Negritos in Malaysia, except in contact areas with the Senoi, who are known to intermarry with the Negritos.

1.4.2.4. Dermatoglyphics

Other physical anthropological investigations have included dermatoglyphics. Although its mechanism of inheritance is not completely under-

stood, finger-prints, a polygenic trait, has long been used for population studies. In Indonesian populations such a study has recently been carried out for the Javanese (Satmoko, 1981). Jacob (1981b) has also reported a finger-print study for the Javanese performed more than three decades ago and compared the result with other Indonesian data available. Though he found a little higher the frequency of arches compared with other Indonesian populations, the result is still comparable with other Mongoloid populations.

1.4.3. Distance analyses based on physical anthropological data

In the South East Asian region, multivariate analyses using craniometric data have been carried out by Pietrusewsky (1974). Comparisons were made between four sets of skulls representing prehistoric populations and 15 sets of skulls from recent populations. The former four populations were originally from mainland South East Asia, while the latter included materials from Java, Sulu, Philippines, New Guinea, Fiji, Hawaii, Guam, Siberia, China and Mongolia.

Mahalanobis generalized D^2 statistics were used to construct a dendrogram using 13 measurements from more than 1100 male and female crania. Somewhat different results were obtained for the two sexes, but both agreed in showing the prehistoric skulls to be different from the modern populations, with the specimens from Non Nok Tha and Ban Kao in Thailand being the most distinctive.

For the more recent populations the dendrogram constructed from the male data gave a major cluster which comprised the Annam (South Vietnam) - Java and the Sulu archipelago cluster, the Laos-Thai cluster, the Cambodia-Philippines cluster and the Tonkin (North Vietnam) - China cluster. The Hawaii and Guam cluster and the Mongolian-Siberian cluster were next attracted to the major cluster. In the dendrogram based on the female data Java and other samples from island and mainland South East Asia form a general cluster. In addition, the Laos-

Thai cluster and the Mongolian-Siberian cluster were also present as in the dendrogram from the male data. Pietrusewsky concludes, therefore, that D^2 values, plots of canonical variates and classification data all indicate similar patterns among the South East Asian and the Pacific groups and that, in addition, the four prehistoric samples are distinctive from one another.

Despite the availability of anthropological data for Indonesian populations, little attempt has been made until recently to comprehend and classify the ethnogenetical relationship of the people in a unified manner until Glinka (1978) carried out such effort. He examined the collective material, encompassing 258 populations from Indonesia, Malaysia, the Philippines, Taiwan and Madagascar. For the purpose of comparison, 62 populations from neighbouring areas were also included. Glinka (1978 and 1981) calculated Penrose's 'Generalized Distance' using data for stature and 8 standard measurements of the head. On the basis of the distance values obtained, he combined the population into two large groups, the first of which encompasses the population of the outer chain of islands of Indonesia and of Taiwan, the second that of Dayaks from Kalimantan, Madagascar and the Philippine 'primitive' population. With successive sub-classification, 9 groups arose; one of 'Proto-Malayids' (Cluster A), one of 'Semang-Senoi' (Cluster B), three of 'Deutero-Malayids' (Cluster C,D and F), one of 'Dayak' together with the Kubu and Igorot (Cluster G), one of 'Malgassy' (Cluster H), one of the Aeta and Mangyan (Cluster I) and one of the Mestizos from Kisar (Cluster E).

Using body height and 3 usual indices (i.e. cephalic, facial and nasal indices) with the clustering technique of Wanke (1954), Glinka described 4 broad types i.e. Europoid-Indoid (Type I), Proto-Malayid (Type II), Deutero-Malayid (Type III) and Negroid.

From the above analysis and comparisons it is concluded that

Semang-Senoi of Malay and the Aeta of Luzon cannot be proven to be related to the same population. In other words, they might be regarded as two separate race complexes of an old stratum which, at that time, probably spread itself over the entire Indonesian area. Through this large extension two separate races developed.

The close relationship of the Proto-Malayids with the Semang-Senoi could suggest an early intermixing of both respectively or mongolidization of a part of the Negrito which contributes to the appearance of the Proto-Malayids. These Proto-Malayids moved in a southeast direction to the Lesser Sunda islands at the time of the existing landbridges. During their migrations they left traces in the Siberut and in the Tengger area of east Java. At approximately the same time a population migration took place in an eastern and northeastern direction via Kalimantan to the Philippines. Glinka (1978) raises a question whether this group had another ethnogenetic origin or whether it was only the result of its own racial development. The remains of this group are the Kubu in Sumatera and the Igorot, Bontok and Nabaloi in North Luzon. He further claims that the last colonization sequence was the Deutero-Malayids, who must already have spread after the disappearance of the landbridges. They spread from continental south-eastern Asia in a southern direction to Sumbawa, in an eastern direction, through Kalimantan to Halmahera and in a northern direction to the Philippines and Taiwan. So far as the Australians are concerned it may be concluded that their racial development occurred later on the Australian continent, as suggested also by Jacob (1967; 1976).

Migration tendencies in a westward direction must also have occurred. One of these tendencies was probably manifest in the migration of the Dayak group to east Africa and Madagascar. As well, in Indonesia itself, by infiltration from the Melanesian area, a secondary Melanesization of several populations occurred in east Indonesia.

In the Lesser Sunda Islands, a larger heterogeneity in physical data exists. Employing anthropological data from Keers (1948), Glinka finds the populations of this area fall into 8 clusters. The Atoni of Timor as well as the east Florinese clearly distinguish themselves from all the other populations.

Glinka (1981) described in further detail some clusters of recent Indonesian population determined by his analysis.

The Negrito

The Negritos are represented by the small statured curly haired forest tribes of Malakka and the Aeta of the Philippines. They have been considered as the dispersed remains of an old stratum and as a morphological racial entity (Schebesta, 1952).

Even though the description of the Negritos in the Indonesian archipelago have been rather vague, Keers (1948) found many Negritos in some places in the Eastern Little Sunda islands. The origin of the Negritos is unknown. Regarding the Indonesian archipelago, it is generally assumed that the Negritos form one of the oldest, if not the very oldest, layer of its population (Keers, 1948). However, no reliable data are available to confirm this opinion.

The Palaeo- or Proto-Malayids

The Palaeo- or Proto-Malayids have a low or medium stature, they are dolicho- to mesocephalic with a long narrow to medium broad head and a very narrow forehead. The frequency of the classic mongoloid characters of the face such as the mongoloid eye-fold is very low. The hair is wavy to crisp, skin colour predominantly dark brown. In general, skin colour becomes darker and the hair more crisp in the eastern direction.

Their settlement in the eastern part of the Little Sunda islands coincides with the district of Nusa Tenggara Timur (NTT); Sumba, Flores, Timor and all the smaller islands of this archipelago up to Kisar. Probably the area extends eastward and northeastward

to the southern Moluccas. In the west the Tenggarese of east Java and the inhabitants of the island Siberut in the west of Sumatera could be considered as remains of the Proto-Malayids. The admixture of other racial elements varies in different regions. In the eastern part of the Little Sunda Islands, the Melanesian influence is remarkable.

The Dayakids

The Dayaks represent the collective name of the aboriginal inhabitants of Kalimantan. The Dayakids have a low stature ($\bar{x}$ = 157. cm), they are mesocephalic (cephalic index $\bar{x}$ = 79.7) and the head is long and medium broad to narrow. As to body build, the Dayakids are rather broad, stocky and robust.

The main part of the Dayakids is represented by the so-called land-Dayaks. The coastal regions of Kalimantan are much interspersed with Malays. Other populations which have to be classified here are the Kubu of Samatera and the so-called Igorots of Luzon. Polunin and Sneath (1956) argued that because the Land Dayak have high gene frequencies of A and B they must have had a different origin from the other tribes. Lie-Injo draws attention also to a study by Kurisu (1970), using a distance analysis based on physical traits, which demonstrated that the Land Dayak in Sarawak are different from the other tribes in the same area, in agreement with the conclusion of Polunin and Sneath.

The Neo- or Deutero-Malayids

These populations differ from the Proto-Malayids particularly by their stronger mongoloid characters. It is to be seen not only in the form of head, face and hair, but also in the fairer yellowish skin colour and in the higher frequency of the mongolian eye-fold. They are small stature ($\bar{x}$ = 159.7 cm) and brachycephalic, the head is medium broad to broad and medium long. Hair form varies very much regionally from straight to a little wavy, skin colour varies from brownish to yellowish.

The area of the Deutero-Malayids extends from Taiwan in the north to Sumbawa in the south, from Sumatera in the west to probably Halmahera in the east. This large distribution may lead, of course, to the development of local variants.

Compared to the detail of population relationships provided by Glinka on the basis of anthropometrics, genetic distance analysis using blood genetic markers so far in the Indonesian and South East Asian area has been very limited, but Lie-Injo (1976) concluded her review by carrying out such a genetic distance analysis. Using blood group data she found that Javanese were genetically closest to Malayans and Thais, and more distant from Chinese, Indians and Burmese. Negritos were not particularly close to any population, but of the comparisons made the genetic distance was smallest between Negritos and Malays and Thais, with Javanese only slightly further away.

The genetic distances based on serum proteins, red cell enzymes and haemoglobin did not include the Javanese, for which no data was available at that time, Indonesian population being represented only by the Batak of northern Sumatera. The Batak were genetically closest to Chinese and Aboriginal Malays and most distant from Senoi, with Negritos being intermediate. Since blood group data were not available for Batak, and serum protein and red cell enzyme data not available for Javanese, it was not possible for Lie-Injo to carry out a genetic distance analysis using all genetic marker data and including an Indonesian population. For the remaining populations, however, when this was done (but excluding Hb and G-6-PD because of the fact they are known to be environmentally sensitive) it is interesting that the Negritos are closest to the Malays, Thais and Chinese, in that order.

They were however quite distinct from the Senoi who, in their turn, were closest to the Aboriginal Malays, Thais and Malays, with Chinese only slightly further away. Lie-Injo's (1976) analysis therefore indicates that blood genetic markers show a large measure of homogeneity between populations in Mainland South East Asia and one of the nearest Indonesian populations, the Batak. Later in this thesis the genetic distance analysis using blood genetic markers will be extended to include many more Indonesian populations as well as others in the Western Pacific.

1.4.4. The pattern of population development of physical characteristics

As noted already it is possible that the first known immigrants into Indonesia were the Negritos (Neill, 1973). It is thought that they moved eastward into the Philippines and Japan as well as southward through Indonesia, although their origin is unknown. Other immigrants who have been also recognized were the Austromelanesians. In this region, Wajak Man (Jacob, 1967) was postulated to be the predecessor of the Austromelanesian and early Malays. The Malays remained in the west or migrated towards the northeast, while the Austromelanesians migrated southward and eastward. In their new place the latter then split into present Melanesians and Australoids. Later, a Mongoloid element migrated southward bringing with them a New Stone culture (Bellwood, 1978). Bellwood states that these last immigrants maintained a slow constant migration up to the present time, that is, he does not accept the separate migration of Proto- and Deutero-Malays. As a result of this continuous migration process, varying degrees of intermixture between the earlier and the later settlers must have taken place. The process of intermixture could have become more complicated, for it is possible that these immigrants, or their offspring, migrated backward to their ancestor's previous settlement where a hybrid genera-

tion may have been in existence.

By the early centuries of the Christian era other influences came from various directions. Though contact between Indonesian people and Indian or Chinese people was in existence by the fourth century A.D. (Chatterji, 1967), the first large Chinese settlement in Indonesia seems to have occurred in the thirteenth century (Van Niel, 1963) and then again during the Dutch occupation (Spruyt and Robertson, 1973).

Intensive contacts with Asiatic Indians probably have taken place since the seventh century A.D. when the Indian religions, Hinduism and Buddhism were practised in the region. At that time the Kingdom of Sriwijaya in southern Sumatera was very important, for it was located midway along the India-China route. However, large scale Indian immigration is quite recent, dating from the late nineteenth century (Osborne, 1979). By the raising of Islam in the fourteenth century, Hinduism faded from the country except in Bali, where Hinduism is still practised.

Among other immigrants were the Arabs. Their first arrival in the archipelago is associated with the spread of Islam. During the period of the Indianized Kingdom, trading routes in the archipelago were busy with merchants coming to South East Asia and the Far East from Western Asia or vice versa. They consisted of Arabs, Persians, Indians, Chinese as well as Indonesians (Peacock, 1973). Although the means by which the Islamization of the people took place is not known precisely, there is no doubt that the Moslem merchants played an important role in its spread.

European contacts took place relatively recently when the Portuguese and the Dutch first came for trading in the sixteenth century. It is worth noting that as far as religion and culture are concerned the Dutch do not seem to have had great influence despite the long period of Dutch occupation. Their influence has only been profound in Minahasa, the northern tip of Sulawesi (Lebar, 1972). On the other hand, the Portu-

guese influence is profound and lasting in Flores, Solor and Adonara (Franca, 1970). Even today the majority of people in these places adopt Portuguese names and practise Catholicism.

It is against this long and varied development of population history in Indonesia that I shall attempt an interpretation of the blood genetic marker studies carried out during the last three years.

CHAPTER 2

POPULATIONS STUDIED AND METHODS

2.1. Introduction

A comprehensive population genetic study of all Indonesian populations is, from a practical point of view, almost impossible. A selection of populations therefore has to be made and in doing this several factors need consideration. In the present work selection was made firstly in an attempt to obtain representative groups from various parts of the country - from the Greater Sundas, the Lesser Sundas, the Moluccas and also from the western part of New Guinea (Irian Jaya).

A second constraint was the availability of transport from capital or main cities to the site of populations to be sampled. For an island country stretching more than 3000 miles, rapid transportation of samples creates some problems. The national capital of Jakarta and each capital city of the Provinces are linked by regular air or sea transport. Within Provinces road or water transports are available, depending on local conditions. Although for Java and Bali there was no such problem in many other Provinces difficulties in transportation were often encountered.

Thirdly, there was the problem of obtaining the willing consent for the drawing of blood from the subjects. Generally speaking in Indonesia bleeding or needle insertion is an experience which is usually avoided, particularly by villagers. Even for medical purposes, due to lack of experience of the practice, it is sometimes not easy to obtain a blood sample. Since during this study blood drawn was basically not for medical purposes, special care to obtain consent had to be taken. For those attending Public Health Centres there were no difficulties as long as the local tradition was appreciated.

2.2. Populations sampled

In this study blood samples were collected by me during four different field trips to Indonesia. All these trips were made possible with the permission of "Badan LITBANGKES" Department of Health and Department of Internal Affairs of the Republic of Indonesia. In the field, assistance was given by the Governors of the Provinces, Heads of the Provincial Health Services, District Health officials and many others. In addition, samples from Bali, Irian Jaya and Bangkok, Thailand were collected in other investigations referred to later.

The first field trip was carried out during the 2nd March to 5th April, 1980, in the Province of Yogyakarta. During this field trip, 407 blood specimens (295 males and 112 females aged 12 to 84 years) were collected in Yogyakarta city and nearby villages. Blood samples from Yogyakarta city were obtained mainly in collaboration with the Indonesian Red Cross (or P.M.I.) Yogyakarta branch. In addition, some specimens were drawn from student nurses at the "P.K.U.MUHAMMADIYAH" Hospital and a few from patients in "PUGERAN" and "BETHESDA" Hospitals. From nearby villages blood samples were drawn from patients and officials of Sleman District Hospital in addition to some samples drawn in collaboration with the P.M.I. Yogyakarta branch.

The second field trip was carried out during the 3rd August to 18th October, 1980, in the Lesser Sunda complex. During this field trip two ethnic groups, Bimanese and Sasak, were sampled from the Western Nusa Tenggara Province and five other ethnic groups were sampled from the Eastern Nusa Tenggara Province. Bimanese were sampled in Raba - Bima. A hundred and ninety-eight blood samples were drawn from villagers attending the out-patient clinic of Bima District Hospital in addition to some samples from the health officials (125 males and 73 females aged 15 to 70 years). Samples from Sasak were collected in Selong, eastern

Lombok. They included 191 individuals attending the outpatient clinic of Selong Hospital (127 males and 64 females aged 14 to 75 years). All blood samples from five other ethnic groups were collected in Kupang. They included 133 Timorese mainly from Kupang city and nearby villages (58 males and 75 females aged 13 to 67), 36 Savunese (17 males and 19 females aged 17 to 64 years), 59 Rotinese (36 males and 23 females aged 13 to 68 years), 26 Alorese (14 males and 12 females aged 17 to 53 years) as well as 76 individuals from Flores regardless of their local group (50 males and 26 females aged 16 to 63 years). All these samples were obtained from individuals attending the outpatient clinic as well as from student nurses at PROF. DR. W.Z. JOHANNES Hospital in Kupang.

The third field trip was carried out during 5th April to 12th May, 1981, in the northern Moluccas. In this northern part of Indonesia, samples from 213 apparently healthy Ternatan villagers were collected in Ternate island (117 males and 96 females aged 15 to 102 years). In addition 30 samples from Makianese (17 males and 13 females aged 17 to 60 years) were also collected in Ternate. They were all leprosy patients in Sorofo Ternate. At the northern tip of Halmahera island, 155 samples were collected from apparently healthy Galelarese high school students and villagers in the sub-district of Galela (109 males and 46 females aged 16 to 100 years).

The last field trip was carried out during 10th January to 19th February, 1982, in the Province of South Sulawesi. All samples were obtained from apparently healthy individuals belonging to two different ethnic groups, Macassarese and Buginese respectively. Blood samples of 199 Macassarese were mainly collected from the students of SMAN Goa in Sungguminasa in addition to some samples collected from the students of SPK, SMAK, SMF, SPK Muhammadiyah, PGAN, MAN, SGP, SPRG as well as from student nurses of Pelamonia Hospital in Ujung Pandang (116 males and 83

females aged 16 to 60 years). Blood samples of 205 Buginese were collected from the students of SPGN and SMAN Pangkep as well as from some officials of Pangkep District Health Service. In addition, some samples were also obtained from the same places as Macassarese in Ujung Pandang (79 males and 126 females aged 16 to 50 years).

In addition to the samples collected during the four field trips detailed above, blood samples from three other ethnic groups were available for this study. Samples from Balinese and Toraja were kindly provided by Drs. G. Breguet and R. Ney from Switzerland: they were collected during a survey on Bali supported by the Swiss National Fund for Scientific Research. Samples from the Asmat area of Irian Jaya were kindly provided from collections made by Dr D.C. Gajdusek and deposited with the Department of Human Biology, John Curtin School of Medical Research. Samples from Bangkok were kindly provided by Dr Dasnayanee Chandanayingyong, Director of Blood Bank Siriraj Hospital, Bangkok, Thailand.

The list of populations and the number of persons tested is shown in table 2.1; a separate list for the Balinese is shown in table 2.2.

2.2.1. Cultural characteristics of the populations

The following is a brief description of cultural characteristics of groups included in the present study. For more detailed information see Lebar (1972).

a. Javanese.

The inhabitants of Java, the Javanese, on the basis of some cultural differences are customarily divided into 3 major groups: the Javanese proper, Sundanese and Madurese. In addition, the Badui and Tenggarese constitute minor groups. The respective numbers in each group are not available since ethnic group was not considered in the recent census. However, it is estimated that, by 1981, the population of Java and Madura was around 94 million (BPS, 1974). Javanese are distinct from the other populations in Indonesia because Java was subjected to strong Hindu and

Table 2.1. List of populations and the number of persons tested.

Population*	Number tested			Age range (years)
	Male	Female	Total	
Javanese (urban)	161	42	203	15 - 84
Javanese (rural)	134	70	204	12 - 70
Balinese	-	-	970	- - -
Sasak	127	64	191	14 - 75
Bimanese	125	73	198	15 - 70
Florenese	50	26	76	16 - 63
Savunese	17	19	36	17 - 64
Rotinese	36	23	59	13 - 68
Timorese	58	75	133	13 - 67
Alorese	14	12	26	17 - 53
Macassarese	116	83	199	16 - 60
Buginese	79	126	205	16 - 50
Toraja	93	8	101	16 - 24
Ternatans	117	96	213	15 - 102
Makianese	17	13	30	17 - 60
Galelarese	109	46	155	16 - 100
Asmat	110	53	163	2 - 49
TOTAL			3162	

* 94 samples from Bangkok, Thailand are also tested.

Table 2.2. List of the populations of Bali from various villages and number of persons tested.*

District and Village	Number tested
Jembrana :	
Dauh Waru	80
Tabanan :	
Penebel	58
Badung :	
Ayunan	69
Renon	95
Serangan	39
Klungkung :	
Banjar Rangkan	48
ed (Nusa Penida)	34
Karangasem :	
Rendang	124
Seraya	85
Bangli :	
Songan	116
Buleleng :	
Julah	49
Sembiran	101
Beratan	32
Gianyar :	
Wanayu	40
TOTAL	970

* see also Fig. II.1

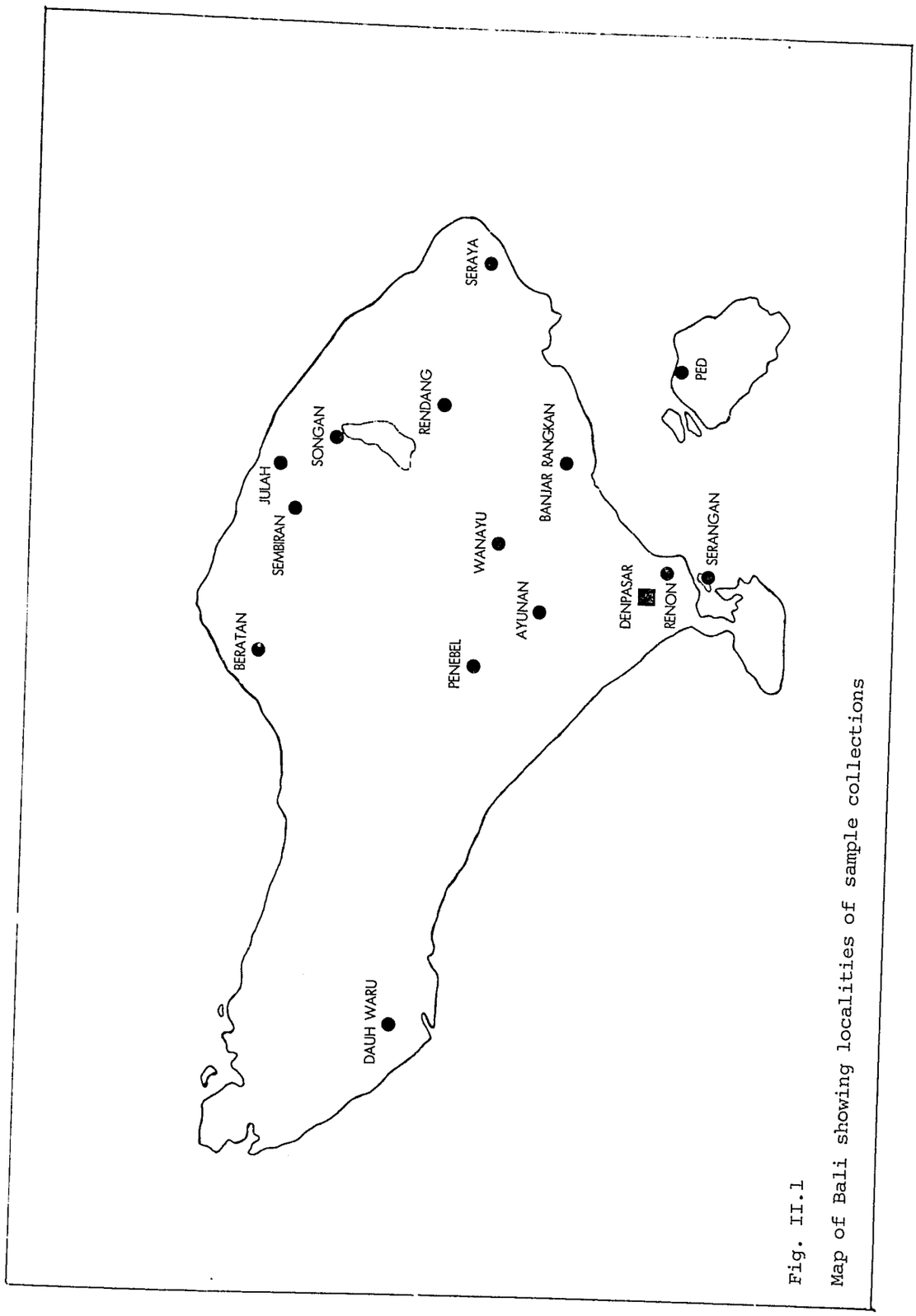


Fig. II.1

Map of Bali showing localities of sample collections

Buddhist influences in the past. Although Islam has now had a major influence on the people, the impact of Hinduism and Buddhism seems to be retained. Some Javanese communities may be found on other islands due either to their migration in the past or to the recent policy of transmigration.

b. Balinese

The inhabitants of Bali island, east of Java are culturally much more homogeneous. Despite many cultural similarities between Bali and Java, the two populations differ greatly because Balinese were not influenced by Islam. After the Islamization of Java in the fifteenth century, a number of noble families from the Hindu Kingdom of Java migrated to Bali. Since then Bali became more or less isolated until the present century and Hinduism is still practised. By 1981 the population of Bali was estimated to be nearly 2.7 million (BPS, 1974). Most of the population is Balinese: other ethnic groups, which are mostly traders, represent only small numbers out of the total population. Some Balinese may be found outside Bali mainly in the western part of Lombok.

c. Sasak

The Sasak are the major population group on Lombok. They mainly occupy the central and eastern part of Lombok and are Islamic in religion. They are physically and culturally related to peoples of Java, Bali and Sumbawa. Balinese influence is mainly in the western part of Lombok and Sumbawanese influence is mainly in the eastern part. Other components of the population includes Arabs, Buginese and Chinese. Relations with Sumbawa and Macassar were in existence during the seventeenth century. By the eighteeneth century the Balinese had become rulers of Lombok and by the end of the nineteenth century the Dutch occupied the island. It is estimated that, by 1981, the population of Lombok was 2 million (BPS, 1974).

d. Bimanese

The population of Sumbawa island are mainly Sumbawanese and Bimanese. Sumbawanese inhabit the western part of the island and are more or less homogeneous culturally. In the eastern part of the island there are Bimanese, Dompu and Sanggar as well as the hill-dwelling Dou Donggo. The indigenous population is said to be a mixture of 'Proto Malay' and 'Deutero-Malay' elements. Contact with Javanese took place during the period of the Hindu Kingdom of Majapahit in the fourteenth century. By the early seventeenth century a great influence came from Macassar and the ruler was converted to Islam and, even now, most Bimanese are Moslem. Although the number of Bimanese is not known exactly, by 1981 the population of the District of Bima was close to 400,000 (BPS, 1974).

e. Flores

The population of Flores was 1.3 million in 1981 (BPS, 1974) and was mainly composed of 5 major ethnolinguistic groups. They are named after the districts, Manggarai, Ngada, Sika, Endeh and Larantuka. People from the western part of the island are Malay in appearance, while those from the central and eastern part show strong Papuan or Melanesian characters. Before the Dutch occupation, the western part, particularly Manggarai was under the control of Bimanese. At present, through missionary activities, the people of Flores are mostly Christian.

f. Savunese

The Savunese inhabit the island of Savu located between Sumba and Timor, but some of them have migrated to Sumba and Timor. They speak a language closely related to the language of Sumba. Historically the people are believed to have had a connection with the Javanese Kingdom of Majapahit in the fifteenth century. At present, the Savunese are mostly Christian.

g. Rotinese

The island of Roti is the southernmost island of Indonesia, and the Rotinese are traditionally divided into the eastern half and the western half of the island. Many Rotinese have migrated to Timor. Small populations of Rotinese are also found on Sumba and Flores. They speak a language closely related to some of the languages in Timor. Both Dutch and Portuguese had an influence in the island.

h. Timorese

As in Flores there are many ethnolinguistic groups in Timor, and at least 14 different languages are spoken, some of them classified as either Austronesian or Non-Austronesian language. In Kupang, the capital city of the Province, the majority of the people are Kupangese (or Helonese) who are said to be the remnants of a cultural group generally regarded as having once inhabited the whole of southwestern Timor. In addition to the Kupangese, the Atoni are the predominant population of most of western Timor. They inhabit the interior hills and mountain slopes throughout the central and western portions of the island. The next major population, the Tetum, are widespread throughout central Timor.

Contact between Timorese and traders from Java occurred long before Europeans arrived. Contact with Europeans, which started in the seventeenth century, has facilitated the spread of Christianity.

i. Alorese

The island of Alor is inhabited by various groups of people. They can be divided between Moslem coastal people constituted by immigrants from Timor, Kisar and Flores and mountain dwellers comprised of pagans and Christians. The latter may be divided further into various ethnolinguistic groups. The past history of the island is not clearly understood.

j. Macassarese and Buginese

These two ethnic groups represent the majority of the people inhabiting the southern peninsula of Sulawesi. Macassarese are more concentrated in the capital city of Ujung Pandang and nearby areas, while Buginese are spread further to the north. They are similar culturally although they speak a different language. Long before the Dutch captured Macassar in the seventeenth century, both peoples are well known as seafarers. Their influence may be seen in coastal areas throughout the archipelago. Nowadays most Macassarese and Buginese are Moslem. Since marriages between members of those two groups are very common and ethnic affinity is not considered in the census, it is not possible to obtain an accurate number for each group. However, by 1981, it is estimated that the population of the Province of Southern Sulawesi, where those two groups are located, is about 6.8 million (BPS, 1974).

k. Toraja

The Toraja people inhabit the high mountain ranges and interior valleys of central Sulawesi. Although because of relative isolation they are composed of a number of groups, they are all similar culturally. Customarily they are divided into three groups: Western Toraja, Eastern Toraja and Southern Toraja. The latter is influenced primarily by Buginese. Although Islam and Christianity have been introduced to the Toraja, the supernatural beliefs in which ancestor cult plays a prominent part, are strongly in practice. The estimated 1.2 million people for the Province of Central Sulawesi in 1981 (BPS, 1974) more or less represents the Toraja population.

l. Ternatans

The majority of people in the northern Moluccas are Ternatans. They inhabit either the island of Ternate with its principal town Ternate or other islands scattered around Halmahera. Islam was intro-

duced by Javanese merchants in the fifteenth century and, since then, the Ternate Sultanate had a great influence throughout the Moluccan area. Portuguese and Dutch arrived in the sixteenth century and facilitated the spread of Christianity in some areas.

m. Galelarese

Together with its closely related ethnic groups, Tobelorese and Tobaru, Galelarese inhabit the northern portion of Halmahera. Galelarese are also found on other islands such as Morotai, Bacan and Obi.

n. Makianese

Makianese originally inhabit the island of Makian, southwest of Halmahera. Many Makianese have migrated to Ternate, Halmahera and Bacan.

o. Asmat

Asmat is one of several population groups inhabiting the southern coastal plain of Irian Jaya. Detailed study of the people and their ecology have been described by Eyde (1967). During his field-work, the population of Asmat was about 23,000 living in a number of villages along the rivers. Although other anthropological studies are very limited, studies on some genetic markers have been done in this population (Gajdusek *et al.* 1978).

It should be noted that all of the populations studied are speakers of Austronesian languages with the exception of Ternatans and Galelarese from the northern Moluccas and the Asmat from Irian Jaya who speak Non-Austronesian languages.

2.3. Blood collection

During the first and second field trips, disposable syringes (Terumo, Japan) were used. 10 ml blood samples taken were then transferred into lithium heparinized plastic tubes and kept in a polystyrene container packed with ice immediately after drawing before they were taken to the

base laboratory. G-6-PD screening test was carried out using these heparinized blood samples before further processing.

During the third and last field trips, EDTAed venoject vacutainers (Terumo - Japan) were used. Blood samples collected in the field were treated in the same manner as those collected by disposable syringes. Blood samples from Galelarese unfortunately had to be stored in a domestic refrigerator of the local ice producer for a period of 3 to 9 days before being air-freighted to Ternate for separation.

Blood smears were prepared from these venous blood samples for red cell morphology examination.

Separation:

At the base laboratory, plasma was separated by centrifugation at 3000 rpm for 5-6 minutes, transferred into glass vials and frozen in the freezer compartment of a domestic refrigerator. The remaining packed red cells were then washed with an equal volume of cold normal saline. During the first field trip this procedure was repeated three times but, in the subsequent field trips, the red cells were washed only once. Supernatants were removed and the packed red cells transferred into glass vials and frozen in the freezer compartment of a domestic refrigerator until transported, with the exception of samples collected during the first field trip which were stored in a deep freezer at $-20^{o}C$ after separation.

Transportation:

During the first field trip, samples were transported in wet-ice by air to Jakarta where the wet-ice was replaced by dry-ice. They were then air-freighted to Canberra under dry-ice conditions.

During the second field trip, samples collected in Bima were air-freighted to Yogyakarta in wet-ice and then stored in a deep freezer

at -20°C until further transportation. Blood samples collected in Selong were transported by bus to Mataram with wet-ice and stored in the freezer compartment of a domestic refrigerator until air-freighted to Yogyakarta under wet-ice conditions. Blood samples collected in Kupang - Timor were air-freighted to Denpasar with wet-ice and stored overnight in a commercial ice-cream freezer available at Denpasar airport. Next morning they were air-freighted to Yogyakarta with wet-ice and then stored in a deep freezer at -20°C. From Yogyakarta they were air-freighted to Jakarta under wet-ice conditions where the wet-ice was replaced by dry-ice. They were further transported by air to Canberra with dry-ice.

During the third and last field trips, the samples were transported by air in wet-ice to Jakarta. In Jakarta they were stored in a commercial cold storage room for about one week before being air-freighted to Canberra under dry-ice conditions.

In Canberra all blood samples were stored in a freezer room at -20°C until used.

2.4. Laboratory techniques

All blood samples collected were examined in the laboratory in Canberra for electrophoretic variation of three serum proteins, 21 red cell enzymes and haemoglobin. In addition, Glucose-6-phosphate dehydrogenase deficiency was screened in the field during the first and second field trips.

Serum Protein systems	Abbreviation
1. Haptoglobin	Hp
2. Transferrin	Tf
3. Ceruloplasmin	Cp

Red cell enzyme systems	Abbreviation
1. Acid Phosphatase	ACP_1
2. Adenosine Deaminase	ADA
3. Adenylate Kinase	AK
4. Carbonic Anhydrase 1	CA_1
5. Carbonic Anhydrase 2	CA_2
6. Esterase D	ESD
7. Glutamic-Pyruvic Transaminase	GPT
8. Glyoxalase I	GLO I
9. Lactate Dehydrogenase	LDH
10. Malate Dehydrogenase	MDH
11. Peptidase A	PEP A
12. Peptidase B	PEP B
13. 6-Phosphogluconate Dehydrogenase	6-PGD
14. Phosphoglucomutase locus 1	PGM_1
15. Phosphoglucomutase locus 2	PGM_2
16. Phosphoglycerate Kinase	PGK
17. Phosphoglycolate Phosphatase	PGP
18. Phosphohexose Isomerase	PHI
19. Phosphoserine Phosphatase	PSP
20. Superoxide Dismutase	SOD
21. Glucose-6-Phosphate Dehydrogenase	G6PD
Others	
1. Haemoglobin	Hb

Electrophoresis was carried out in horizontal starch gels based on the technique described by Smithies (1955a). In general, gels were prepared using 11.5% - 12% (w/v) hydrolyzed starch (Connaught Medical Research Laboratory, Toronto, Canada) and were electrophoresed between metal ccoling plates at 8-10^{o}C in buffer systems and for times and

voltage gradients as indicated below.

2.4.1. Serum protein systems

Electrophoresis for haptoglobin, transferrin and ceruloplasmin were carried out in the same gel using the discontinuous buffer system described by Ashton and Braden (1961).

Plasma samples treated with haemoglobin solution (one drop of 1% haemoglobin solution in three drops of plasma) were inserted into the gel on 4 x 7 mm strips of Whatman 3 MM chromatography paper.

Buffer system:

Solution A:

Lithium hydroxide	1.2 gm)	
	)	in 1 litre of distilled water
Boric acid	11.8 gm)	

Solution B:

Citric acid	1.6 gm)	
	)	in 1 litre of distilled water
Tris	6.2 gm)	

Solution A was used for electrode buffer. One part of solution A and nine parts of solution B was used for the preparation of gels. Gels were electrophoresed for 4-5 hours at 15 volts/cm (until the "borate front" almost reaches the anode end of the gel).

Following electrophoresis gels were sliced into top and bottom halves; one was stained for ceruloplasmin and haptoglobin and the other for transferrin employing the following methods:

a. Ceruloplasmin and haptoglobin.

2 gm O-dianisidine added to 1 litre of 0.5% acetic acid in water, stirred overnight then filtered.

The gels were incubated in the O-dianisidine solution for 30-60 minutes at 37°C to develop the ceruloplasmin bands which appear on the anodal side of the origin. The addition of 100 volume hydrogen peroxide in the ratio of 1 ml into approximately 150 ml of the

O-dianisidine solution will detect the haptoglobin bands between ceruloplasmin and the origin.

b. Transferrin.

2 gm amido black was stirred into a mixture of 135 ml methanol, 135 ml water and 30 ml glacial acetic acid and then filtered.

Transferrin was stained in the amido black solution for about 5 minutes at room temperature and the gels were decolorized in methanol/water/glacial acetic acid solution in proportions 50 : 50 : 10 (v/v). Dark blue bands of transferrin appear above the haptoglobin zone. For further phenotype discrimination, special agarose gels, 2 mm thick, poured onto 17 x 15 cm glass plates were employed. Gels were prepared using 1% agarose type II (Sigma Chemical Company, St. Louis, USA) in the Ashton-Braden buffer desc-ibed above and 2 µl of sample was applied into slots cut in the gel. Electrophoresis was carried out on cooling plates at a voltage gradient of 20 volts/cm for approximately 3-5 hours.

Following electrophoresis, the gel was put into concentrated picric acid solution for 20 minutes. Excess picric acid was rinsed off with tap water and the gel was then squashed (1 layer wet filter paper, wad paper towel, plate and weight) for 10 minutes and fan dried. The gel was stained with 0.2% Coomassie blue dissolved in methanol/acetic acid/water solution in proportion 9 : 2 : 9 (v/v) for 5 minutes and followed by destaining in 2.5% acetic acid and 2.5% teepol in distilled water. The transferrin bands are clearly recognized as blue bands approximately in the centre of the gel or a little more anodally.

Alternatively vertical gel electrophoresis in E-C vertical gel electrophoresis cell (E-C Apparatus Corporation, Philadelphia) was also employed. Electrophoresis was carried out in acrylamide using

Tris-Glycine buffer system described by Sutton (personal communication).

Buffer system:

Tris 1.60 gm)
Glycine 3.73 gm) bring to 1 litre with distilled water

Sample preparation:

2 drops of plasma

2 drops of 0.25% Ferrous Ammonium Sulphate

5 drops of 10% sucrose with blue dye (Bromo-phenol blue)

Gel preparation:

9.99 gm acrylamide (Sigma, A. 8887)

0.525 gm N,N'-methylenebisacrylamide (Sigma, M. 7256) dissolved in 200 ml above buffer. Add 0.2 gm ammonium persulphate and mix by swirling and let stand about 2-5 minutes. Filter through any medium grade filter paper then add 0.5 ml N,N,N',N'-tetramethyl ethylenediamine (TMED), swirl gently and immediately pour into gel cell prepared with slot former ready. Turn ice water on to circulate through gel cell.

After at least 20 minutes, place cell in vertical position, add buffer to cover electrodes. Prerun at constant voltage (350 volts) with a maximum of 125 mA per gel for 30 minutes. Remove slot former with care, apply 20 µl sample with disposable microsampling pipettes. Run the gel at constant voltage (350 volts) for 3.5-4 hours.

Following electrophoresis the gel was stained in the amido black solution for 15-30 minutes at 37°C and then decolorized overnight in methanol/water/glacial acetic acid solution in proportions 50 : 50 : 10 (v/v). The transferrin bands are clearly recognized as blue bands approximately in the centre of the gel or a little more anodally.

2.4.2. Red cell enzyme systems

Electrophoresis for all red cell enzyme systems was carried out with buffer systems and substrate procedures appropriate to each system.

Haemolysates prepared from thawed frozen packed red cells were inserted into the gels on 4 x 7 mm strips of Whatman 3 MM chromatography paper, except for GPT, PGP and PSP where 7 x 10 mm No. 17 chromatography paper was used. In some systems such as ADA, AK, ESD, GLO 1, GPT, Pep A, Pep B, PGM and G6PD, haemolysates were treated with 1% 2-mercaptoethanol (1 drop in 3 drops of haemolysate) 30 minutes prior to insertion.

Acid Phosphatase.

Electrode buffer:

Citrate-phosphate buffer pH 5.9 as described by Karp and Sutton (1967) with the following composition:

0.245 M $NaH_2PO_4.2H_2O$	38.22 gm
0.150 M Trisodium citrate.$2H_2O$	44.12 gm
0.005 M Na_2 EDTA	1.86 gm

dissolved in 1 litre distilled water

Gel buffer:

Electrode buffer diluted 1 in 100. 0.5 ml 2-mercaptoethanol per 2 gels was added just after degassing. Gels were electrophoresed for 17 hours at a voltage gradient of 6 volts/cm.

Staining:

Following electrophoresis gels were sliced and substrated using 2 mg methyl umbelliferyl phosphate dissolved in a small amount of acetone and 10 ml 1% citric acid (adjusted to pH 5.7 with 10 N NaOH), applied on to the gel using a filter paper overlay. After incubation at 37°C for about 10-15 minutes, gels were read under U.V. light.

Adenosine Deaminase and Adenylate Kinase.

Electrophoresis for red cell ADA and AK was carried out in the same gel, using the buffer system of Kirchberg and Wendt (1970) at pH 6.5.

Electrode buffer:

Solution A: 0.5 M KH_2PC_4 68.045 gm/L H_2O

Solution B: 0.5 M Na_2HPO_4 70.98 gm/L H_2O

For electrode buffer, mix 93 ml solution A and 38 ml solution B in 1 litre of distilled water.

Gel buffer:

Electrode buffer diluted 1 in 10.

ls were electrophoresed for 17 hours at a voltage gradient of 3.5 volts/cm, and the gels were then sliced, one slice being used to stain for ADA and the other for AK.

Substrate.

a. ADA : Adenosine 9 mg

MTT 2 mg

PMS 2 mg

dissolved in 15 ml 0.02 M phosphate buffer pH 7.5.

Nucleoside phosphorylase (130 u/ml) 4 µl

Xanthine oxidase (8.5 u/ml) 10 µl

2% warm agar solution 15 ml

Mixed well and poured over the gel. Gel was then incubated at 37°C for 2 hours.

b. AK : 10 mM Glucose 40 mg

20 mM $MgCl_2.6H_2O$ 80 mg

1 mM ADP 25 mg

0.4 mM NADP 15 mg

PMS 5 mg

MTT 5 mg

dissolved in 10 ml of 0.1 M Tris HCl buffer pH 8.2.

G6PD (370 u/ml)	5 µl
Hexokinase (500 u/ml)	2 µl
2% warm agar solution	10 ml

Mixed well and poured onto the gel. Gel was then incubated for 10-15 minutes.

Carbonic Anhydrase 1 and Carbonic Anhydrase 2.

Carbonic Anhydrase 1 and 2 were electrophoresed in the same gel. The buffer system was that described by Hopkinson *et al.* (1974) and modified by Blake (1978a).

Electrode buffer:

Tris	109.00 gm
EDTA (acid)	5.85 gm
Boric acid	30.9 gm

dissolved in 1 litre distilled water, to be used as a stock solution.

For electrode buffer, the above stock solution was diluted 1 in 14.

Gel buffer:

Stock solution diluted 1 in 60.

Gels were electrophoresed for 1 hours at 5 volts/cm.

Note: Haemolysate samples on 3MM chromatography paper were inserted at centre of the gel, since CA_1 bands run anodally and CA_2 bands run cathodally.

Staining:

Prepare ADA/AK electrode buffer (93 ml of solution A and 38 ml solution B in 1 litre of distilled water) as described in p.40. Use 1 in 5 dilution of this buffer for CA_1 and CA_2 substrate buffer.

a. CA_1:

1 mg of 4-methyl umbelliferyl acetate dissolved in a small quantity of acetone and added 10 ml of substrate buffer. A filter paper was soaked in this substrate and overlayed onto the gel. Gel was incubated at 37°C for 10-15 minutes and read under U.V. light. Fluorescent bands due to CA_1 activity appear at the anodal side of the origin.

b. CA_2:

2mg of fluorescein diacetate dissolved in a small quantity of acetone and added 10 ml of substrate buffer. Mixed with 10 ml of 2% warm agar and poured over the gel. Gel was incubated at 37°C for 10-15 minutes and read under U.V. light. Fluorescent yellow bands due to CA_2 activity appear at the cathodal side of the origin.

Esterase D, Phosphoglucomutase locus 1 and locus 2.

Electrophoresis for red cell ESD was carried out in the same gel with red cell PGM_1 and PGM_2 using buffer system described by Spencer *et al.* (1964).

Electrode buffer:

0.1 M Tris	12.11 gm
0.1 M Maleic acid	11.6 gm
0.01 M Na_2-EDTA	3.72 gm
0.01 M Magnesium sulphate	2.46 gm

dissolved in 1 litre of distilled water and adjusted to pH 7.4 with NaOH (approximately 4.5 gm/litre)

Gel buffer:

Electrode buffer diluted 1 in 15.

Gels were electrophoresed for 17 hours at a voltage gradient of 6.5 volts/cm.

Staining:

Following electrophoresis gel was sliced. The top half was used for ESD staining and the bottom half was used for PGM_1 and PGM_2 staining.

a. ESD.

2.5 mg of 4-methyl umbelliferyl acetate dissolved in a small quantity of acetone and added 10 ml of 0.025 M acetate buffer pH 5.2. A filter paper was soaked in this substrate and overlayed onto the gel. Gel was incubated at 37°C for 10-15 minutes and read under U.V. light. Fluorescent bands due to ESD activity appear at the anodal side of the gel.

b. PGM_1 and PGM_2.

Glucose-1-phosphate*	30 mg
Magnesium sulphate	5 mg
NADP	7 mg
PMS	5 mg
MTT	5 mg
G6PD (370 u/ml)	4 µl

dissolved in 10 ml of 0.1 M Tris HCl buffer pH 8.2. Mixed with 10 ml of 2% warm agar and poured over the gel. The gel was then incubated for 15-30 minutes at 37°C. Dark blue bands due to PGM_1 and PGM_2 activity appear at the anodal side of the origin.

Glutamic-Pyruvic Transaminase.

Electrode buffer:

The PGM buffer system of Spencer *et al.* (1964) is also used for GPT.

* The glucose-1-phosphate used was the Sigma product (Catalogue number G. 1259) which contains 1% glucose-1,6-diphosphate, an essential component in the reaction of PGM..

Gel buffer:

Electrode buffer diluted 1 in 10.

Gels were electrophoresed for 17 hours at a voltage gradient of 7 volts/cm.

Staining:

Following electrophoresis gels were sliced and stained with the following substrate:

0.28 M Alanine	250 mg
0.03 M α-ketoglutarate	44 mg
1.3 mM NADH	9 mg
LDH (10600 u/ml)	20 µl

dissolved in 10 ml 0.3 M Tris HCl buffer pH 8.3.

A Whatman No. 3 filter paper was soaked in this subtrate and overlayed onto the gel for 1-3 hours at 37°C, read under U.V. light. Blue bands due to GPT activity appear at the anodal side of the origin.

Glyoxalase I

Electrode buffer:

The buffer system of Hashimoto *et al.* (1978) was used.

0.1 M Tris	12.11 gm
0.034 M Citric acid	7.14 gm
0.039 M Boric acid	2.14 gm
0.025 M Lithium hydroxide	1.05 gm

dissolved in 1 litre of distilled water and adjusted to pH 7.2.

Gel buffer:

Electrode buffer diluted 1 in 10.

Gels were electrophoresed for 17 hours at a voltage gradient

of 2.3 volts/cm.

Staining:

Following electrophoresis gels were sliced and stained in a two step procedure (Kompf *et al.* 1975).

Step one.

Methyl glyoxal (Pyruvic aldehyde) 0.23 ml

Glutathione (reduced form) 7.5 mg

dissolved in 10 ml of 0.2 M phosphate buffer pH 6.8 (prepared from 5.5 ml of 0.2 M KH_2PO_4 and 4.5 ml of 0.2 M K_2HPO_4).

A Whatman No. 3 filter paper was soaked in this substrate and overlayed onto the gel for 30 minutes at 37°C.

Step two.

18 mg of MTT dissolved in 15 ml of 0.1 M Tris HCl buffer pH 8.5. Dichlorophenol indophenol solution was added until the colour was distinctly blue. Mixed with 15 ml of 2% warm agar and poured over the gel after removing the filter paper. Gel was then incubated at 37°C for 10-15 minutes. Light coloured bands in a dark blue background appear at the anodal side of the gel.

Lactate Dehydrogenase, Malate Dehydrogenase, 6-Phosphogluconate Dehydrogenase and Superoxide Dismutase.

Electrophoresis for red cell LDH was carried out in the same gel with red cell MDH, 6-PGD and SOD in the buffer system of Fildes and Parr (1963).

Electrode buffer:

0.2 M Na_2HPO_4 28 gm

Citric acid 4.6 gm

dissolved in 1 litre of distilled water. pH = 7.0

Gel buffer:

Electrode buffer diluted 1 in 20.

Gels were electrophoresed for 17 hours at a voltage gradient of 4 volts/cm.

Staining:

Following electrophoresis gels were sliced. The top half was used for LDH and MDH staining and the bottom half was used for 6-PGD and SOD staining.

a. MDH.

PMS	2 - 3 mg
MTT	5 mg
NAD	5 mg
0.1 M Na Malate pH 7.2	0.1 ml

dissolved in 10 ml of 0.1 M Tris HCl buffer pH 8.2. Mixed with 10 ml of 2% warm agar and poured over the gel. Gel was incubated for 10-15 minutes at 37°C. Dark blue bands due to MDH activity appear at the anodal side of the origin.

b. LDH.

0.1 ml of 70% Na-Lactate solution dissolved in 10 ml of 0.1 M Tris HCl buffer pH 8.2. Poured over the MDH substrate, incubated for 5 minutes at 37°C. Regions of LDH activity appear as dark blue bands at cathodal and anodal side of the origin.

c. 6-PGD and SOD.

NADP	2 mg
PMS	1 mg
MTT	2 - 3 mg
6-Phosphogluconic acid (trisodium salt)	10 mg

dissolved in 10 ml of 0.1 M Tris HCl buffer pH 8.2

Mixed with 10 ml of 2% warm agar and poured over the gel. Gel was then incubated for 10 minutes at 37°C. Dark blue bands due to 6-PGD activity appear at the anodal side of the origin and colourless bands in the yellow background indicate areas of SOD activity, more anodal than 6-PGD.

Peptidase A and Peptidase B.

The buffer system of Povey *et al.* (1972) was used.

Electrode buffer:

0.1 M Tris	12.11 gm
0.1 M $NaH_2PO_4.2H_2O$	15.6 gm

dissolved in 1 litre of distilled water and adjusted to pH 7.4

Gel buffer:

Electrode buffer diluted 1 in 20.

Gels were electrophoresed for 17 hours at a voltage gradient of 4 volts/cm.

Staining:

Following electrophoresis gels were sliced. The top half was used for PEP B staining and the bottom half was used for PEP A staining. Spread 0.5 ml of 0.1 M $MnCl_2$ on the wet surface of each gel before staining.

a. PEP A.

L-amino acid oxidase	5 mg
Peroxidase	10 mg
O-dianisidine	5 mg
L-valyl leucine	20 mg

dissolved in 10 ml of 0.1 M Tris HCl buffer pH 8.2.

Mixed with 10 ml of 2% warm agar and poured over the gel, incubated at 37°C for 30-60 minutes. Brownish bands due to PEP A activity appear at the anodal side of the origin.

b. PEP B.

L-amino acid oxidase	5 mg
Peroxidase	10 mg
O-dianisidine	5 mg
L-leucyl glycyl glycine	20 mg

dissolved in 10 ml of 0.1 M Tris HCl buffer pH 8.2

Mixed with 10 ml of 2% warm agar and poured over the gel, incubated at 37°C for 10-15 minutes. Brownish bands due to PEP B activity appear at the anodal side of the origin.

Phosphoglycerate Kinase and Phosphohexose Isomerase.

Electrophoresis for red cell PGK and PHI was carried out in the same gel. Samples were inserted at the centre of the gel and the buffer system of Chen *et al.* (1971) was used.

Electrode buffer:

0.1 M Tris	12.11 gm
Citric acid	6.15 gm

dissolved in 1 litre of distilled water. pH = 7.5

Gel buffer:

Electrode buffer diluted 1 in 10.

Gels were electrophoresed for 17 hours at 8 volts/cm

Staining:

Following electrophoresis gels were sliced. Either the top half or the bottom half was used for PGK or PHI.

a. PGK.

3-Phosphoglyceric acid (sodium salt)	12 mg
ATP	25 mg

Magnesium chloride	12 mg
Na_2EDTA	22 mg
Glyceraldehyde-3-phosphate dehydrogenase (880 u/ml)	10 µl

dissolved in 10 ml of 0.1 M Tris HCl buffer pH 8.2

A filter paper was soaked in this substrate and placed over the gel, incubated at 37°C for 15 minutes and read under U.V. light. Blue bands due to PGK activity appear anodal to the origin.

b. PHI.

Fructose-6-phosphate	6 mg
MTT	1.5 mg
PMS	1 mg
NADP	1-2 mg
G-6-PD (370 u/ml)	1 µl

dissolved in 10 ml of 0.1 M Tris HCl buffer pH 8.2

Mixed with 10 ml of 2% warm agar and poured over the gel, incubated at 37°C for 15 minutes. Dark blue bands due to PHI activity appear at the cathodal side of the gel, more cathodally than haemoglobin bands.

Phosphoglycolate Phosphatase and Phosphoserine Phosphatase.

Electrophoresis of red cell PGP and PSP was carried out in the same gel using the buffer system described by Barker and Hopkinson (1978).

Electrode buffer:

0.1 M Tris	12.11 gm
0.1 M Maleic acid	11.6 gm
0.01 M Na_2EDTA	3.72 gm
0.01 M Magnesium sulphate	2.46 gm

dissolved in 1 litre of distilled water and adjusted to pH 7.2 with NaDH (approximately 4.5 gm NaOH/litre).

Gel buffer:

Electrode buffer diluted 1 in 10. 1 ml of 2-mercaptoethanol per 2 gels was added just after degassing. Gels were electrophoresed for 17 hours at a voltage gradient of 6 volts/cm.

Staining:

Following electrophoresis gels were sliced. The top half was used for PSP staining and the bottom half was used for PGP staining. Both systems were stained in 2 steps.

Step one.

a. PGP.

Phosphoglycolic acid	5 mg
Magnesium sulphate	2 mg

dissolved in 10 ml of 0.1 M Tris HCl buffer pH 7.5.

A Whatman No. 3 filter paper was soaked in this substrate and placed over the gel, incubated for 37°C for 2-3 hours.

b. PSP.

Phospho-L-serine	25 mg
Magnesium sulphate	2 mg

dissolved in 10 ml of 0.1 M Tris HCl buffer pH 7.5.

A Whatman No. 3 filter paper was soaked in this substrate and placed over the gel, incubated at 37°C for 2-3 hours.

Step two.

0.75 g ascorbic acid dissolved in 10 ml of 2.5% (w/v) ammonium molybdate in 4 N H_2SO_4. A filter paper was soaked in this substrate and placed over PGP or PSP gel after removing filter paper of the first step staining. Dark blue bands due to PGP or PSP activity appear at the anodal side of the gel.

2.4.3. Other systems

Haemoglobin and Glucose-6-Phosphate Dehydrogenase.

Electrophoresis for Hb and G6PD was carried out in the same gel using the same tris-EDTA-borate stock buffer described under Carbonic Anhydrase (p.41).

Electrode buffer:

For the anode side, stock buffer diluted 1 in 7.

For the cathode side, stock buffer diluted 1 in 5 and 4 mg of NADP was added.

Gel buffer:

Stock buffer solution diluted 1 in 20. 4 mg of NADP per gel was added just after degassing. Gels were electrophoresed for 17 hours at 4 volts/cm.

Staining:

a. G6PD.

Glucose	10-15 mg
PMS	0.5-0.7 mg
MTT	5 mg
NADP	2 mg

dissolved in 10 ml of Tris HCl buffer pH 8.2.

Mixed with 10 ml of 2% warm agar and poured over the gel, incubated at 37°C for 30 minutes. Dark blue bands due to G6PD activity appear more anodally than haemoglobin A bands.

b. Haemoglobin.

Haemoglobin bands were visualised without staining.

G6PD screening test.

(Beutler and Mitchell, 1968)

Reaction mixture.

The following reaction mixture was prepared and stored at -20°C in a frozen state in quantities of 10 ml per vial:

1 part 0.01 M Glucose-6-phosphate

1 part 0.0075 M NADP

2 parts 1.0% Saponin

1 part 0.008 M Oxidised gluathione

3 parts 0.75 M Tris HCl buffer pH 7.8

2 parts Distilled water,

Screening procedure:

10 µl of blood was added to 100 µl of the reaction mixture and mixed well. The mixture was spotted on Whatman No. 1 filter paper at various intervals of time (0, 5, 10, 15 and 20 minutes) and dried in a warm current of air after each spotting. It was then examined visually under long wave U.V. light. Normal G6PD samples show a bright fluorescence, while G6PD deficient samples show little or no fluorescence.

2.5. Statistical methods

2.5.1. Gene frequency calculation

With the exception of PGK and G6PD which are under X-linked genetic control, all other loci studied are controlled by autosomal codominant alleles. Therefore, gene frequencies were calculated directly by gene counting from phenotype frequencies available. The Chi-square test was used to determine any deviation from the assumption of Hardy-Weinberg equilibrium for a random mating population (Cavalli-Sforza and Bodmer, 1971). For the two X-linked loci, PGK was invariant in the populations studied, whilst for G6PD the gene frequency for males was taken as the proportion of males with deficiency. Because of the difficulty of

detecting heterozygote females no estimate of gene frequency in females was made.

2.5.2. Genetic distance

The genetic information collected was used to estimate genetic distance between populations. Several methods are available for this purpose (see Nei, 1975 for a recent review). Since the results of the various methods have been shown to give very similar results in practice (Chakraborty, 1976 and Kirk, 1977), I have employed the genetic distance measure devised by Nei (1972).

Nei's genetic distances were estimated using the computer program NEIDIS available in the Department of Human Biology. Similarly, dendrograms and eigenvector diagrams were drawn using the computer program TOPOL ARBOR also available in the Department of Human Biology. I am indebted to Dr S.W. Serjeantson for help in using these programs.

CHAPTER 3

RED CELL ENZYME AND SERUM PROTEIN VARIATIONS IN INDONESIA

SECTION A. RED CELL ENZYME MARKERS

3.1. Introduction

During the present study, red cell enzyme data for a number of Indonesian populations will be reported. Out of 20 red cell enzyme loci investigated nine loci were found to be polymorphic, i.e. acid phosphatase, adenosine deaminase, adenylate kinase (in one population only), esterase D, glyoxalase I, glutamic-pyruvic transaminase, 6-phosphogluconate dehydrogenase, phosphoglucomutase locus 1 and phosphoglucomutase locus 2. Five loci had rare variants, i.e. carbonic anhydrase 1, carbonic anhydrase 2, malate dehydrogenase, peptidase B and phosphohexose isomerase. The other six loci were monomorphic.

3.2. Red cell enzymes with polymorphic variation

3.2.1. Acid phosphatase

Acid phosphatase (ACP; E.C. 3.1.3.2) catalyses the reaction of an ortho-phosphate monoester + H20 $\rightleftarrows$ an alcohol + orthophosphate. This type of enzyme activity has been found in red cells as well as in other tissues (Lundin and Allison, 1966; Beckman and Beckman, 1967; Swallow and Harris, 1972). The particular form of activity of the human 'red cell' acid phosphatase (ACP_1) has also been detected in some other tissues but in much lower concentration (Blake *et al.* 1973c; Swallow *et al* 1973).

Human red cell ACP_1 polymorphism was first discovered by Hopkinson

et al. (1963, 1964) in Caucasians and they suggested the variation was controlled by three autosomal alleles $\underline{ACP_1}^A$, $\underline{ACP_1}^B$ and $\underline{ACP_1}^C$. Five out of the six expected phenotypes were observed, A, AB, B, BC and AC respectively. Further discovery of the homozygous C phenotype (Lai *et al.* 1964; Radam and Strauch, 1966) confirmed the three codominant allele hypothesis. Since then the phenotype distribution has been reported for various populations (Bhasin and Fuhrman, 1972). Sorensen (1973) compared the distribution of ACP gene frequencies among Caucasian populations. The relatively high frequency of the $\underline{ACP_1}^C$ gene in Caucasians suggests that this gene is a Caucasian gene (Giblett and Scott, 1965; Scott *et al.* 1966) and its occurrence in other populations may be due to admixture with Caucasians.

In addition to these three alleles, two other rare alleles have been reported. These alleles which were found in combination with the common allele $\underline{ACP_1}^B$ were $\underline{ACP_1}^D$ (Karp and Sutton, 1967) and $\underline{ACP_1}^E$ (Sorensen, 1975). Another allele which was first reported from a Negro woman, $\underline{ACP_1}^R$, in combination with $\underline{ACP_1}^A$ (Giblett and Scott, 1965) was later found to be common in Negro populations of South Africa (Jenkins and Corfield, 1972), among the Khoisan peoples or related populations in particular. Herbich *et al.* (1970) reported a silent allele, $\underline{ACP_1}^O$, discovered in a Caucasian family from Vienna. Its occurrence was supported by family study as well as by quantitative enzyme assay.

The locus controlling ACP_1 has been assigned to chromosome 2 (Ferguson-Smith *et al.* 1973).

The ACP_1 gene frequencies in the population of Indonesia have been reported for Irian Jaya populations (Gajdusek *et al.* 1978 and Kirk *et al.* 1973), Toba Batak (McDermid *et al.* 1973) and Balinese (Breguet *et al.* 1982b). Unpublished results are also available for Niasans (Blake, personal communication).

In the present study the common alleles of the ACP_1 locus, $\underline{ACP_1}^A$ and $\underline{ACP_1}^B$ were found in all populations. The gene frequencies and phenotype distributions are shown in table 3.1.a. The $\underline{ACP_1}^B$ gene frequency varies from 53.4% in Rotinese to 73.4% in Macassarese. The observed phenotype distribution is in agreement with Hardy-Weinberg equilibrium expectation, except for Javanese who show a deviation at the $P = 0.05$ level. As shown in table 3.1.b, in Balinese the $\underline{ACP_1}^B$ gene frequency variation is greater, ranging from 56.3% in Dauh Waru to 87.5% in Songan. The phenotype distribution is also in agreement with Hardy-Weinberg equilibrium except for Dauh Waru and Beratan which show significant deviations each with $P<0.02$. The latter sample, however, is very small and may not be random.

Compared to the data from neighbouring populations (see table 3.1.c) it seems that $\underline{ACP_1}^B$ gene frequency in Indonesian populations is in the range of frequencies for other South East Asian populations from Malaysia, Thailand and the Philippines, except for the Negrito population whose $\underline{ACP_1}^B$ gene frequency reaches 85.6% (Omoto *et al.* 1978). High frequencies of $\underline{ACP_1}^B$ also are found in Irian Jaya towards the east and south (e.g. Dani), Manus island, Santa Cruz and Western Caroline islands as well as among the Aborigines of Australia.

It is worth noting that only a single $\underline{ACP_1}^C$ allele was found in a single Macassarese in the heterozygous form with $\underline{ACP_1}^B$. It has not been found in other populations of Indonesia. So far, in the South East Asia and Pacific regions, $\underline{ACP_1}^C$ allele has only been found in Malays (Lai and Kwa, 1968) and in New Guinea natives from Oksapmim and Trobriand island (Lai, 1966). Among east Asians, higher frequencies of $\underline{ACP_1}^C$ have been reported for the Japanese (Shinoda, 1967). However, this finding was later questioned since it was not found in the subsequent investigation by the same author (Shinoda, 1969). A rather surprising discovery of a

Table 3.1.a.

Distribution of Acid Phosphatase (ACP) phenotypes and gene frequencies in Indonesian populations

Population	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1 *
		A	AB	B	ACP^A	ACP^B	
Javanese (urban)	203	42	86	75	.4187	.5813	3.4167
Javanese (rural)	204	37	92	75	.4069	.5931	0.8781
Javanese (total)	407	79	178	150	.4128	.5872	3.8971
Balinese	970	101	414	455	.3175	.6825	0.2254
Sasak	191	22	85	84	.3377	.6623	0.0050
Bimanese	198	16	86	96	.2980	.7020	0.2884
Florenese	76	9	35	32	.3487	.6513	0.0147
Savunese	36	3	15	18	.2917	.7083	0.0025
Rotinese	59	12	31	16	.4661	.5339	0.1830
Timorese	133	11	60	62	.3083	.6917	0.4442
Alorese	26	5	10	11	.3846	.6154	0.9141
Macassarese	199†	17	71	110	.2638	.7337	1.6284
Buginese	205	25	92	88	.3463	.6537	0.0160
Toraja	101	6	43	52	.2723	.7277	0.5581
Ternatans	213	21	85	107	.2981	.7019	0.4591
Makianese	30	4	14	12	.3667	.6333	0.0007
Galelarese	155	30	75	50	.4355	.5645	0.0391
Asmat	163	24	87	52	.4141	.5859	1.6281

* d.f=2 for Macassarese

† including 1 ACP BC; ACP^C = 0.0025

Table 3.1.b.

Distribution of Acid Phosphatase (ACP)

phenotypes and gene frequencies in

Balinese populations

Village	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1
		A	AB	B	ACP^A	ACP^B	
Dauh Waru	80	10	50	20	.4375	.5625	5.8251
Penebel	58	7	31	20	.3879	.6121	0.9136
Ayunan	69	5	28	36	.2754	.7246	0.0196
Renon	95	8	54	33	.3684	.6316	4.6579
Serangan	39	7	17	15	.3974	.6026	0.3153
Banjar Rangkan	48	9	21	18	.4062	.5938	0.4162
Ped	34	0	13	21	.1912	.8088	1.8995
Rendang	124	17	52	55	.3468	.6532	0.6857
Serava	85	8	38	39	.3176	.6824	0.0832
Songan	116	2	25	89	.1250	.8750	0.0253
Julah	49	4	15	30	.2347	.7653	1.0708
Sembiran	101	12	45	44	.3416	.6584	0.0091
Beratan	32	7	8	17	.3437	.6563	6.3621
Wanayu	40	5	17	18	.3375	.6625	0.0985

Table 3.1.c.

Additional Acid Phosphatase (ACP) gene frequency data in Indonesia and neighbouring areas

Population	Number Tested	Gene Frequency ACP^A	Gene Frequency ACP^B	Reference
Indonesia				
Batak (Toba)	187	.2406	.7594	McDermid *et al.* 1973
Niasans	192	.2917	.7083	Blake (unpublished)
Balinese	316	.3592	.6408	Breguet *et al.* 1982b
Asmat	650	.231	.769	Gajdusek *et al.* 1978
Awyu	501	.342	.658	-ditto-
Kayagar	71	.247	.754	-ditto-
Moni	320	.204	.796	-ditto-
Ekagi	26	.385	.615	-ditto-
Lake Plain	245	.212	.788	-ddito-
Dani	257	.1809	.8191	Kirk *et al.* 1973
Singapore, Malaysia and Philippines				
Malays (Singapore)	259	.3282	.6718	Blake *et al.* 1973a
Chinese "	378	.2143	.7857	-ditto-
Malays "	260	.346	.654	Lai and Kwa, 1968
Chinese "	620	.222	.778	-ditto-
Kadazans	264	.3693	.6307	Tan *et al.* 1979
Negrito	129	.1445	.8555	Omoto *et al.* 1978
Tagalog	253	.2800	.7200	-ditto-
Thailand				
Bangkok	94	.2606	.7394	Present study

Table 3.1.c. Cont'd

Population	Number Tested	Gene Frequency ACP^A	Gene Frequency ACP^B	Reference
Australia				
Aborigines				
Elcho Is.	35	.0000	1.0000	Kirk *et al.* 1971
Bathurst Is.	104	.014	.986	-ditto-
Mowanjum	244	.0184	.9815	Blake, 1979
Papua New Guinea				
Manus Is.	183	.1749	.8251	Malcolm *et al.* 1972
Motu	754	.1479	.8521	Blake (unpublished)
Western Pacific area				
Solomon Islands				
Santa Cruz Is.	350	.1029	.8971	Mazzur and Blake, 1981
Trust Territory of Pacific Islands				
Western Caroline Is.	382	.0602	.9398	Blake *et al.* 1973b
Fiji				
Fijians	332	.2485	.7515	Blake (unpublished)

very high frequencies of $\underline{ACP_1^R}$ and $\underline{ACP_1^C}$ in the Philippines was reported by Bayani-Sioson (1971). Again this result seems to be doubtful since Omoto *et al.* (1978) found neither $\underline{ACP_1^C}$ or $\underline{ACP_1^R}$ in Negrito or non-Negrito populations of the Philippines.

Since the $\underline{ACP_1^C}$ gene is regarded to be a Caucasian gene (Giblett and Scott, 1965; Scott *et al.* 1966), its occurrence in the Macassarese may also be related to Caucasian influence. This assumption is not impossible since the region has experienced European and/or Indian contact for a long period of time. In the case of the $\underline{ACP_1^R}$ gene, which is very common in South Africa, its absence in the populations of Indonesia is not surprising since no evidence of contact between those populations has been recorded.

3.2.2. Adenosine deaminase

Adenosine deaminase (ADA; E.C. 3.5.4.4) catalyses the deamination of adenine to inosine:

$$\text{Adenosine} + H_2O \rightleftarrows \text{inosine} + NH_3$$

Its activity has been detected in various human tissues (Edwards *et al.* 1971) and the most active tissues are the spleen, intestine and red blood cells.

In human red cells, three common electrophoretic phenotypes were described by Spencer *et al.* (1968) and designated ADA 1-1, ADA 2-2 and ADA 2-1, determined by codominant autosomal allelic genes called $\underline{ADA^1}$ and $\underline{ADA^2}$. There is evidence that the locus is located on chromosome 20 (Tischfield *et al.* 1974).

A rare phenotype, ADA 3-1, has been described in an English family (Hopkinson *et al.* 1969) and in a Danish family (Dissing and Knudsen, 1969), but the two variants showed different electrophoretic patterns. In the phenotype ADA 3-1 from the English family, in addition to all

components normally seen in type ADA 1-1, there was a very weak slower isozyme migrating in about the same position as the slowest isozyme of the usual ADA 2-1 or ADA 2-2 pattern. In the Danish variant, in addition to all components normally seen in type ADA 2-1, there were bands slightly slower and slight faster but with less intensity than the slowest isozyme of the ADA 2 pattern.

Another phenotype, ADA 5-1, was reported in two Black Americans living in Seattle (Detter *et al.* 1970) showing a different electrophoretic pattern to the two variants previously reported. Another rare variant, ADA 6-1, was also reported (Radam *et al.* 1974) and later the heterozygous form of $\underline{ADA}^5$ and $\underline{ADA}^2$ gene product (ADA 5-2) was reported in a Czechoslovakian family (Radam *et al.* 1975). Berg *et al.* (1975) reported $\underline{ADA}^7$ in a Bavarian family and Kirk *et al.* (1977) reported a single example of $\underline{ADA}^8$ in a Turkoman speaking person from Iran.

It has been observed that when a haemolysate is stored at $4^{\circ}C$ for some days, changes in the ADA isozyme pattern take place. It was suggested that each ADA isozyme had at least one free reactive sulphhydryl (-SH) group which was responsible for the change of electrophoretic pattern when it bound to oxidized glutathione, which increases on storage of haemolysates (Hopkinson and Harris, 1969).

Giblett *et al.* (1972) reported the presence of immunological deficiency with no measurable red cell ADA enzyme activity in two young unrelated girls. More severe combined immunodeficiency syndrome with lack of red cell ADA activity was also reported in a baby girl (Dissing and Knudsen, 1972). Since then the association between combined immunodeficiency disease and the absence of red cell ADA activity, has been reported by various authors (Ochs *et al.* 1973; Yount *et al.* 1974; Chen *et al.* 1974) and a method for large scale screening has been developed (Moore and Meuwissen, 1974). Brinkmann *et al.* (1973), however, des-

cribed a silent allele, $\underline{ADA}^{O}$ in a German family with no clinical abnormalities and Jenkins (1973) reported red cell ADA deficiency with only a slight impairment of cellular immunity. However, a workshop on this matter indicated that the coexistence of ADA deficiency and combined immunodeficiency disease represented a highly significant relationship (Meuwissen *et al.* 1975).

Apart from this clinical aspect of ADA, the phenotype distributions in different populations is also of interest. When the ADA polymorphism was first described (Spencer *et al.* 1968) the $\underline{ADA}^{2}$ gene frequency distribution varied in three different populations i.e. 0.06, 0.03 and 0.11 in Europeans, Negroes and Asiatic Indians respectively. It seems that the $\underline{ADA}^{2}$ gene frequency in Asians is the highest in comparison with other populations.

In Indonesia, the ADA gene frequency has been reported previously for the population of Irian Jaya (Kirk *et al.* 1973; Omoto, 1972), Toba batak (McDermid *et al.* 1973) and Bali (Breguet *et al.* 1982b). An unpublished result is also available for Niasans (Blake, personal communication).

In the present study the ADA gene frequency and phenotype distribution in various Indonesian populations is shown in table 3.2.a. Two of the series, Timorese and Balinese, show departure from Hardy-Weinberg equilibrium. The departure in both cases is due to the excess of ADA 2-2 over expected homozygotes in a cell where the numbers are small. Further study would be needed to assess the real validity of these departures from expectation. The $\underline{ADA}^{2}$ gene frequency distribution ranges from 4.8% in Bimanese to 15.3% in Rotinese. As shown in table 3.2.b, its distribution in the Balinese varies even more, from 0.7% in Ayunan village to 15.4% in Serangan village. However, the average for Balinese (5.2%) falls within the range of Indonesian populations. The

Table 3.2.a.

Distribution of Adenosine Deaminase (ADA)

phenotypes and gene frequencies in Indonesian populations

Population	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1
		1-1	2-1	2-2	ADA^1	ADA^2	
Javanese (urban)	203	173	30	-	.9261	.0739	1.2945
Javanese (rural)	204	176	28	-	.9314	.0686	1.1081
Javanese (total)	407	349	58	-	.9287	.0713	2.3956
Balinese	970	876	87	7	.9479	.0521	8.0939
Sasak	191	165	24	2	.9267	.0733	1.0761
Bimanese	198	179	19	-	.9520	.0480	0.5029
Florenese	76	64	12	-	.9211	.0789	0.5584
Savunese	36	31	5	-	.9306	.0694	0.2005
Rotinese	59	43	14	2	.8475	.1525	0.3989
Timorese	133	116	13	4	.9211	.0789	14.2992
Alorese	26	23	3	-	.9423	.0577	0.0975
Macassarese	199	169	27	3	.9171	.0829	2.3145
Buginese	205	182	22	1	.9415	.0585	0.1422
Toraja	101	85	14	2	.9109	.0891	2.1569
Ternatans	213	166	44	3	.8826	.1174	0.0019
Makianese	30	27	3	-	.9500	.0500	0.0831
Galelarese	155	127	26	2	.9032	.0968	0.2539

Table 3.2.b.

Distribution of Adenosine Deaminase (ADA)

phenotypes and gene frequencies in Balinese populations

Village	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1
		1-1	2-1	2-2	ADA^1	ADA^2	
Dauh Waru	80	70	9	1	.9313	.0687	1.1793
Penebel	58	52	5	1	.9397	.0603	3.3364
Ayunan	69	68	1	-	.9928	.0072	0.0037
Renon	95	86	9	-	.9526	.0474	0.2349
Serangan	39	29	8	2	.8462	.1538	1.7548
Banjar Rangkan	48	42	6	-	.9375	.0625	0.2133
Ped	34	27	7	-	.8971	.1029	0.4477
Rendang	124	115	9	-	.9637	.0363	0.1758
Seraya	85	79	6	-	.9647	.0353	0.1138
Songan	116	107	8	1	.9569	.0431	3.1186
Julah	49	43	5	1	.9286	.0714	2.6095
Sembiran	101	95	6	-	.9703	.0297	0.0947
Beratan	32	30	1	1	.9531	.0469	13.5314
Wanayu	40	33	7	-	.9125	.0875	0.3678

Table 3.2.c.

Additional Adenosine Deaminase (ADA) gene frequency data in Indonesia and neighbouring areas

Population	Number Tested	Gene Frequency ADA^1	Gene Frequency ADA^2	Reference
Indonesia				
Batak (Toba)	188	.9122	.0878	McDermid *et al.* 1973
Niasans	192	.9505	.0495	Blake (unpublished)
Balinese	316	.8908	.1092	Breguet *et al.* 1982b
Dani	258	.9031	.0969	Kirk *et al.* 1973
West Irian	149	.8524	.1477	Omoto, 1972
Malaysia and Philippines				
Malays	256	.885	.115	Welch *et al.* 1975
Kadazans	283	.9417	.0583	Tan *et al.* 1979
Land Dayaks	283*	.840	.154	Ganesan *et al.* 1976
Sea Dayaks	188†	.907	.090	-ditto-
Negrito	129	.8953	.1047	Omoto *et al.* 1978
Tagalog	253	.9200	.0800	-ditto-
Thailand				
Bangkok	94	.8883	.1117	Present study
Australia				
Aborigines				
Elcho Is.	232	.976	.024	Omoto, 1972
Mowanjum	244	.998	.002	Blake, 1979
Papua New Guinea				
Manus Is.	181	.9337	.0663	Malcolm *et al.* 1972
Motu	754	.9688	.0312	Blake (unpublished

Table 3.2.c. Cont'd

Population	Number Tested	Gene Frequency ADA[1]	Gene Frequency ADA[2]	Reference
Western Pacific area				
Micronesia				
Ulithi Atoll	374	.9826	.0174	Omoto, 1972
Trust Territory of Pacific Islands				
Western Caroline Is.	382	.9829	.0171	Blake *et al.* 1973b

* 1 ADA 5-1 variant

† 1 ADA 5-1 and 1 other variant

phenotype distributions in Bali fit well to Hardy-Weinberg equilibrium except for Beratan, where the departure is due entirely to the very small expectation in the ADA 2-2 category.

As shown in table 3.2.c, in other South East Asian populations from Malaysia, Thailand and the Philippines, $\underline{ADA}^2$ frequencies are comparable to those in Indonesian populations. Very low frequencies are found in Micronesia and in the Aborigines of Australia. From the viewpoint of the $\underline{ADA}^2$ frequency, apart from most Negro populations studied so far, $\underline{ADA}^2$ in the Aborigines of Australia is among the lowest in the world. In the Indonesian populations its frequency is still comparable to other Asian populations studied so far (see Bhasin and Fuhrman, 1972).

3.2.3. Adenylate kinase

Adenylate kinase (AK; E.C. 2.7.4.3) or myokinase, catalyses the reversible reaction of ATP + AMP $\rightleftharpoons$ 2 ADP. The enzyme occurs in various tissues; however, there are at least two different sets of isozymes found within an individual. The AK from muscle, erythrocytes and brain are different from those from liver, kidney, spleen and heart (Khoo and Russel, 1972). Further study revealed that there were at least six different adenylate kinase isozymes from six different organs which showed an organ specificity (Russel *et al.* 1974). Studies by Wilson *et al.* (1976) suggested the presence of a third locus, AK_3, in addition to two loci described by Khoo and Russel (1972) designated as AK_1 and AK_2 loci.

Human variation of the red cell AK was characterized on starch gel electrophoresis by Fildes and Harris (1966). Three phenotypes, AK 1, AK 2-1 and AK 2 were reported, determined by codominant autosomal alleles, $\underline{AK}^1$ and $\underline{AK}^2$ (Bowman *et al.* 1967). The AK_1 locus has been assigned to chromosome 9 together with the AK_3 locus (Povey *et al.* 1976).

Rare variants, AK 3-1 and AK 4-1, were later reported by Bowman *et al.* (1967). The electrophoretic pattern of AK 3-1 is characterized

by a strong more anodal band in addition to the normal AK 1 band and the AK 4-1 variant is characterized by a single band in the AK 1 location and another band approximately half way between the AK 1 and AK 2 position. Seger *et al.* (1978) reported a polymorphic frequency for the $\underline{AK}^3$ allele in the Wayampi population of French Guiana.

Another rare variant, AK 5-1, described by Santachiara-Benerecetti *et al.* (1972) was found in a non-tribal Indian population. It was characterized by a strong band more cathodal than the AK 2 band. The existence of a possible silent allele has also been suggested (Singer and Brock, 1971) in a patient with half normal specific activity of red cell adenylate kinase.

So far, very limited data has been reported for AK variation in Indonesian populations.

In the present study only 2 phenotypes, AK 1 and AK 2-1, are found restricted to some populations as shown in table 3.3.a. The $\underline{AK}^2$ allele reaches polymorphic frequency only in Ternatans with a frequency of 1.9%. In the other populations it ranges from 0.3% in Javanese to 0.7% in Florenese. In Bali (table 3.3.b) variation on this locus is found in five villages, $\underline{AK}^2$ being polymorphic in Serangan (1.3%), Wanayu (1.3%) and Banjar Rangkan (1.0 %). The phenotype distribution in Balinese as well as in other Indonesian populations is in agreement with Hardy-Weinberg equilibrium expectation. The average $\underline{AK}'$ gene frequency for Balinese as a whole is 0.26%. In the previous study, Breguet *et al.* (1982b) reported an $\underline{AK}^2$ gene frequency of 0.3% in Balinese from Tenganan Pageringsingan, which is comparable to the present study.

The $\underline{AK}^2$ allele also has been regarded as a Caucasian marker (Tills *et al.* 1970a) and its highest frequency is achieved in populations from the Indian subcontinent where a gene frequency of 13.1% was reported for the population of Sugali in Andhra Pradesh (Blake *et al.*1981).

Table 3.3.a.

Distribution of Adenylate Kinase (AK) phenotypes and gene frequencies in Indonesian populations

Population	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1
		1-1	2-1	2-2	AK^1	AK^2	
Javanese (rural)	204	202	2	-	.9951	.0049	0.0049
Javanese (total)	407	405	2	-	.9975	.0025	0.0025
Balinese	970	965	5	-	.9974	.0026	0.0068
Sasak	191	189	2	-	.9948	.0052	0.0053
Bimanese	198	197	1	-	.9975	.0025	0.0013
Florenese	76	75	1	-	.9934	.0066	0.0033
Ternatans	213	205	8	-	.9812	.0188	0.0780
Galelarese	155	154	1	-	.9968	.0032	0.0016
Buginese	205	204	1	-	.9976	.0024	0.0012

Table 3.3.b.

Distribution of Adenylate Kinase (AK)

phenotypes and gene frequencies in Balinese populations

Village	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1
		1-1	2-1	2-2	AK^1	AK^2	
Ayunan	69	68	1	-	.9928	.0072	0.0037
Serangan	39	38	1	-	.9872	.0128	0.0066
Banjar Rangkan	48	47	1	-	.9896	.0104	0.0053
Seraya	85	84	1	-	.9941	.0059	0.0030
Wanayu	40	39	1	-	.9875	.0125	0.0064
Others	689	689	-	-	1.0000	.0000	0.0000
Total	970	965	5	-	.9974	.0026	0.0016

Table 3.3.c.

Additional Adenylate Kinase (AK) gene frequency data in Indonesia and neighbouring areas

Population	Number Tested	Gene Frequency AK^1	Gene Frequency AK^2	Reference
Indonesia				
Batak (Toba)	188	.9974	.0026	McDermid *et al.* 1973
Niasans	192	1.0000	-	Blake (unpublished)
Balinese	316	.9968	.0032	Breguet *et al.* 1982b
Asmat	653	1.0000	-	Gajdusek *et al.* 1978
Awyu	505	1.0000	-	-ditto-
Kayagar	71	1.0000	-	-ditto-
Moni	320	1.0000	-	-ditto-
Ekagi	26	1.0000	-	-ditto-
Lake Plain	245	1.0000	-	-ditto-
Dani	258	1.0000	-	Kirk *et al.* 1973
Singapore, Malaysia and Philippines				
Malays (Singapore)	259	.9846	.0154	Blake *et al.* 1973 a
Chinese "	378	1.0000	-	-ditto-
Malayan Aborigines	483	.987	.013	Welch *et al.* 1971
Malays	324	.985	.015	-ditto-
Malays	385	.9813	.018	Chan, 1971
Kadazans	257	1.0000	-	Tan *et al.* 1979
Negrito	129	.9302	.0698	Omoto *et al.* 1978
Tagalog	253	.9960	.0040	-ditto-
Thailand				
Bangkok	94	.9840	.0160	Present study

Table 3.3.c. Cont'd

Population	Number Tested	Gene Frequency AK^1	Gene Frequency AK^2	Reference
Australia				
Aborigines				
Groote Eylandt	90	1.0000	-	Kirk *et al.* 1971
Elcho Is.	35	1.0000	-	-ditto-
Bathurst Is.	104	1.0000	-	-ditto-
Mowanjum	244	1.0000	-	Blake, 1979
Papua New Guinea				
Manus IS.	183	1.0000	-	Malcolm *et al.* 1972
Motu	752	1.0000	-	Blake (unpublished)
Western Pacific area				
Fiji				
Fijians	332	1.0000	-	-ditto-

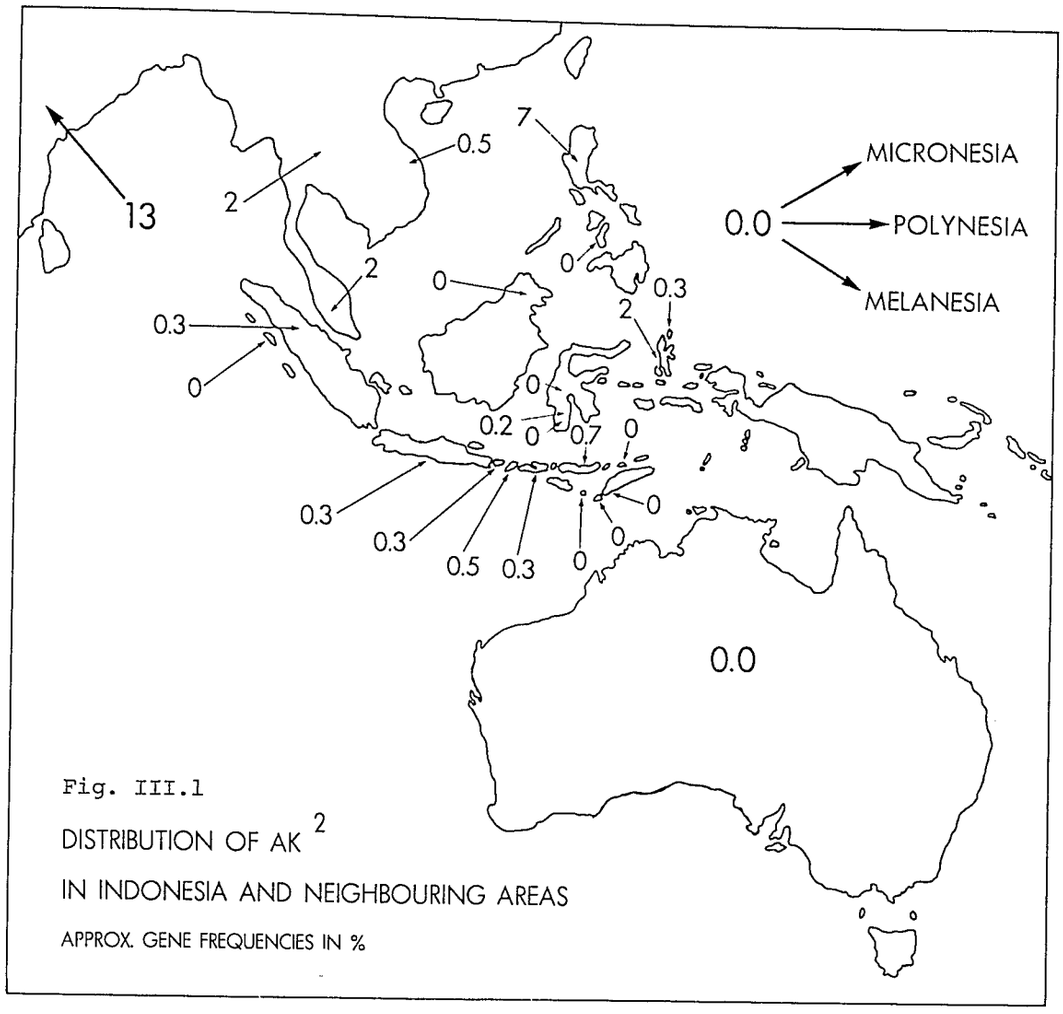


Fig. III.1

DISTRIBUTION OF AK ^{2}

IN INDONESIA AND NEIGHBOURING AREAS

APPROX. GENE FREQUENCIES IN %

The $\underline{AK}^2$ allele is absent in Oceania and absent or found in very low frequencies in other neighbouring populations of Indonesia (see Fig. III.1). As shown in table 3.3.c, Blake *et al.* (1973a) found no $\underline{AK}^2$ in Singapore Chinese. Similarly, Tan *et al.* (1979) found no $\underline{AK}^2$ allele in the Kadazans of Sabah. However, McDermid *et al.* (1973) reported an $\underline{AK}^2$ frequency of 0.3% in the Toba Batak of Sumatra, though Lie-Injo *et al.* (1974) found no variation in a sample of Batak from Medan. In the Philippines, a frequency of 0.4% was reported for Tagalog (Omoto *et al.* 1978) and, during my present study, $\underline{AK}^2$ also has been found in Bangkok with a gene frequency of 1.6%.

The most interesting finding in the region is an unexpectedly high frequency of $\underline{AK}^2$ (7%) in the Aetas of Luzon, reported by Omoto *et al.* (1978) who raises the possibility that this allele is a basic character of this Negrito population. However, it seems unlikely that the Caucasian $\underline{AK}^2$ gene was contributed to the Negritos. To clarify this point further investigations need to be carried out on other Negrito populations and also to determine whether the AK 2 gene product in Negritos is identical to that in Caucasians. It is possible that the $\underline{AK}^2$ allele found in the Negritos of the Philippines was once common in some populations of South East Asia and it was scattered by diffusion. But in the case of the Indonesian populations, the presence of the $\underline{AK}^2$ gene may either derive from Indian or European sources since Indonesian populations have experienced Indian or European contact over a long period of time.

3.2.4. Esterase D

Esterases (ESD; E.C. 3.1.1.1): a large number of different carboxylic ester hydrolases have been found in various human tissues. There are at least nine structural gene loci, in addition to choline esterase and carbonic anhydrase loci, determining human tissue esterase isozymes

(Coates *et al.* 1975).

Genetic variation of human red cells esterase D was demonstrated by Hopkinson *et al.* (1973) after electrophoresis using the fluorogenic substrates, 4-methyl umbelliferyl acetate and 4-methyl umbelliferyl butyrate. Three types of ESD isozyme patterns were reported, ESD 1, ESD 2-1 and ESD 2 respectively determined by two alleles. $\underline{ESD}^1$ and $\underline{ESD}^2$ at an autosomal locus. Further study revealed that this locus is situated on chromosome 13 (Bender and Grzeschik, 1976b).

Studies of three different populations showed the gene frequency for $\underline{ESD}^2$ was 9.8% in Europeans and Black Africans and 22.7% in Asiatic Indians living in England (Hopkinson *et al.* 1973). These three phenotypes were later also reported from Japan (Ishimoto *et al.* 1974) where the $\underline{ESD}^2$ gene frequency was 35%, higher than that of European and Black Africans but closer to the Asiatic Indian value reported previously. Welch and Lee (1974) reported a higher frequency of $\underline{ESD}^2$ (24.3%) for Lapland and a low frequency (5.5%) in Cameroon, Africa.

A new variant, ESD 3-1 has been reported by Bender and Frank (1974) in an individual from south west Germany and by Rittner and Muller (1975) in an individual living in Bonn, north Germany. The variant pattern is a three banded pattern like the common ESD 2-1, but the variant isozyme moves cathodal to the ESD 1 position. Blake (1976) reported ESD 3-1 in a single individual from northern New Guinea. Another rare variant allele, $\underline{ESD}^4$ was reported by Berg *et al.* (1976) and described as controlling an even slower electrophoretic variant. Evidence for the existence of a "silent" allele, $\underline{ESD}^O$, has also been reported (Koziol and Stepien, 1980; Marks *et al.* 1977; Sparkes *et al.* 1979).

One of the most interesting findings is the report of a high frequency of a unique esterase D allele called $\underline{ESD}^{3N}$ in a Philippines

Negrito population, the Aetas of Luzon, where the frequency of $\underline{ESD}^{3N}$ was 10.1% (Omoto *et al.* 1978). This variant was not detected in three non-Negrito populations of the Philippines.

During the present study the common phenotypes ESD 1-1, ESD 2-1 and ESD 2-2 were present in all populations. As shown in table 3.4.a, with the exception of the Asmat in which the $\underline{ESD}^2$ gene frequency is 4.9%, the distribution of the $\underline{ESD}^2$ gene varies from 15.4% in the Alorese to 40.9% in the urban Javanese. These are comparable to values for the Toba Batak and Nias ans studied by Blake (1976) and to Balinese from Tenganan Pageringsingan studied by Breguet *et al.* (1982b). From the phenotype distribution, four populations show significant departure from Hardy-Weinberg equilibrium at the P = 0.05 level, i.e. the Sasak, Florenese, Alorese and Galelarese. In three of these populations there is an excess of ESD 2-2 persons over expectation, but a deficiency in Florenese. This cell makes the main contribution to the χ^2 value. If Yates' correction is applied only Sasak remains significantly different from Hardy-Weinberg expectation.

As shown in table 3.4.b, the distribution of $\underline{ESD}^2$ gene frequency for the Balinese varies from 26.5% in Julah village to 52.1% in Banjar Rangkan village, which is comparable to the other Indonesian populations. The phenotype distribution is in agreement with Hardy-Weinberg equilibrium with the exception of Rendang in which significant deviation from equilibrium is observed ($P<0.05$).

In the case of the Asmat in Irian Jaya, the low $\underline{ESD}^2$ frequency is similar to the values in the previous study of Asmat by Blake (1976) and by Gajdusek *et al.* (1978). It is also comparable to other Papua New Guineans so far studied (Blake, 1976) with the exception of Motu from the Central District, in which the $\underline{ESD}^2$ gene frequency is the highest (30.9%) among New Guineans. As shown in table 3.4.c, the $\underline{ESD}^2$ gene

Table 3.4.a.

Distribution of Esterase D (ESD) phenotypes

and gene frequencies in Indonesian populations

Population	Number Tested	Phenotypes			Gene Frequencies		χ^2
		1-1	2-1	2-2	ESD^1	ESD^2	d.f=1
Javanese (urban)	203*	71	96	34	.5862	.4089	0.0035
Javanese (rural)	204†	71	99	33	.5907	.4069	0.0499
Javanese (total)	407ϕ	142	195	67	.5885	.4079	0.0212
Balinese	969#	335	488	141	.5975	.3999	3.4922
Sasak	191	89	72	30	.6545	.3455	5.2978
Bimanese	198*	83	83	30	.6288	.3662	1.1181
Florenese	76	33	40	3	.6974	.3026	4.6338
Savunese	36	16	14	6	.6389	.3611	0.8895
Rotinese	59	36	20	3	.7797	.2203	0.0106
Timorese	133	84	42	7	.7895	.2105	0.3325
Alorese	26	20	4	2	.8462	.1538	4.3512
Macassarese	199*	96	74	27	.6683	.3266	3.4570
Buginese	205*	95	87	21	.6756	.3195	0.0005
Toraja	101	52	38	11	.7030	.2970	0.9911
Ternatans	213	91	97	25	.6549	.3451	0.0121
Makianese	30	16	11	3	.7167	.2833	0.2830
Galelarese	155	107	39	9	.8161	.1839	4.0497
Asmat	163	147	16	0	.9509	.0491	0.4342

* Including 2 "ESD 5-2"

† " 1 "

ϕ " 3 "

\# " 5 "

Table 3.4.b.

Distribution of Esterase D (ESD) phenotypes and gene frequencies in Balinese populations

Village	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1
		1-1	2-1	2-2	ESD^1	ESD^2	
Dauh Waru	80	29	40	11	.6125	.3875	0.2275
Penebel	58	23	31	4	.6638	.3362	2.2617
Ayunan	69	32	29	8	.6739	.3261	0.1319
Renon	95	30	45	20	.5526	.4474	0.1677
Serangan	39	15	14	10	.5641	.4359	2.8442
Banjar Rangkan	48	10	26	12	.4792	.5208	0.3486
Ped	34	9	17	8	.5147	.4853	0.0000
Rendang	123	41	70	12	.6179	.3821	5.1795
Seraya	85	25	46	14	.5647	.4353	0.8635
Songan	116	37	59	20	.5733	.4267	0.1816
Julah	49*	27	16	4	.7142	.2653	0.1631
Sembiran	101†	29	54	15	.5545	.4307	2.2976
Beratan	32	10	20	2	.6250	.3750	3.5556
Wanayu	40	18	21	1	.7125	.2875	3.1689

* Including 2 "ESD 5-2"

† " 3 "

Table 3.4.c.

Additional Esterase D (ESD) gene frequency data in Indonesia and neighbouring areas

Population	Number Tested	Gene Frequency ESD^1	Gene Frequency ESD^2	Reference
Indonesia				
Batak (Toba)	147	.7074	.2925	Blake, 1976
Niasans	192	.7682	.2317	-ditto-
Awyu	222	.9864	.0135	-ditto-
Asmat	146	.9760	.0239	-ditto-
Moni	264	1.0000		-ditto-
Balinese	316	.7009	.2991	Breguet *et al.* 1982b
Singapore, Malaysia and Philippines				
Malays (Singapore)	198	.6439	.3560	Blake, 1976
Chinese "	262	.5648	.4351	-ditto-
Kadazans	214	.4720	.5280	Tan *et al.* 1979
Negrito*	129	.7791	.1201	Omoto *et al.* 1978
Tagalog	253	.6919	.3081	-ditto-
Thailand				
Bangkok	94	.5851	.4149	Present study
Australia				
Aborigines				
Elcho Is.	142	.9612	.0387	Blake, 1976
Mowanjum	244	.975	.025	Blake, 1979
Papua New Guinea				
Motu	204	.6911	.3088	Blake, 1976

Table 3.4.c. Cont'd

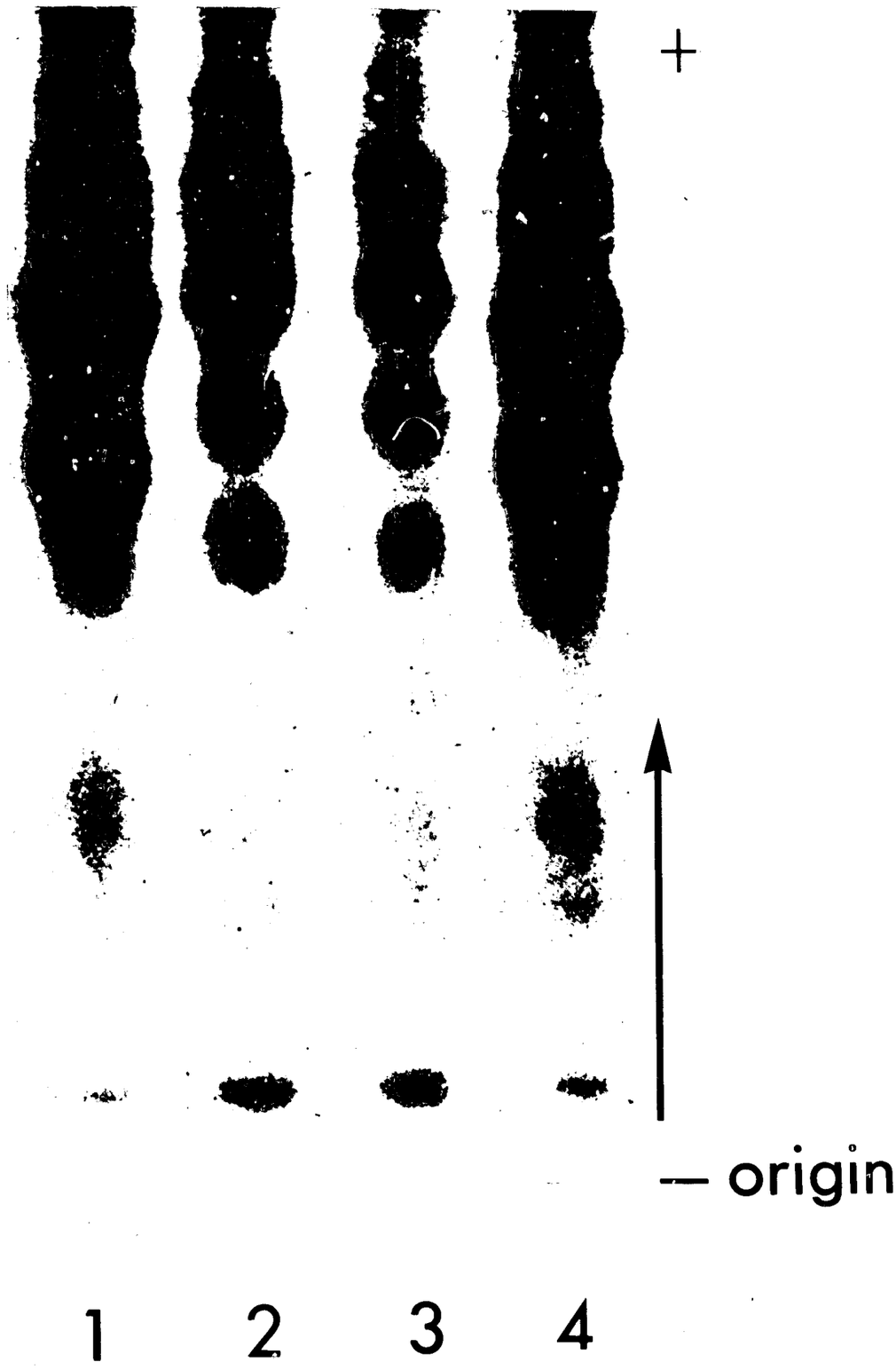
Population	Number Tested	Gene Frequency ESD^1	Gene Frequency ESD^2	Reference
Western Pacific area				
Solomon Islands				
Santa Cruz Is.	253	.8478	.1522	Blake, 1976
" " "	351	.8177	.1823	Mazzur and Blake, 1981
Trust Territory of Pacific Island-				
Western Caroline Is.	273	.5549	.4450	Blake, 1976
Eastern Caroline Is.	952	.5715	.4286	-ditto-
Fiji				
Fijians	54	.8148	.1851	-ditto

* ESD^{3N} gene frequency = .1008

Fig. III.2 Variant of esterase D (ESD)

1 and 4. ESD 2-1

2 and 3. ESD 5-2


+
origin
1
2
3
4

frequency in other neighbouring populations is very low in the Aborigines of Australia, low in the Negritos of the Philippines and high in the Kandazans of Sabah: other values fall in the range of Indonesian populations.

In this study none of the other known rare variants, including the $\underline{ESD}^{3N}$ variant common in Negritos of the Philippines, have been encountered. However, another ESD phenotype which can be distinguished from the common 2-1 was found in the Javanese (both urban and rural), Balinese, Bimanese, Macassarese and Buginese. The symmetrical, three banded electrophoretic pattern of this presumptive heterozygote has its most anodal isozyme in the position of the normal ESD 2 band and its most cathodal isozyme occupies an intermediate position between the normal ESD 1 band and the hybrid band of ESD 2-1 (see Fig. III.2). It is proposed to call this new phenotype ESD 5-2 and the allele $\underline{ESD}^{5}$. It is of interest that the 14 examples of this new allele were all in combination with $\underline{ESD}^{2}$. The position of the ESD 5 band is such that the pattern for the ESD 5-1 phenotype would be extremely difficult to discriminate with certainty. Whilst a careful search for this phenotype was carried out, none could be detected. Since it has been found in several population groups, further studies on the distribution of this variant will be of interest. It is unfortunate that after this variant phenotype was discovered it has not been possible, so far, to carry out a family study to confirm it as another codominant allele at the ESD locus.

3.2.5. Glyoxalase I

The glyoxalase system consists of two enzymes, glyoxalase I and II. Glyoxalase I or Lactoyl-glutathione-lyase (GLO I; E.C. 4.4.1.5) catalyses the combination of methylglyoxal and reduced glutathione (GSH) to form s-lactoyl glutathione. Glyoxalase II catalyses the

breakdown of s-lactoyl glutathione into lactic acid and GSH.

Red cell GLO I was first reported to be polymorphic in the German population (Kömpf *et al.* 1975). Using horizontal starch gel electrophoresis, 3 common phenotypes were reported, GLO 1, GLO 2-1 and GLO 2, being determined by two codominant autosomal alleles, $\underline{GLO}^1$ and $\underline{GLO}^2$. The $\underline{GLO}^1$ gene frequency in Germany was later reported to be 42.7% (Kömpf and Bissbort, 1975). Using cellulose acetate electrophoresis, Meera Khan and Doppert (1976) confirmed the autosomal allele hypothesis. Subsequent studies revealed that the $\underline{GLO}^1$ gene frequency varies between populations. Bagster *et al.* (1975) reported a $\underline{GLO}^1$ gene frequency difference between an English population and the Jali of Gambia (Africa) and Olaisen *et al.* (1976) reported $\underline{GLO}^1$ gene frequency differences between Norwegians and Lapps.

In addition to the common alleles $\underline{GLO}^1$ and $\underline{GLO}^2$, the existence of a silent allele has also been reported (Rittner and Weber, 1978; Rubinstein and Suciu-Foca, 1979). A new allele, $\underline{GLO}^3$ has also been described (Ranzani *et al.* 1979).

Perhaps the most interesting finding in the GLO I polymorphism is that the enzyme is located on chromosome 6 linked to the HLA regio. . Bender and Grzeschik (1976a) using man-mouse somatic cell hybrids demonstrated that the GLO I locus is located on chromosome 6 and provided evidence for synteny of the GLO and PGM_3 genes. The possible linkage of GLO I and HLA was first suggested by Mayr *et al.* (1976) and was further confirmed by Kömpf *et al.* (1976) and Weitkamp (1976).

In this study the common phenotypes GLO 1-1, GLO 2-1 and GLO 2-2 were found in all populations screened except the Asmat in which this enzyme was monomorphic (Table 3.5.a). Apart from the Alorese in which the $\underline{GLO}^1$ gene frequency is low, 5.8%, its distribution in the other

populations varies from 10.5% in the Timorese to 27.8% in the Savunese. The phenotype distribution shows significant deviation from Hardy-Weinberg equilibrium in the Javanese, Savunese and Toraja, although only the Javanese and Savunese series remain significant at the P = 0.05 and 0.02 level respectively after applying Yates' correction. For the Javanese there is deficiency of GLO 1-1 persons, but there is an excess in Savunese. This suggests random factors rather than systematic typing errors. Although the $\underline{GLO}^1$ gene frequency in the Balinese is 14.9%, as shown in table 3.5.b, it varies greatly from 3.7% in Wanayu to 24.6% in Songan. The phenotype distribution is in agreement with Hardy-Weinberg equilibrium.

Previous studies in Indonesian populations show the $\underline{GLO}^1$ gene frequency in the Toba Batak, Niasans (Ghosh, 1977a) and Balinese (Breguet *et al.* 1982b) to fall within the range of the present study.

In general, with the exception of the Alorese, the $\underline{GLO}^1$ gene frequencies for the Indonesian populations fall within the range of Asia and Africa (see Busi *et al.* 1979). Among Asian populations, a lower $\underline{GLO}^1$ gene frequency of less than 10% has been reported for the Japanese (Hashimoto *et al.* 1978). The highest frequencies so far reported are found in Caucasian populations.

In the western Pacific area, the $\underline{GLO}^1$ gene frequency varies from 4.5% in the Eastern Carolines to 36.1% in the Tokelau islands (Ghosh, 1977a). In the case of the Asmat, the absence of the $\underline{GLO}^1$ allele is comparable with the very low to complete absence of this allele in Papua New Guineans except the Motu and the Waritsian, who have comparable $\underline{GLO}^1$ gene frequency to the Indonesian populations. A very low $\underline{GLO}^1$ frequency also is found in the Aborigines of Australia with the exception of Western Desert and at Yarrabah (Ghosh, 1977a) though there is a possibility of European admixture at the two latter localities.

Table 3.5.a.

Distribution of Glyoxalase I (GLO I) phenotypes and gene frequencies in Indonesian populations

Population	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1
		1-1	2-1	2-2	GLO^1	GLO^2	
Javanese (urban)	203	1	57	145	.1453	.8547	3.4548
Javanese (rural)	204	3	67	134	.1789	.8211	2.8329
Javanese (total)	407	4	124	279	.1622	.8378	5.9798
Balinese	970	22	246	702	.1495	.8505	0.0067
Sasak	191	6	47	138	.1545	.8455	0.6398
Bimanese	198	5	41	152	.1288	.8712	1.1812
Florenese	76	2	15	59	.1250	.8750	0.7261
Savunese	36	6	8	22	.2778	.7222	7.1659
Rotinese	59	2	12	45	.1356	.8644	1.0335
Timorese	133	3	22	108	.1053	.8947	1.9747
Alorese	26	-	3	23	.0577	.9423	0.0975
Macassarese	199	4	63	132	.1784	.8216	1.2731
Buginese	205	8	51	146	.1634	.8366	1.6649
Toraja	101	5	20	76	.1485	.8515	4.7584
Ternatans	213	13	61	139	.2042	.7958	3.0117
Makianese	30	1	6	23	.1333	.8667	0.5436
Galelarese	155	2	31	122	.1129	.8871	0.0004
Asmat	161	-	-	161	.0000	1.0000	0.0000

Table 3.5.b.

Distribution of Glyoxalase I (GLO I) phenotypes and gene frequencies in Balinese populations

Village	Number Tested	Phenotypes			Gene Frequencies		χ^2
		1-1	2-1	2-2	GLO^1	GLO^2	d.f=1
Dauh Waru	80	1	26	53	.1750	.8250	1.2608
Penebel	58	-	18	40	.1552	.8448	1.9567
Ayunan	69	-	19	50	.1377	.8623	1.7590
Renon	95	4	24	67	.1684	.8316	0.9143
Serangan	39	-	3	36	.0385	.9615	0.0624
Banjar Rangkan	48	1	5	42	.0729	.9271	2.5289
Ped	34	-	9	25	.1324	.8676	0.7912
Rendang	124	2	27	95	.1250	.8750	0.0026
Seraya	85	-	23	62	.1353	.8647	2.0808
Songan	116	8	41	67	.2457	.7543	0.2499
Julah	49	-	6	43	.0612	.9388	0.2084
Sembiran	101	5	30	66	.1980	.8020	0.4243
Beratan	32	1	12	19	.2187	.7813	0.3020
Wanayu	40	-	3	37	.0375	.9625	0.0607

Table 3.5.c.

Additional Glyoxalase I (GLO I) gene frequency data in Indonesia and neighbouring areas

Population	Number Tested	Gene Frequencies GLO^1	Gene Frequencies GLO^2	Reference
Indonesia				
Batak (Toba)	148	.1486	.8514	Ghosh, 1977a
Niasans	192	.2187	.7813	-ditto-
Balinese	316	.1835	.8165	Breguet *et al.* 1982b
Singapore, Malaysia and Philippines				
Chinese (Singapore)	149	.1544	.8455	Ghosh, 1977a
Kadazans	218	.3000	.7000	Tan *et al.* 1979
Negrito	129	.2442	.7558	Omoto *et al.* 1978
Thailand				
Bangkok	93	.1452	.8548	Present study
Australia				
Aborigines				
Elcho Is.	151	-	1.0000	Chosh, 1977a
Mowanjum	244	.012	.988	Blake, 1979
Papua New Guinea				
Motu	154	.1558	.8442	Ghosh, 1977a
Western Pacific area				
Solomon Islands				
Santa Cruz Is.	351	.0342	.9658	Mazzur and Blake, 1981
Trust Territory of Pacific Islands				
Eastern Caroline Is.	748	.0455	.9545	Ghosh, 1977a
Fiji				
Fijians	137	.1679	.8321	-ditto-

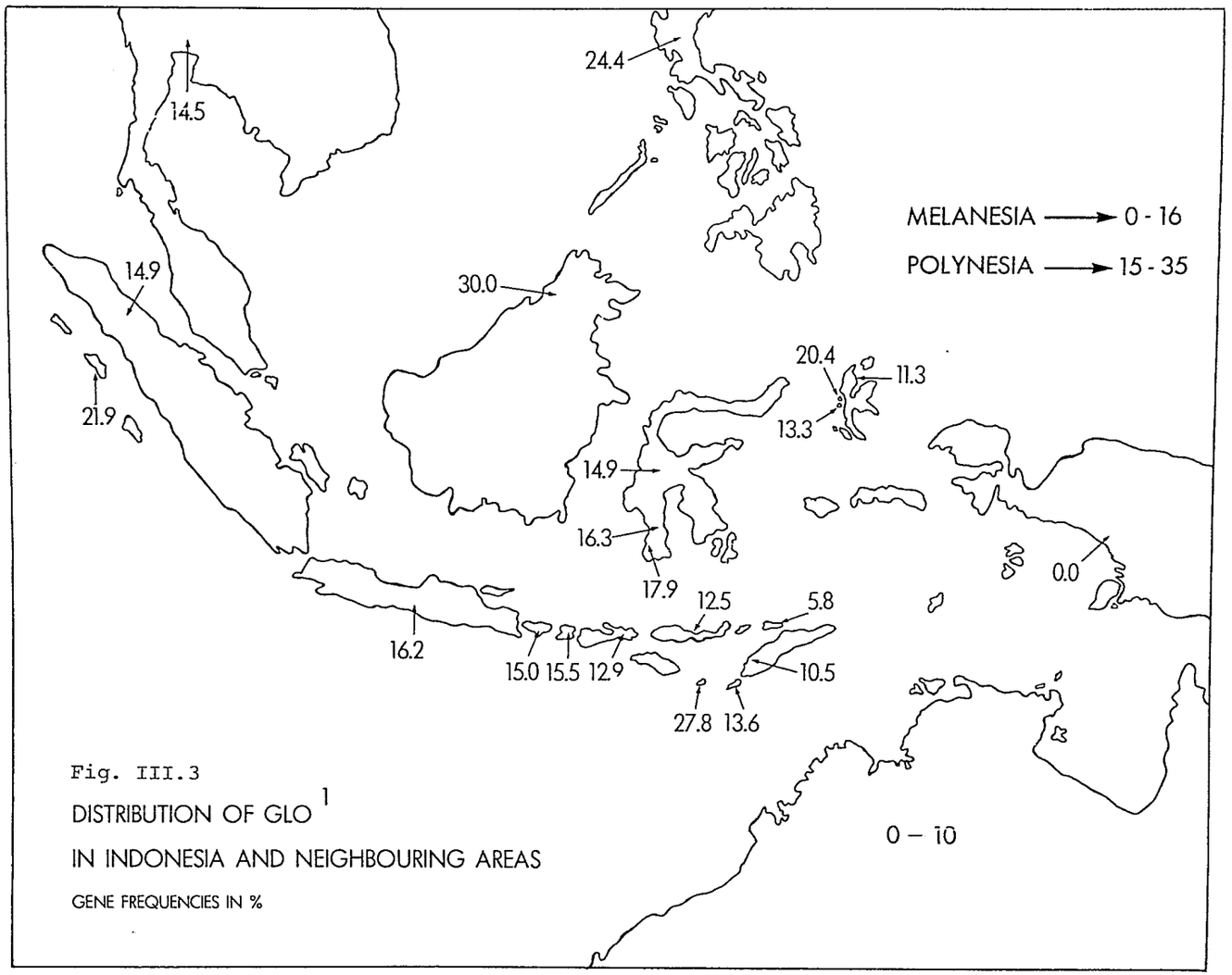


Fig. III.3

DISTRIBUTION OF GLO [1]

IN INDONESIA AND NEIGHBOURING AREAS

GENE FREQUENCIES IN %

As shown in Fig. III.3, with the exception of Savunese in the Lesser Sunda and Ternatans in Moluccas, the $\underline{GLO}^1$ gene frequency seems to decrease as one moves from the mainland South East Asia through the Indonesian archipelago towards the east.

3.2.6. Glutamic-pyruvic transaminase

Glutamic-pyruvic transaminase (GPT; E.C.2.6.1.2) catalyses the reversible reaction of L-alanine and α-ketoglutarate to L-glutamate and pyruvate. The enzyme exists in two different forms, the cytoplasmic (soluble) and the mitochondrial forms, though the latter is only found at a relatively low concentration (Swick *et al.* 1965).

Chen and Giblett (1971) reported that human red cell GPT (soluble GPT) exhibited genetic polymorphism in Caucasian, Afro-American and Oriental-American populations. Three phenotypes, GPT 1, GPT 2-1 and GPT 2 were detected electrophoretically. Family studies indicated that the three phenotypes were the expression of two autosomal codominant alleles designated $\underline{GPT}^1$ and $\underline{GPT}^2$. The frequency of the two genes varies considerably between populations. In their original paper, Chen and Giblett (1971) showed the gene frequency for $\underline{GPT}^1$ in Black Americans was higher than that for other groups tested and this was confirmed by subsequent reports (Chen *et al.* 1972; Welch *et al.* 1975). Quantitative studies demonstrated that the mean value of GPT activity was GPT 1 > GPT 2-1>GPT 2 (Welch, 1972).

In addition to the common phenotypes, a number of rare variants have been described. During their study in various populations, Chen *et al.* (1972) observed the presence of GPT 3-1 and 3-2 phenotypes in Caucasians, GPT 4-1 in Afro-Americans, GPT 5-1 in a Canadian Eskimo family, GPT 6-1 and 6-2 in samples from New Guinea and the Philippines. Other authors also reported the presence of the $\underline{GPT}^3$ allele in Caucasians (Anger *et al.* 1974; Brinkmann *et al.* 1972; Scozzari *et al.* 1975;

Spielmann *et al.* 1973; Welch *et al.* 1975; Wiebecke and Brackebusch, 1974). Ishimoto and Kuwata (1974) reported the presence of $\underline{GPT}^3$ and $\underline{GPT}^6$ in the Japanese and Blake (1976) has shown that both alleles, $\underline{GPT}^3$ and $\underline{GPT}^6$, are present in the western Pacific area. Further studies reported the existence of a silent allele, $\underline{GPT}^0$, (Olaisen, 1973a; Spielmann *et al.* 1973), a rare $\underline{GPT}^7$ allele (Olaisen, 1973b) and a $\underline{GPT}^8$ allele (Santachiara-Benerecetti *et al.* 1975).

As shown in table 3.6.a, the common phenotypes, GPT 1-1, GPT 2-1 and GPT 2-2 are found in all populations tested in the present survey. With the exception of the Ternatans, Alorese and Asmat, who have $\underline{GPT}^1$ gene frequencies 63.8%, 69.2% and 85.4% respectively, the GPT^1 distribution in the other populations varies from 25.7% in the Toraja to 52.7% in Florenese. A previous report on the Toba Bat. and Niasans of Indonesia (Blake, 1976) also show their $\underline{GPT}^1$ gene frequency falls within this range. The phenotype distribution shows significant departure from Hardy-Weinberg equilibrium in the Balinese ($P<0.05$), Sasak ($P<0.05$) and Ternatans ($P<0.02$). Close examination of the data suggests there may be a technical problem associated with these significant departures from Hardy-Weinberg equilibrium in the GPT system. The discrepancies in the Balinese, Sasak and Ternatans are all associated with an excess of both homozygote classes and a deficiency of the heterozygote 2-1's. This underscoring of GPT 2-1 phenotypes is understandable when it is recognized that the level of enzyme activity of the $\underline{GPT}^2$ gene product is very much less than that of the $\underline{GPT}^1$ gene product. Although attempts were made to guard against this source of error by reading gels at several times during a 3-4 hour period, it seems that, in some cases, heterozygotes were incorrectly scored as homozygotes.

In the Balinese (table 3.6.b), a slightly lower $\underline{GPT}^1$ gene frequency varies from 17.4% in Ayunan to 32.7% in Sembiran. Significant deviation

Table 3.6.a.

Distribution of Glutamic Pyruvic Transaminase (GPT) phenotypes and gene frequencies in Indonesian populations

Population	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1*
		1-1	2-1	2-2	$\underline{CPT}^1$	$\underline{GPT}^2$	
Javanese (urban)	203	17	82	104	.2857	.7143	0.0219
Javanese (rural)	204	27	86	91	.3431	.6569	0.8569
Javanese (total)	407	44	168	195	.3145	.6855	0.7412
Balinese	970**	91	356	521	.2773	.7217	7.5785
Sasak	191	39	79	73	.4110	.5890	4.0549
Bimanese	198	37	84	77	.3990	.6010	2.6374
Florenese	75	22	35	18	.5267	.4733	0.3072
Savunese	36	3	13	20	.2639	.7361	0.1790
Rotinese	59	13	30	16	.4746	.5254	0.0226
Timorese	133	35	57	41	.4774	.5226	2.6483
Alorese	26	13	10	3	.6923	.3077	0.2458
Macassarese	198†	35	79	76	.3813	.5985	3.8010
Buginese	205††	29	85	90	.3488	.6488	1.6753
Toraja	101ϕ	6	38	48	.2574	.6980	0.3058
Ternatans	213	95	82	36	.6385	.3615	5.8738
Makianese	30	7	17	6	.5167	.4833	0.5435
Galelarese	155ϕϕ	32	73	48	.4419	.5516	0.8549
Asmat	161	118	39	4	.8540	.1460	0.1298

* d.f=2 for Balinese, Macassarese, Buginese, Toraja and Galelarese

** 2 GPT 6-2; $\underline{GPT}^6$=0.0010

† 2 GPT 6-1; 6 GPT 6-2; $\underline{GPT}^6$ = 0.0202

†† 1 GPT 6-2; $\underline{GPT}^6$ = 0.0024

ϕ 2 GPT 6-1; 7 GPT 6-2; $\underline{GPT}^6$ = 0.0446

ϕϕ 2 GPT 6-2; $\underline{GPT}^6$ = 0.0065

Table 3.6.b.

Distribution of Glutamic Pyruvic Transaminase (GPT)

phenotypes and gene frequencies in Balinese population

Village	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1 *
		1-1	2-1	2-2	GPT^1	GPT^2	
Dauh Waru	80**	7	28	44	.2625	.7312	1.0374
Penebel	58	2	20	36	.2069	.7931	0.1492
Ayunan	69	3	18	48	.1739	.8261	0.5854
Renon	95	8	41	46	.3000	.7000	0.0722
Serangan	39	2	21	16	.3205	.6795	2.1763
Banjar Rangkan	48	5	20	23	.3125	.6875	0.0441
Ped	34	3	15	16	.3088	.6912	0.0380
Rendang	124†	15	36	72	.2661	.7298	8.3837
Seraya	85	8	30	47	.2706	.7294	0.9531
Songan	116	15	40	61	.3017	.6983	3.8280
Julah	49	6	16	27	.2857	.7143	1.9600
Sembiran	101	10	46	45	.3267	.6733	0.1252
Beratan	32	3	8	21	.2187	.7813	2.3082
Wanayu	40	4	17	19	.3125	.6875	0.0048

* d.f=2 for Dauh Waru and Rendang

** 1 GPT 6-2; $GPT^6 = 0.0062$

† 1 GPT 6-2; $GPT^6 = 0.0040$

Table 3.6.c.

Additional Glutamic-Pyruvic Transaminase (GPT) gene frequency data in Indonesia and neighbouring areas

Population	Number Tested	Gene Frequencies * GPT[1]	GPT[2]	Reference
Indonesia				
Batak (Toba)	148	.3885	.6114	Blake, 1976
Niasans	191	.4293	.5706	-ditto-
Asmat	145	.8758	.1241	-ditto-
Asmat	114	.89	.11	Gajdusek *et al.* 1978
Awyu	223	.969	.031	-ditto-
Moni	188	.822	.178	-ditto-
Ekagi	23	.870	.130	-ditto-
Singapore, Malaysia and Philippines				
Malays (Singapore)	214	.3434	.6471	Blake, 1976
Chinese (")	303	.5000	.4900	-ditto-
Kadazans	249	.2650	.7350	Tan *et al.* 1979
Negrito	129	.1434	.8566	Omoto *et al.* 1978
Tagalog	253	.3241	.6709	-ditto-
Thailand				
Bangkok	94	.3564	.6436	Present study
Australia Aborigines				
Elcho Is.	144	.6112	.3889	Blake, 1976
Mowanjum	244	.758	.242	Blake, 1979
Papua New Guinea				
Motu	469	.3507	.6493	Blake, 1976

Table 3.6.c. Cont'd

Population	Number Tested	Gene Frequencies * GPT[1]	GPT[2]	Reference
Western Pacific area				
Solomon Islands				
Santa Cruz Is.	250	.6400	.3600	Blake, 1976
-ditto-	351	.5484	.4516	Mazzur and Blake, 1981
Trust Territory of Pacific Islands				
Western Caroline Is.	266	.5563	.4436	Blake, 1976
Eastern Caroline Is.	908	.7064	.2924	-ditto-
Fiji				
Fijians	54	.5370	.4629	-ditto-

* GPT[6] = .0093, .0099, .0050 and .0011 for Singapore Malays, Singapore Chinese, Tagalog and Eastern Carolines respectively.

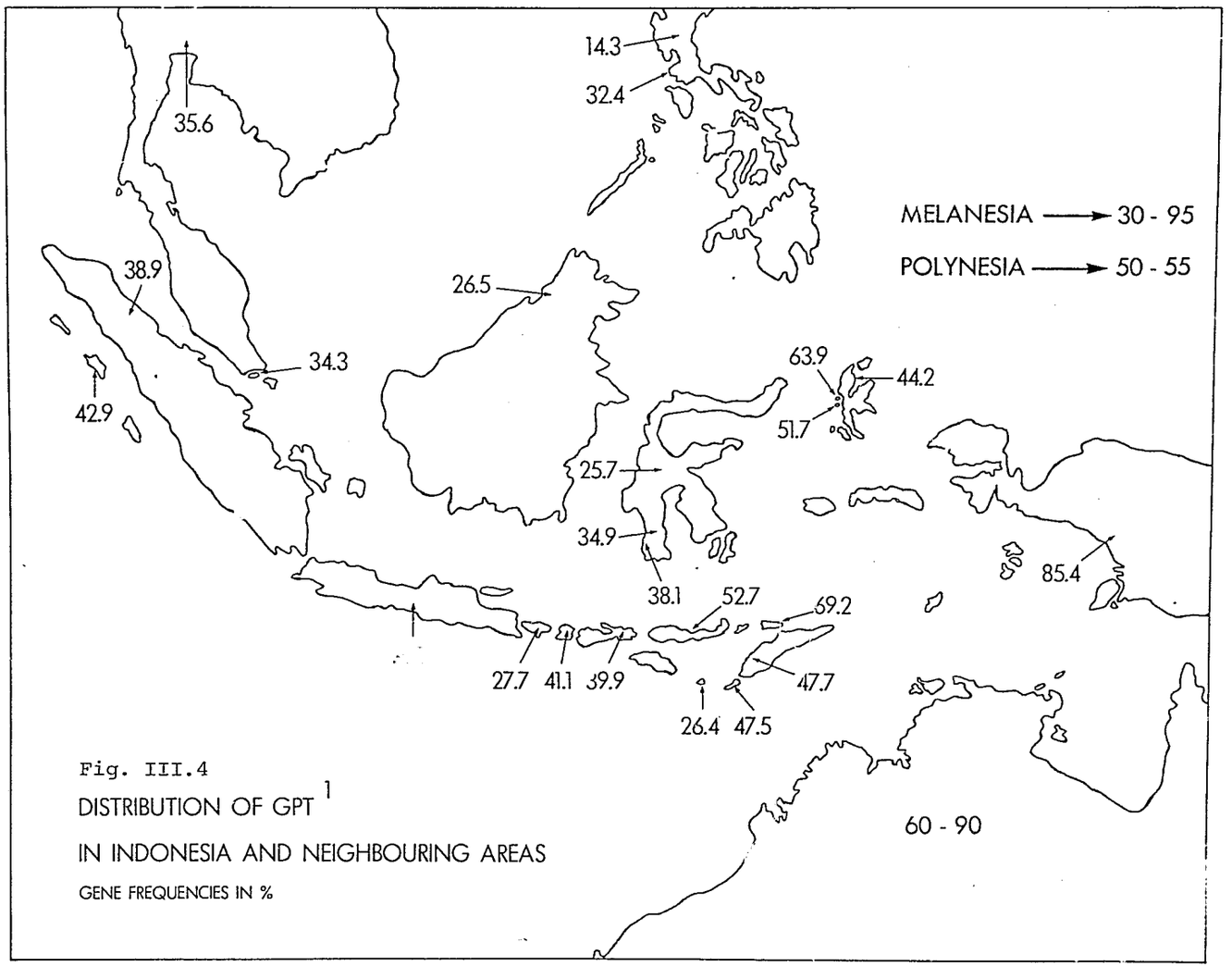


Fig. III.4

DISTRIBUTION OF GPT [1]

IN INDONESIA AND NEIGHBOURING AREAS

GENE FREQUENCIES IN %

from the random mating expectation is observed in Rendang ($P<0.02$): in this case also the deviation is due to deficiency of heterozygotes.

The high $\underline{GPT}^1$ gene frequency in the Asmat is comparable to the previous reports on other Irian Jaya populations (Blake, 1976; Gajdusek *et al.* 1978). As shown in table 3.6.c, the $\underline{GPT}^1$ gene frequency in other South East Asian populations, except for the Negrito (Luzon Aetas) of the Philippines, falls within the range of the Indonesian populations. A slightly higher $\underline{GPT}^1$ gene frequency is found in the western Pacific area including the Aborigines of Australia, but not in the Motu in Papua New Guinea in whom the $\underline{GPT}^1$ gene frequency falls within the range for Indonesian populations.

As shown in Fig. III.4, in the Indonesian archipelago there is a cline of increasing $\underline{GPT}^1$ gene frequency towards the east, being highest among the Asmat of Irian Jaya.

In addition to the two common alleles described above, another allele, $\underline{GPT}^6$ has been found in the Balinese, Macassarese, [illegible]ginese, Toraja and Galelarese. The $\underline{GPT}^6$ gene frequency of 4.5% in the Toraja is among the highest so far reported. The existence of this allele in three populations of Sulawesi is not surprising, since they are more or less interrelated. Similarly, high frequencies for $\underline{GPT}^6$ have been found in the Ifugao from the Philippines (3.2%, Omoto *et al.* 1978), and in Guam (5.4%, Blake *et al.* 1982b). In other neighbouring populations, $\underline{GPT}^6$ has been found at low frequencies in Singapore Malays and Chinese. It has also been found in the Eastern Caroline islands and the Tokelau islands of the western Pacific, Karkar island and the Morobe District of Papua New Guinea (Blake, 1976) and also in the Japanese (Ishimoto and Kuwata, 1974). Since this allele has only been found to occur in the Asia-Pacific region, it is very likely that it was once common in some populations of the region and was later scattered by population diffusion.

3.2.7. 6-Phosphogluconate dehydrogenase

6-phosphogluconate dehydrogenase or D-glucose-1-phosphate phosphotransferase (6-PGD; E.C. 1.1.1.44) catalyses the conversion of 6-phosphogluconate to ribulose-5-phosphate and the coenzyme nicotinamide adenine dinucleotide phosphate (NADP) being simultaneously reduced to NADPH in the pentose - phosphate pathway. The enzyme occurs in many tissues, but the red cell enzyme may conveniently be studied.

Fildes and Parr (1963) were the first to demonstrate the existence of genetically determined variants for the human red cell 6-PGD. Two phenotypes were described, one with a single A band and the other with an A and B band. Later, Parr (1966) described eight different electrophoretic patterns of 6-PGD from human red cells. They included the normal phenotype with the A band, the common variant with A and B bands, the Canning variant, Richmond variant, Hackney variant and three other phenotypes characterized by lower enzyme activity which were later designated as the Newham variant, the Ilford variant and the Whitechapel variant (Parr and Fitch, 1967). The control of the normal, common and Canning variants is by two codominant autosomal alleles, $\underline{PGD}^{A}$ and $\underline{PGD}^{C}$ (American authors favour $\underline{PGD}^{A}$ and $\underline{PGD}^{B}$).

In addition to the phenotype variation noted above, deficiency of this enzyme has also been reported (Brewer and Dern, 1964; Parr and Fitch, 1964; Dern *et al.* 1966; Parr, 1966; Parr and Fitch, 1967). More electrophoretic variants were later reported such as the Friendship variant (Davidson, 1967), Elcho variant (Blake and Kirk, 1969; Radam and Strauch, 1971), Thai variant (Tuchinda, 1968; Lie Injo and Welch, 1972), Singapore variant (Blake *et al.* 1973a), Oshakati variant (Jenkins and Nurse, 1974).

Tills *et al.* (1970b,1971) provide the geographical distribution of alleles for this enzyme. Among the variants, the Elcho variant is found

to be polymorphic in some populations of Aborigines in Australia. On the other hand, the $\underline{PGD}^{R}$ allele (Richmond variant) though rare, has been found in most populations where a large number of samples has been tested.

A shown in table 3.7.a, the common phenotypes PGD A and PGD AC are found in all the Indonesian populations tested, while PGD C occurs only in some populations. The phenotype distribution agrees well with the Hardy-Weinberg equilibrium, the apparent exception of the Timorese being non-significant with Yates' correction for small numbers ($\chi^2_1 = 3.67$). The gene frequency for the $\underline{PGD}^{C}$ allele ranges from as low as 2.2% in the Balinese to 28.8% in the Asmat. For the Balinese themselves, four villages i.e., Ayunan, Banjar Rangkan, Julah and Sembiran show no $\underline{PGD}^{C}$ at all and in the other villages the $\underline{PGD}^{C}$ gene frequency varies from a non-polymorphic level (0.4% in Rendang and Songan) to 10.3% in Ped (table 3.7.b). Therefore, the low average of 2.2% for the Balinese is mainly due to the absence of $\underline{PGD}^{C}$ in those four villages.

In previous studies of Indonesian populations, Breguet *et al.* (1982b) report a $\underline{PGD}^{C}$ gene frequency of 3.8% in Balinese from Tenganan Pageringsingan, Lie Injo *et al.* (1974) report 1.6% in Minangkabau, 6% in the Atjehnese and 5.1% in the Medan Batak, and McDermid *et al.* (1973) report 4.7% in the Toba Batak. Unpublished results show 11.4% in the Niasans (Blake, personal communication).

As shown in table 3.7.c, a very high frequency of $\underline{PGD}^{C}$ is found in Papua New Guinea and other Melanesian populations. Lower frequencies are found in the South East Asian populations and among the Aborigines of Australia. Tills *et al.* (1970b,1971) show that the $\underline{PGD}^{C}$ gene has been found to be low in Europeans and slightly higher in Africans and Asiatic populations. It is virtually absent from the Aboriginal American populations.

Table 3.7.a.

Distribution of 6-Phosphogluconate Dehydrogenase (6PGD) phenotypes and gene frequencies in Indonesian populations

Population	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1*
		A	AC	CC	$\underline{PGD}^A$	$\underline{PGD}^C$	
Javanese (urban)	203	188	15	-	.9631	.0369	0.3029
Javanese (rural)	204	184	20	-	.9510	.0490	0.5429
Javanese (total)	407	372	35	-	.9570	.0430	0.8216
Balinese	970	929	40	1	.9784	.0216	0.6968
Sasak	191†	160	30	-	.9188	.0785	1.4899
Bimanese	198	166	30	2	.9141	.0859	0.2394
Florenese	76	66	10	-	.9342	.0658	0.3769
Savunese	36	33	3	-	.9583	.0417	0.0681
Rotinese	59	49	9	1	.9068	.0932	0.5632
Timorese	133	109	20	4	.8947	.1053	5.4098
Alorese	26	18	7	1	.8269	.1731	0.0918
Macassarese	199	169	27	3	.9171	.0829	2.3145
Buginese	205	171	34	-	.9171	.0829	1.6762
Toraja	101	90	11	-	.9455	.0545	0.3350
Ternatans	213	179	30	4	.9108	.0892	3.7795
Makianese	30	21	8	1	.8333	.1667	0.0480
Galelarese	155	144	11	-	.9645	.0355	0.2098
Asmat	163	78	76	9	.7117	.2883	3.0191

* d.f=2 for Sasak

† 1 $\underline{PGD}^{A\ Lombok}$; $\underline{PGD}^{Lbk}$ = 0.0026

Table 3.7.b.

Distribution of 6-Phosphogluconate Dehydrogenase (6PGD) phenotypes and gene frequencies in Balinese populations

Village	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1
		A	AC	CC	PGD^A	PGD^C	
Dauh Waru	80	76	4	-	.9750	.0250	0.0526
Penebel	58	51	7	-	.9397	.0603	0.2392
Ayunan	69	69	-	-	1.0000	.0000	0.0000
Renon	95	90	5	-	.9737	.0263	0.0694
Serangan	39	35	4	-	.9487	.0513	0.1140
Banjar Rangkan	48	48	-	-	1.0000	.0000	0.0000
Ped	34	27	7	-	.8971	.1029	0.4477
Rendang	124	123	1	-	.9960	.0040	0.0020
Seraya	85	81	4	-	.9765	.0235	0.0494
Songan	116	115	1	-	.9957	.0043	0.0022
Julah	49	49	-	-	1.0000	.0000	0.0000
Sembiran	101	101	-	-	1.000	.0000	0.0000
Beratan	32	26	6	-	.9063	.0937	0.3425
Wanayu	40	38	1	1	.9625	.0375	-

Table 3.7.c.

Additional 6-Phosphogluconate Dehydrogenase (6-PGD) gene frequency data in Indonesia and neighbouring areas

Population	Number Tested	Gene Frequencies*		Reference
		PGD^A	PGD^C	
Indonesia				
Batak (Toba)	182	.9533	.0467	McDermid *et al.* 1973
Batak (Medan)	264	.949	.051	Lie-Injo *et al.* 1974
Minangkabau	65	.984	.016	-ditto-
Atjehnese	25	.940	.060	-ditto-
Niasans	192	.8828	.1146	Blake (unpublished)
Balinese	315	.9619	.0381	Breguet *et al.* 1982b
Asmat	654	.784	.216	Gajdusek *et al.* 1978
Awyu	508	.804	.196	-ditto-
Kayagar	71	.669	.331	-ditto-
Moni	320	.917	.083	-ditto-
Ekagi	26	.962	.038	-ditto-
Lake Plain	245	.892	.108	-ditto-
Dani	258	.7481	.2519	Kirk *et al.* 1973
Singapore, Malaysia and Philippines				
Malays (Singapore)	259	.9382	.0598	Blake *et al.* 1973a
Chinese (")	378	.9325	.0661	-ditto-
Malays (Malaya)	581	.967	.032	Lie-Injo and Welch, 1972
Chinese (")	435	.969	.031	-ditto-
Malayan Aborigines				-ditto-
Miscellaneous	253	.945	.055	-ditto-
Semai	214	.963	.037	-ditto-

Table 3.7.c. Cont'd

Population	Number Tested	Gene Frequencies* PGD^A	PGD^C	Reference
Malayan aborigines				
Temiar	86	.936	.064	Lie-Injo and Welch, 1972
Kadazans	284	.981	.019	Tan *et al.* 1979
Land Dayaks	132	.955	.045	Ganesan *et al.* 1976
Sea Dayaks	127	.953	.047	-ditto-
Negrito	128	.9766	.0234	Omoto *et al.* 1978
Tagalog	253	.9121	.0879	-ditto-
Thailand				
Bangkok	94	.9309	.0691	Present study
Australia				
Aborigines				
Elcho Is.	35	.986	-	Kirk *et al.* 1971
Groote Eylandt	90	.911	.028	-ditto-
Bathurst Is.	104	.928	.072	-ditto-
Mowanjum	244	.932	.049	Blake, 1979
Papua New Guinea				
Manus Is.	183	.7951	.2049	Malcolm *et al.* 1972
Motu	754	.8833	.1167	Blake (unpublished)
Western Pacific area				
Solomon Islands				
Santa Cruz Is.	349	.8195	.1805	Mazzur and Blake, 1981
Trust Territory of Pacific Islands				
Western Caroline Is.	382	.9385	.0615	Blake *et al.* 1973b
Fiji				
Fijians	332	.8012	.1988	Blake (unpublished)

Table 3.7.c. Cont'd

* $\underline{PGD}^{Richmond}$ = .0026 and .0019 for Niasans and Singapore Malays.

$\underline{PGD}^{Singapore}$ = .0013 for Singapore Chinese

$\underline{PGD}^{Thai}$ = .001 for Malayan Malays

$\underline{PGD}^{Elcho}$ = .014, .061 and .019 for Elcho Island, Groote Eylandt and Mowanjum respectively.

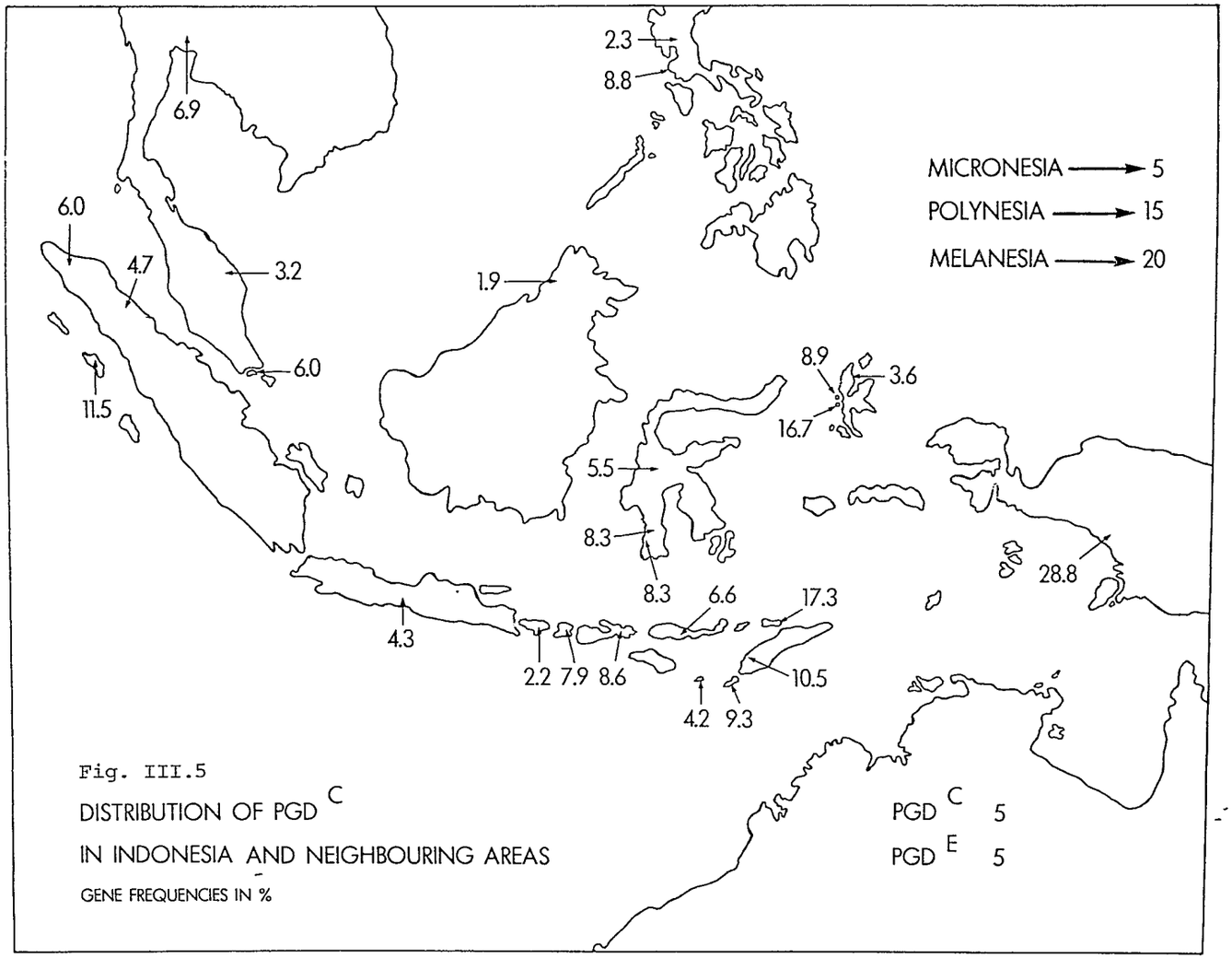


Fig. III.5

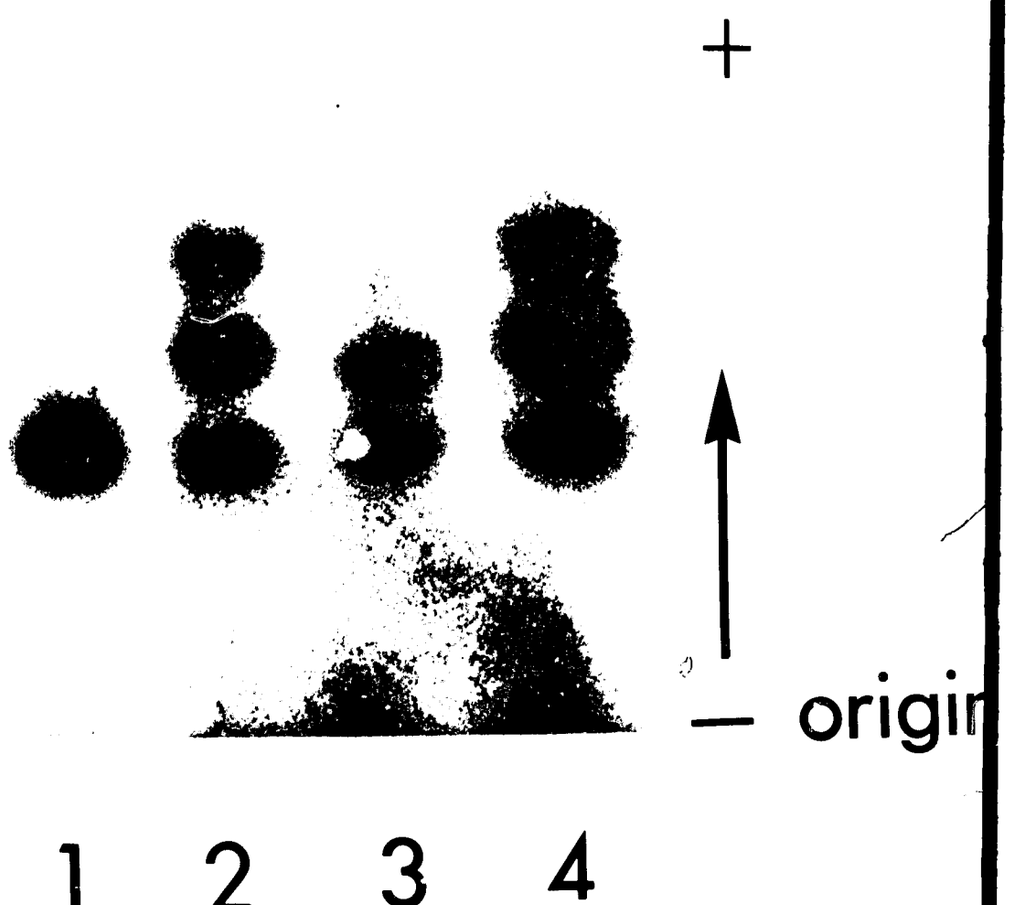
DISTRIBUTION OF PGD ^{C}

IN INDONESIA AND NEIGHBOURING AREAS

GENE FREQUENCIES IN %

Fig. III.6 Variant of 6-phosphogluconate dehydrogenase (6-PGD)

1. 6-PGD A
2 and 4 6-PGD A-Elcho
3. 6-PGD A-Lombok


+
origin
1 2 3 4

In this stud it seems that higher frequencies are found in the population of the eastern part of Indonesia being highest in Asmat (28.8%) which is comparable to other populations of Irian Jaya studied by Gajdusek *et al.* (1978) and Kirk *et al.* (1973). In general, except for a high frequency for Niasans in the west and a low frequency for Galelarese in the east, the 6-$\underline{PGD}^C$ allele seems to decrease in frequency towards the west (Fig. III.5).

In addition to the common phenotypes described above, during this study a single variant was found in a Sasak individual from Lombok. The variant is characterized by a three-banded pattern with the most cathodal band located at the normal $\underline{PGD}^A$ position and the other two bands more anodal. Electrophoretically the variant is distinguishable from a variant available in this laboratory, the Elcho variant (Fig. III.6). It has been designated PGD A-Lombok after the place where it was found.

3.2.8. Phosphoglucomutase

Phosphoglucomutase or α-D glucose 1,6-diphosphate; α-D glucose -1-phosphate phosphotransferase (PGM; E.C. 2.7.5.1) catalyses the transfer of a phosphate group from the first to the sixth position of glucose.

Since the red cell PGM polymorphism was first described by Spencer *et al.* (1964), it has been established that this enzyme is controlled by autosomal codominant alleles at several distinct loci, PGM_1, PGM_2 and PGM_3. the PGM_1 and PGM_2 loci are easily detected in the red cells, while PGM_3 may be detected in other tissues.

At the PGM_1 locus, three common phenotypes, $PGM_1 1$, $PGM_1 2\text{-}1$ and $PGM_1 2$, are found in all populations so far studied. By starch gel electrophoresis it appears that these phenotypes are controlled by two codominant autosomal alleles, $\underline{PGM_1^1}$ and $\underline{PGM_1^2}$. However, by isoelectric focussing technique in polyacrylamide gels, four common alleles at the

PGM_1 locus can be detected (Kuhnl and Spielmann, 1978a Kuhnl *et al.* 1977; Sutton and Burgess, 1978).

At the PGM_2 locus, only a few populations possess alleles which reach polymorphic frequency. The third locus, PGM_3 was first reported to be polymorphic by Hopkinson and Harris (1968) and variation in its activity was observed in different tissues. With the exception of red cells and fibroblasts, about 85-95% of the total PGM activity is determined by the PGM_1 locus, about 2-15% is contributed by the PGM locus and 1-2% is determined by the PGM_3 locus (McAlpine *et al.* 19[illegible]).

More recently a fourth locus, PGM_4, has been described (Cantu and Ibarra, 1982). Its variation was detected in human milk and eight phenotypes determined by four alleles were described.

So far only the PGM_1 and PGM_2 loci have been extensively studied, since their activity is easily detected in red cells (McAlpine *et al.* 1970). Gene frequencies have been reported from various populations. With special reference to the Asia-Pacific region, Blake and Omoto (1975) described eight alleles at the PGM_1 locus and 10 alleles at the PGM_2 locus. One of the alleles at the PGM_2 locus, $\underline{PGM_2^9}$, reaches high frequencies in some populations in New Guinea.

$\underline{PGM_1}$

During the present study the three common phenotypes PGM_1 1-1, PGM_1 2-1 and PGM_1 2-2 were observed in almost all populations. As shown in table 3.8.a, no PGM_1 2-2 phenotypes were found in the Savunese, Rotinese or Asmat. However, the phenotype distribution is in agreement with Hardy-Weinberg equilibrium except for the Ternatans although this is non-significant when Yates' correction is applied ($\chi_1^2 = 3.15$). A very low gene frequency of $\underline{PGM_1^2}$ was found in the Asmat (1.5%) and in the Savunese (6.9%). In the other populations it ranged from 13.6% in the Rotinese to 28.8% in the Sasak.

In addition to the common alleles $\underline{PGM_1^1}$ and $\underline{PGM_1^2}$, some rare variants were observed in this study. In the Javanese five individuals possessed the PGM_1 7-1 phenotype, in the Buginese one individual the PGM_1 6-1 phenotype and in the Asmat the $\underline{PGM_1^3}$ allele had a polymorphic frequency, higher than that of the $\underline{PGM_1^2}$ gene frequency in the same population.

Among the Balinese, as shown in table 3.8.b, a greater range of $\underline{PGM_1^2}$ gene frequency was observed, ranging from 11.8% in Ped to 40.6% in Dauh Waru where significant deviation from Hardy-Weinberg equilibrium occurs ($P<0.05$). The average $\underline{PGM_1^2}$ gene frequency for the Balinese as a whole is 26.5%. No rare variants were found in this population.

Previous studies of PGM_1 in some Indonesian populations have been carried out by Blake (personal communication), Breguet *et al.* (1982b), Gajdusek *et al.* (1978); Kirk *et al.* (1973) and Lie Injo *et al.* (1974). A very low $\underline{PGM_1^2}$ gene frequency was found in some Irian Jaya populations (Gajdusek *et al.* 1978; Kirk *et al.* 1973), which is comparable to the result from the Asmat in the present study. In the other Indonesian populations, the $\underline{PGM_1^2}$ gene frequency falls within the range of the present study (see table 3.8.c).

In neighbouring areas the $\underline{PGM_1^2}$ gene frequencies for populations in South East Asia fall within the range of the Indonesian populations except for the Aeta Negritos of the Philippines who have a higher $\underline{PGM_1^2}$ gene frequency (49.6%). Low $\underline{PGM_1^2}$ frequencies have been observed in the Aborigines of Australia and some populations of Papua New Guinea. In the western Pacific area, the $\underline{PGM_1^2}$ frequency is comparable to that in Indonesian populations (table 3.8.c).

In the case of rare alleles, it seems that $\underline{PGM_1^7}$ is slightly more common than the others. Its frequency is high in Santa Cruz island (Mazzur and Blake, 1981) and in the Western Carolines (Blake *et al.* 1973b). Though in low frequency, it has also been reported in the

Table 3.8.a.

Distribution of Phosphoglucomutase locus 1 (PGM_1) phenotypes and gene frequencies in Indonesian populations

Population	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1*
		1-1	2-1	2-2	$\underline{PGM}_1^1$	$\underline{PGM}_1^2$	
Javanese (urban)	203**	109	78	13	.7365	.2562	1.1071
Javanese (rural)	204†	132	58	12	.7941	.2010	3.0921
Javanese (total)	407††	241	136	25	.7654	.2285	2.4911
Balinese	970	526	373	71	.7345	.2655	0.1890
Sasak	191	98	76	17	.7120	.2880	0.1682
Bimanese	198	133	59	6	.8207	.1793	0.0311
Florenese	76	46	28	2	.7895	.2105	0.8919
Savunese	36	31	5	0	.9306	.0694	0.2005
Rotinese	59	43	16	0	.8644	.1356	1.4518
Timorese	133	85	41	7	.7932	.2068	0.4825
Alorese	26	14	11	1	.7500	.2500	0.4274
Macassarese	199	126	60	13	.7839	.2161	2.4087
Buginese	205ϕ	108	85	11	.7366	.2610	1.5757
Toraja	101	70	25	6	.8168	.1832	3.0162
Ternatans	213	148	54	11	.8216	.1784	3.8927
Makianese	30	17	9	4	.7167	.2833	2.0481
Galelarese	155	110	42	3	.8452	.1548	0.1932
Asmat	163ϕϕ	151	5	0	.9632	.0153	0.2381

* d.f=2 for Javanese (urban, rural and total), Buginese and Asmat

** including 3 PGM 7-1

† " 2 PGM 7-1

†† " 5 PGM 7-1

ϕ including 1 PGM 6-1

ϕϕ " 7 PGM 3-1

Table 3.8.b.

Distribution of Phosphoglucomutase locus 1 (PGM_1) phenotypes and gene frequencies in Balinese populations

Village	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1
		1-1	2-1	2-2	$\underline{PGM}_1^1$	$\underline{PGM}_1^2$	
Dauh Waru	80	33	29	18	.5937	.4063	4.9435
Penebel	58	27	29	2	.7155	.2845	3.0200
Ayunan	69	40	26	3	.7681	.2319	0.2304
Renon	95	55	34	6	.7579	.2421	0.0581
Serangan	39	21	16	2	.7436	.2564	0.2244
Banjar Rangkan	48	25	17	6	.6979	.3021	1.2297
Ped	34	26	8	-	.8824	.1176	0.6044
Rendang	124	67	45	12	.7218	.2782	1.1530
Seraya	85	40	39	6	.7000	.3000	0.7263
Songan	116	57	48	11	.6983	.3017	0.0375
Julah	49	33	14	2	.8163	.1837	0.1093
Sembiran	101	62	38	1	.8020	.1980	3.4406
Beratan	32	20	11	1	.7969	.2031	0.1224
Wanayu	40	20	19	1	.7375	.2625	2.0575

Table 3.8.c.

Additional Phosphoglucomutase 1 (PGM_1) gene frequency data in Indonesia and neighbouring areas

Population	Number Tested	Gene Frequencies PGM_1^1	PGM_1^2	Others	Reference
Indonesia					
Batak (Toba)	188	.7819	.2181	-	McDermid *et al.* 1973
Niasans	192	.7969	.2031	-	Blake (unpublished)
Batak (Medan)	272	.761	.239	-	Lie-Injo *et al.* 1974
Minangkabau	65	.769	.231	-	-ditto-
Atjehnese	26	.808	.192	-	-ditto-
Balinese	316	.6899	.3101	-	Breguet *et al.* 1982b
Asmat	653	.942	.026	.032*	Gajdusek *et al.* 1978
Awyu	505	.769	.148	.083*	-ditto-
Kayagar	71	.951	.049	-	-ditto-
Moni	320	.978	.005	.017*	-ditto-
Ekagi	26	.981	-	.019*	-ditto-
Lake Plain	245	.982	.018	-	-ditto-
Dani	257	.9669	.0214	.0117*	Kirk *et al.* 1973

Table 3.8.c. Cont'd

Population	Number Tested	Gene Frequencies PGM_1^1	PGM_1^2	Others	Reference
Singapore, Malaysia and Philippines					
Malays (Singapore)	259	.7625	.2336	.0039*	Blake *et al.* 1973a
Chinese "	378	.7315	.2632	.0053†	-ditto-
Malaysian Aborigines	920§	.6821	.3076	.0103ϕ	Welch *et al.* 1972
Land Dayaks	285	.716	.284	-	Ganesan *et al.* 1976
Sea Dayaks	240	.779	.221	-	-ditto-
Kadazans	219	.744	.256	-	Tan *et al.* 1979
Negrito	129	.5000	.4961	.0039#	Omoto *et al.* 1978
Tagalog	253	.7225	.2700	.0025# .0050ϕ	-ditto-
Thailand					
Bangkok	94	.7394	.2553	.0053#	Present study
Australia					
Aborigines					
Elcho Is.	35	1.0000	-	-	Kirk *et al.* 1971
Groote Eylandt	90	.95	.05	-	-ditto-

Table 3.8.c. Cont'd

Population	Number Tested	Gene Frequencies PGM_1^1	PGM_1^2	Others	Reference
Aborigines					
Bathurst Is.	104	.93	.07	-	Kirk *et al.* 1971
Mowanjum	244	.887	.114	-	Blake, 1979
Solomon Is.					
Santa Cruz Is.	351	.7293	.2108	.0085* .0514$^{\phi}$	Mazzur and Blake, 1981
Papua New Guinea					
Manus Is.	183	.9016	.0984	-	Malcolm *et al.* 1972
Motu	754	.9297	.0703	-	Blake (unpublished)
Western Pacific Area Trust Territory of Pacific Islands					
Western Carolines	382	.7068	.1662	.0681* .0589$^{\phi}$	Blake *et al.* 1973b
Fiji					
Fijians	332	.6687	.3298	.0015*	Blake (unpublished)

* PGM_1^3 † PGM_1^8 ϕ PGM_1^7

\# PGM_1^6 § Gene frequencies are calculated from available phenotype

Tagalog in the Philippines (Omoto *et al.* 1978), in Malaysian Aborigines (Welch *et al.* 1972), in Thais (Sanpitak *et al.* 1972) and in Javanese (Lie-Injo and Poey-Oey, 1970).

$\underline{PGM_2}$

At this locus, the $\underline{PGM_2^9}$ allele is widespread in Papua New Guinea and Irian Jaya, reaching a frequency of almost 15% in the Pit River Dani (Blake and Omoto, 1975; Gajdusek *et al.* 1978). This allele has also been found, often at polymorphic frequencies, in much of Vanuatu and in some of the Polynesian Outliers of the Solomon islands (Blake *et al.* 1982a). Another allele, $\underline{PGM_2^{10}}$, has a somewhat similar distribution though the frequency does not achieve the high values of $\underline{PGM_2^9}$ (Blake and Omoto, 1975; Gajdusek *et al.* 1978; Blake *et al.* 1982a).

Previous studies in Indonesia (apart from Irian Jaya) revealed no PGM_2 variation in Toba Batak (McDermid *et al.* 1973) or in Niasans (Blake, personal communication). However, Breguet *et al.* (1982b) reported a $\underline{PGM_2^9}$ gene frequency of 0.5% in Balinese from Tenganan Pageringsingan.

This present study indicates that $\underline{PGM_2^9}$ is widespread in Indonesian populations (table 3.9.a). It was found in all of the populations studied except the Savunese, Toraja, Makianese and Galelarese; it reaches polymorphic frequencies in the rural Javanese (1.2%), Sasak (1.6%), Alorese (1.9%) and the Asmat (11.7%) and, in all cases, the phenotype distribution is in agreement with Hardy-Weinberg equilibrium.

In the Balinese villages, $\underline{PGM_2^9}$ occurs at Seraya and Ayunan (table 3.9.b), and the average gene frequency for Balinese as a whole is 0.4%.

The other allele at this locus, $\underline{PGM_2^{10}}$, detected in this study was found in the Asmat of Irian Jaya, but only one example was found in the Indonesian archipelago, in the Balinese village of Penebel.

Table 3.9.a.

Distribution of Phosphoglucomutase locus 2 (PGM_2) phenotypes and gene frequencies in Indonesian populations

Population	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1
		1-1	9-1	9-9	$\underline{PGM}_2^1$	$\underline{PGM}_2^9$	
Javanese (urban)	203	201	2	-	.9951	.0049	0.0052
Javanese (rural)	204	199	5	-	.9877	.0123	0.0303
Javanese (total)	407	400	7	-	.9914	.0086	0.0306
Balinese	970*	963	4	2	.9954	.0041	-
Sasak	191	185	6	-	.9843	.0157	0.0486
Bimanese	198	197	1	-	.9975	.0025	0.0013
Florenese	76	75	1	-	.9934	.0066	0.0033
Savunese	36	36	-	-	1.0000	.0000	0.0000
Rotinese	59	58	1	-	.9915	.0085	0.0043
Timorese	133	132	1	-	.9962	.0038	0.0019
Alorese	26	25	1	-	.9808	.0192	0.0100
Macassarese	198	197	2	-	.9950	.0050	0.0051
Buginese	205	204	1	-	.9976	.0024	0.0012
Toraja	101	101	-	-	1.0000	.0000	0.0000
Ternatans	213	209	4	-	.9906	.0094	0.2002
Makianese	30	30	-	-	1.0000	.0000	0.0000
Galelarese	155	155	-	-	1.0000	.0000	0.0000
Asmat	163*	128	30	4	.8804	.1166	1.9521†

* 1 PGM_2 10-1

† d.f=2

Table 3.9.b.

Distribution of Phosphoglucomutase locus 2 (PGM_2) phenotypes and gene frequencies in Balinese populations

Village	Number Tested	Phenotypes			Gene Frequencies	
		1-1	9-1	9-9	$\underline{PGM_2^1}$	$\underline{PGM_2^9}$
Seraya	85	80	4	1	.9647	.0353
Ayunan	69	68	-	1	.9855	.0145
Penebel	58	57	1*	-	.9914	.0086†
Others	758	758	-	-	1.0000	-
Total	970	963	4 1*	2	.9954	.0041 .0005†

* PGM_2 10-1

† PGM_2^{10}

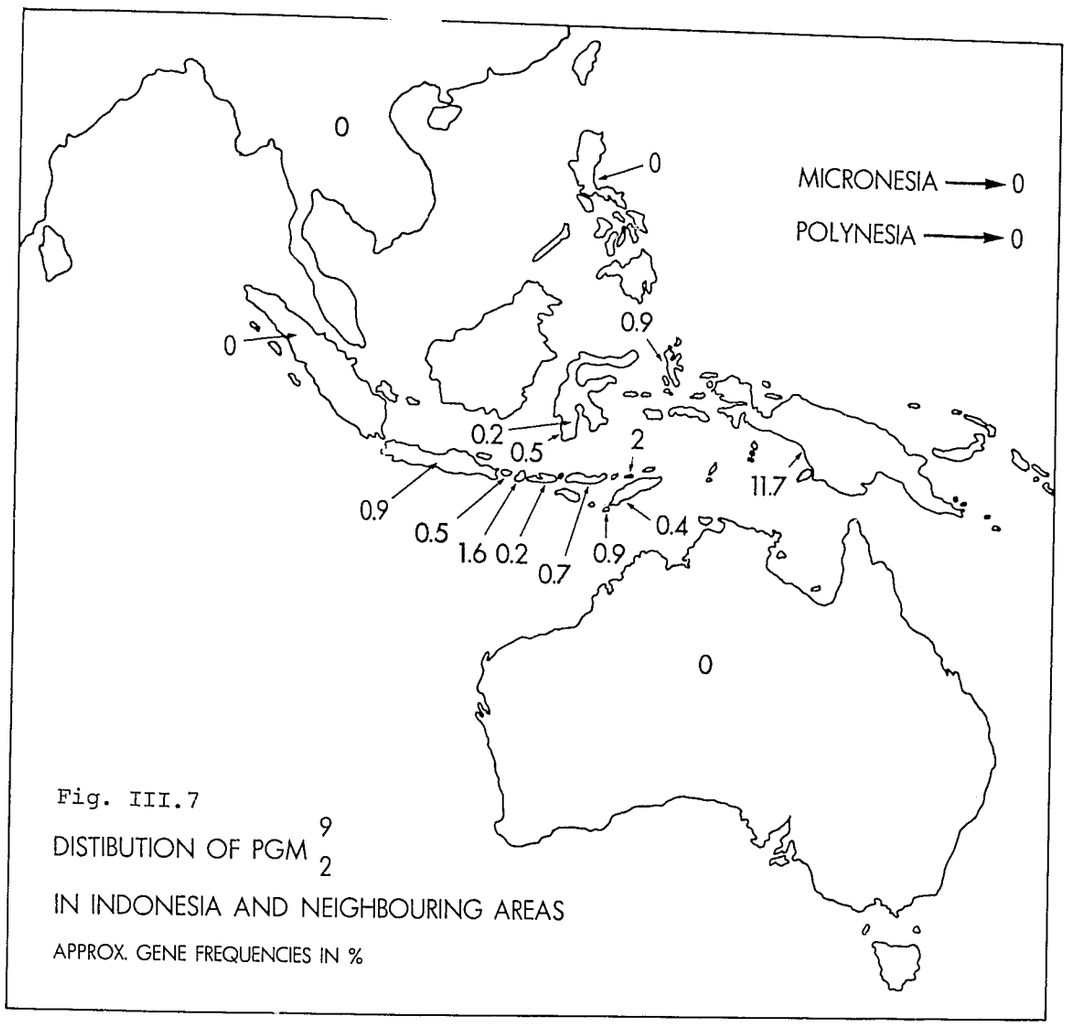


Fig. III.7

DISTIBUTION OF PGM $^{9}_{2}$

IN INDONESIA AND NEIGHBOURING AREAS

APPROX. GENE FREQUENCIES IN %

Since both $\underline{PGM_2^9}$ and $\underline{PGM_2^{10}}$ are widespread in New Guinea and the western Pacific region, the occurrence of these two alleles, and particularly the former, in Indonesia is of great interest. As shown in Fig. III.7, $\underline{PGM_2^9}$ occurs as far west as Java, but it is absent in Sumatera and Kalimantan. The distribution of both $\underline{PGM_2^9}$ and $\underline{PGM_2^{10}}$ over the western Pacific region suggests that the populations show some genetic relationship which may trace back to past common ancestors.

3.3. Red cell enzyme with rare variants

3.3.1. Carbonic anhydrase 1 and 2

Carbonic anhydrase, or carbonic hydro-lyase (CA; E.C. 4.2.1.1), a zinc metalloenzyme, is the second major protein component of red cells. It catalyses the reversible conversion of carbon dioxide and water to hydrogen and bicarbonate ion ($CO_2 + H_2O \rightleftarrows H^+ + HCO_3^-$). It occurs in the red blood cell as well as in many other tissues.

In human red cells, two forms of carbonic anhydrase are known to occur, determined by two autosomal loci. They have been called variously, "low activity" and "high activity", CA B and CA C, CA I and CA II or CA_1 and CA_2 respectively. The latter nomenclature is used here. In starch gel electrophoresis at pH 8.6, CA_1 migrates anodally, whereas CA_2 migrates cathodally. However, although a number of genetic variants of human CA have been described they are, in general, not common.

Carbonic anhydrase 1

At the CA_1 locus, the common allele is $\underline{CA_1^1}$ and a number of rare alleles have been described (see summaries by Blake, 1978a; Tashian *et al.* 1980).

In the present study, a number of examples of a CA_1 electrophoretic variant were found in several populations as shown in table 3.10.a.

Table 3.10.

Distribution of carbonic anhydrase variants in Indonesian populations

a. Carbonic anhydrase 1 (CA_1)

	Number Tested	Phenotypes			Gene Frequencies	
		1-1	3-1	Others	$\underline{CA}_1^1$	$\underline{CA}_1^{3*}$
Javanese	407	400	7	-	.9914	.0086
Balinese	970	965	4	1†	.9969	.0031
Sasak	191	190	-	1ϕ	.9974	.0026
Bimanese	198	196	2	-	.9949	.0051
Ternatans	213	210	3	-	.9930	.0070
Galelarese	155	154	1	-	.9968	.0032
Macassarese	199	197	2	-	.9950	.0050
Buginese	205	203	2	-	.9951	.0049

* CA_1^{10} for Sasak

† CA_1 3-3

ϕ CA_1 10-1

b. Carbonic anhydrase 2 (CA_2)

Population	Number Tested	Phenotypes			Gene Frequencies	
		1-1	6-1	7-1	$\underline{CA}_2^1$	Others
Javanese	407	404	1	2	.9963	.0012*
						.0024†
Bimanese	198	197	1	-	.9975	.0025*

* $\underline{CA}_2^6$

† $\underline{CA}_2^7$

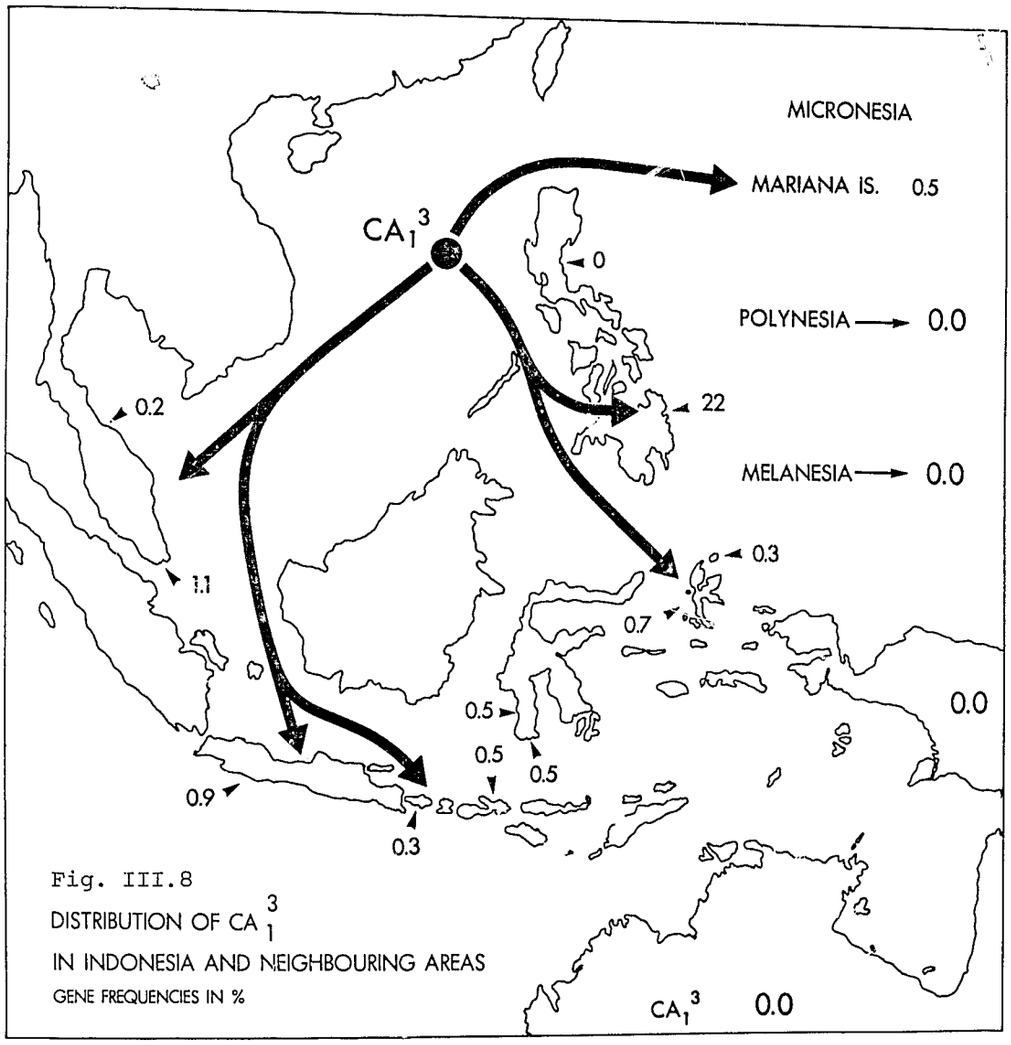


Fig. III.8
DISTRIBUTION OF CA_1^3
IN INDONESIA AND NEIGHBOURING AREAS
GENE FREQUENCIES IN %

Careful testing against known sub-standards showed these to have the same mobility as the Guam variant first detected by Tashian *et al.* (1963) and now designated CA_1 3. Similar variants have also been reported from Bali by Breguet *et al.* (1982b), in Filipinos living in the U.S.A. (Lie-Injo, 1967), in Malaysia (Lie-Injo *et al.* 1971a) and in Indonesia (Lie-Injo and Poey-Oey, 1970). The corresponding allele, $\underline{CA}_1^3$, has been shown to be highly polymorphic in Negritos from the Philippines (the Mamanwas of Mindanao) (Omoto, 1979, 1980; Omoto *et al.* 1981). Omoto *et al.* (1981) have shown recently that the amino acid substitution in the Negrito variant from the Philippines is identical to that in the original Guam variant (Tashian *et al.* 1963). Thus, as shown in Fig. III. 8, there is a centre of high frequency for $\underline{CA}_1^3$ focussed in the Philippines with scattered occurrences at low frequencies towards the east, west and south; this suggests that the variant was once common in some population groups in South East Asia and has spread from these by diffusion.

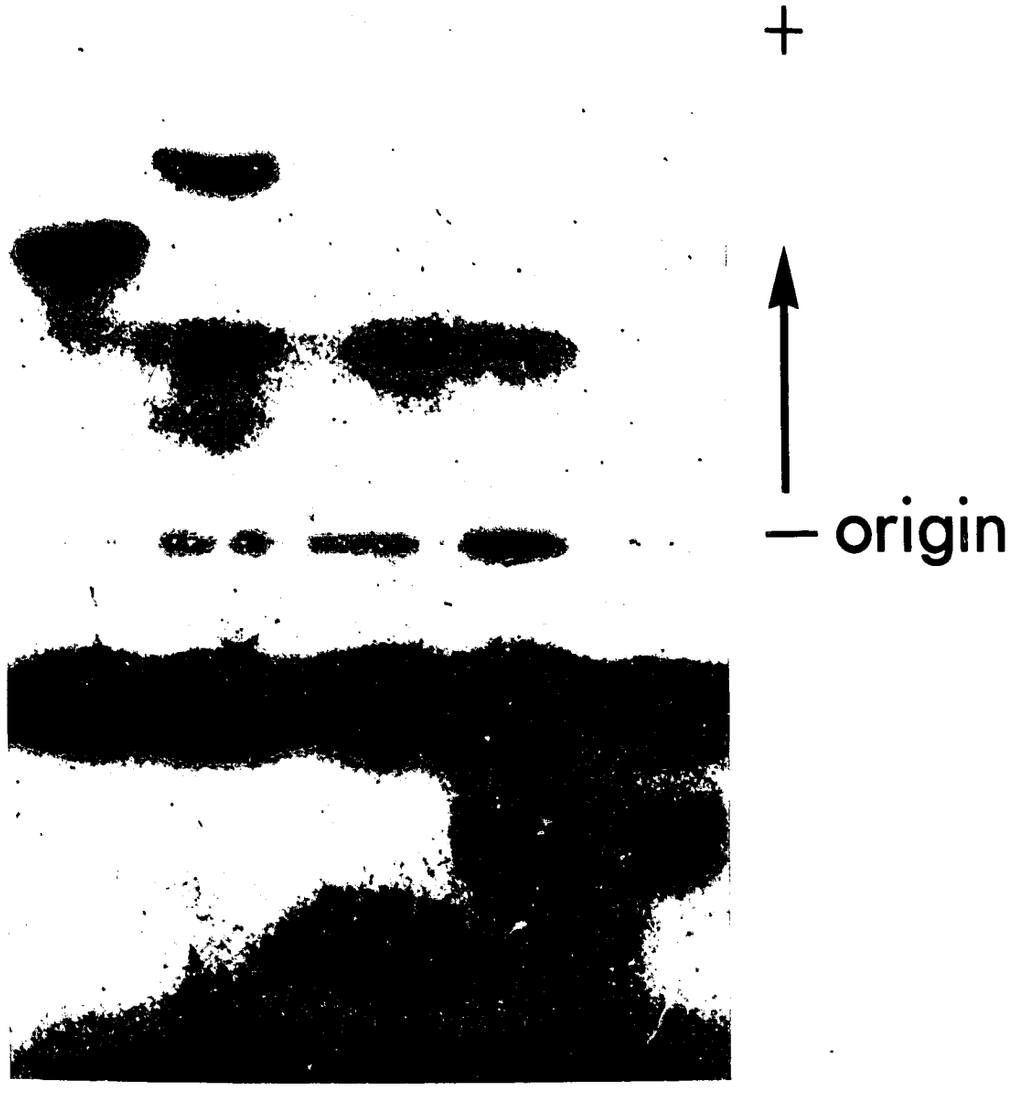
Two other CA_1 alleles, $\underline{CA}_1^9$ and $\underline{CA}_1^{10}$, are both polymorphic in Aborigines in Australia, the former being widespread whilst $\underline{CA}_1^{10}$ has so far only been detected in Nunggubuyu-speaking people from Arnhem Land (Blake, 1978a). However, during the present investigation an unusual phenotype with the variant isozyme, more anodal than the common isozyme, was detected in a Sasak from Lombok. This presumptive heterozygote was electrophoretically indistinguishable from the Australian Aboriginal CA_1 10 1 and it is proposed to designate this phenotype CA_1 10-1 Lombok.

Carbonic anhydrase 2

At the CA_2 locus, $\underline{CA}_2^2$ is polymorphic in Black Africans and Black Americans (Moore *et al.* 1971; Carter, 1972), $\underline{CA}_2^3$ is polymorphic in Parsis (Ghosh, 1977b) and $\underline{CA}_2^4$ has been detected at frequencies up to

Fig. III.9 Variants of carbonic anhydrase (CA_2)

1. CA_2 6-1 2. CA_2 5-1
3. CA_2 4-1 4 and 5. CA_2 7-1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5

8% in Australian Aborigines (Blake, 1978a). Mohrenweiser *et al.* (1979) have reported another allele, CA II^{BAN-1}, to be polymorphic in two Amerindian tribes. Apart from these few populations where polymorphic variation occurs at the CA_2 locus, only a single rare CA2 allele has been so far detected. Ghosh *et al.* (1979) described a family in Bengal in which the father was homozygous and his three daughters heterozygous for a new allele, CA_2^5. In the present study, a further two new alleles at the CA_2 locus have been detected (see table 3.10.b). The first of these was found in a Javanese and a further example of the same pattern was detected in a Bimanese. Electrophoresis of these haemolysates showed a band in the normal CA_2 1 position with the variant isozyme present with a mobility slightly less anodal than the Parsi CA_2 3 isozyme (Fig. III. 9). This presumptive heterozygote has been designated CA_2 6-1; the variant isozyme found in this Javanese blood sample has been subjected to purification and peptide mapping and the amino acid subsitution has been determined as 17 lys→glu (Jones *et al.* 1982). In this publication the variant phenotype has been referred to as CA II$^{Jogjakarta-1}$.

The second new variant, of which two examples were found in the Javanese population, also shows a band in the normal CA_2 1 position. In addition, a further band was present at approximately half the cathodal migration distance of the Australian CA_2 4 isozyme (Fig. III. 9). This presumptive heterozygote has been designated CA_2 7-1. No further purification and characterization of this particular variant has been carried out so far.

3.3.2. Malate dehydrogenase

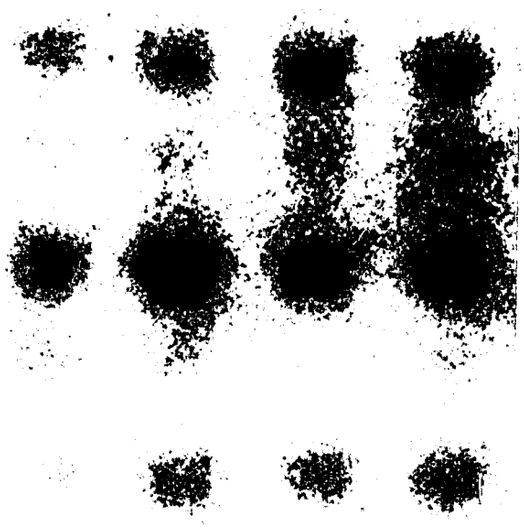
Malate dehydrogenase or L-malate : NAD oxidoreductase (MDH; E.C. 1.1.1.37) catalyses the reversible oxidation of malate to oxaloacetate. In mammalian tissues two different forms of enzyme, the cyto-

Fig. III.10 Variant of soluble malate dehydrogenase (MDH_1)

1. MDH_1 1-1 2. MDH_1 3-1 from Toraja

3 and 5. MDH_1 3-1 from New Guinea

4. MDH_1 3-1 from Macassarese



+

— origin

1 2 3 4 5

plasmic and the mitochondrial forms can be distinguished electrophoretically. Both enzymes are autosomally determined, the cytoplasmic form (MDH_1) being located on chromosome 2 (Creagan *et al.* 1974) and the mitochondrial form (MDH_2) being located on chromosome 7 (van Heyningen *et al.* 1975).

The majority of population genetic studies have been carried out on MDH_1. Davidson and Cortner (1967) detected a single slow electrophoretic variant of MDH_1 in an American Black and Blake *et al.* (1970b) described another variant allele, $\underline{MDH_1^3}$, in the Eastern Highlands of Papua New Guinea. A comprehensive review of its distribution is presented in Blake (1978b). Papiha *et al.* (1977) have documented the presence of $\underline{MDH_1^3}$ in the northeast of England although the identity of this and the New Guinea allele have not been shown by amino acid analysis of the two gene products. Another allele, $\underline{MDH_1^6}$ is polymorphic in the Western Caroline Islands and, to date, has not been detected elsewhere (Blake, 1978b).

In the present study, four examples of an MDH_1 variant were found, one in a Macassarese, one in a Toraja and two in the Asmat population. As shown in Fig. III. 10, these were electrophoretically indistinguishable from the MDH_1 3-1 found widespread in New Guinea populations.

3.3.3. Peptidase B

A number of peptidases are detectable in red cells (Lewis and Harris, 1967). These enzymes are able to hydrolyse a large number of dipeptides and tripeptides with a wide range of relative activities. Genetic polymorphism of peptidase A (PEP A), peptidase B (PEP B) and peptidase D (PEP D) has been demonstrated by Lewis and Harris (1967, 1969) and Santachiara-Benerecetti (1970) has described a peptidase C (PEP C) polymorphism in Babinga Pygmies.

Table 3.11

Distribution of rare Peptidase B (PEP B)

variants in Indonesia

Population	Number Tested	Phenotypes			Gene frequencies		
		1-1	2-1	6-1	PEP B^1	PEP B^2	PEP B^6
Timorese	133	131	2	-	.9925	.0075	-
Florenese	76	75	1	-	.9934	.0066	-
Rotinese	59	58	1	-	.9915	.0085	-
Asmat	163	154	-	9	.9724	-	.0276

Blake *et al.* (1970b) have described two PEP B alleles which are polymorphic in Australian Aborigines, PEP B^6 in the north of the country and PEP B^7 in the centre, and Blake (1979) has extended the distribution of PEP B^6 and has shown it to be restricted to northern tribes. Gajdusek *et al.* (1978) have shown PEP B^6 and also another allele, PEP B^2, to be present in the southern coastal plain of Irian Jaya.

In the present study, the allele PEP B^6 was found at a frequency of 2.8% in the Asmat, but was not detected in any of the populations from the Indonesian archipelago. This is of particular interest since Welch (1973) has reported PEP B^6 in West Malaysian aborigines and Tan *et al.* (1979) showed the presence of this allele in the Kadazans of Sabah.

Another allele, PEP B^2, was also detected in the present study in the Timorese, Florenese and Rotinese but only at low frequency (Table 3.11). PEP B^2 was not detected in the Asmat, in contrast to Gajdusek *et al.* (1978) who reported a frequency of 0.2% for the Awyu of Irian Jaya, a language family adjacent to the Asmat.

3.3.4. Phosphohexose isomerase

Phosphohexose isomerase (PHI) or phosphoglucose isomerase (PGI) or D-glucose-6-phosphate ketol isomerase (E.C. 5.3.1.9) catalyses the reversible conversion of glucose-6-phosphate to fructose-6-phosphate in the glycolytic pathway.

Several inherited variants of red cell PHI have been detected by starch gel electrophoresis in samples collected in London and Seattle (Detter *et al.* 1968). In addition to the common phenotype, ten other phenotypes were reported, most of them being rare. The alleles PHI^3 and PHI^5 appear to be more common in Indian populations, the former being polymorphic in Gujarat Vania Soni (Undevia *et al.* 1978) and in

the Koya (Blake *et al.* 1981) whilst PHI^5, in addition to being present at a low frequency in the Surat Vania Soni (Undevia *et al.* 1978), is highly polymorphic in the Chenchu (Ramesh *et al.* 1980). In the Western Pacific region, the occurrence and distribution of PHI alleles has been reviewed by Ishimoto (1975).

In contrast to the above populations, in this Indonesian survey only two PHI variants were detected, both of them identical electrophoretically with Indian examples of PHI 3-1, one in a Javanese and the other in a Sasak.

3.4. Red cell enzymes showing no variation

During the present study, six red cell enzyme systems showed no variation, i.e. lactate dehydrogenase, peptidase A, phosphoglycerate kinase, phosphoglycolate phosphatase, phosphoserine phosphatase and superoxide dismutase (see table 3.12).

3.4.1. Lactate dehydrogenase

Electrophoretic variants of lactate dehydrogenase (LDH; E.C. 1.1.1.27), a glycolytic enzyme catalysing the conversion of L-lactate to pyruvate, have been identified in various human populations (see Giblett, 1969; Mourant *et al.* 1976). The enzyme is constituted by two different subunits, LDH A and LDH B, the product of genes located on chromosome 11 and 12 respectively (Boone *et al.* 1972; Chen *et al.* 1973). Therefore, the LDH variants may be due to mutations at either the A or B loci. It appears that mutability is higher in the A subunit (Blake *et al.* 1969).

There are at least ten different electrophoretic variants known to occur, most of them in non-polymorphic frequencies (Mourant *et al.* 1976). But one of the variants, LDH Calcutta-1 (LDH CAL-1), a B subunit variant, has been found to be polymorphic in some population

Table 3.12

Systems showing no variation and number of persons tested

Population	SYSTEMS						
	LDH	PEP A*	PGK	PGP	PSP	SOD	Cp
Javanese (total)	407	407	407	407	407	407	407
Balinese	970	n.t.	970	n.t.	n.t.	970	n.t.
Sasak	191	191	191	191	191	191	191
Bimanese	198	198	198	198	198	198	198
Florenese	76	76	76	76	76	76	76
Savunese	36	36	36	36	36	36	36
Rotinese	59	59	59	59	59	59	59
Timorese	133	133	133	133	133	133	133
Alorese	26	26	26	26	26	26	26
Macassarese	199	199	199	199	199	199	199
Buginese	205	205	205	205	205	205	205
Toraja	101	101	101	101	101	101	101
Ternatans	213	213	213	213	213	213	213
Makianese	30	30	30	30	30	30	30
Galelarese	155	155	155	155	155	155	155
Asmat	163	163	163	163	n.t.	163	156

* see also Table 3.13.

n.t.: not tested

groups in India (Das *et al.* 1970; Mukherjee and Das, 1974; Cutten, 1981). In the South East Asia and Pacific region, LDH variants have been reported in a New Guinea Highland population (Blake *et al.* 1969), in Chinese, Indian or Malays from Malaysia (Lie-Injc *et al.* 1973b) and in Chinese and Malays from Singapore (Blake *et al.* 1973a).

In the present study, no LDH variants were detected, not even in the Balinese, in contrast to the finding of LDH CAL-1 at polymorphic frequency (2.9%) reported by Breguet *et al.* (1982b).

3.4.2. Peptidase A

Electrophoretic variation of red cell peptidase A (PEP A; E.C. 3.4.11) first reported by Lewis and Harris (1967), is determined by a series of alleles at an autosomal locus. Later, several rare alleles have been identified in Europeans (Harris and Hopkinson, 1976). However, the common variants, PEP A $^{-}$, PEP A 8-1 and PEP A 8 have only been described in Europeans in extracts of leucocytes (Lewis, 1973). These variants, determined by autosomal codominant alleles, PEP A^1 and PEP A^8 were confirmed by Kömpf *et al.* (1979). In red blood cells, wide variation in the level of the enzyme activity between different individuals with the common electrophoretic phenotype PEP A 1 has been demonstrated, unlike the enzyme activity in leucocytes which shows less variation (Sinha *et al.* 1970). Furthermore, the mean value of the enzyme activity is also different between the sexes. Based on enzyme activity levels, it is not possible to classify the individuals into clearly separate phenotypes.

During this study, in addition to the common high activity of PEP A 1 phenotype, the 1-1 weak and zero phenotypes were also observed in almost all populations tested (see table 3.13). No other rare variants have been encountered.

Table 3.13.

Distribution of Peptidase A phenotypes

in Indonesia

Population	Number Tested	Phenotypes		
		1-1	1-1 weak	0
Javanese (total)	407	375	29	3
Sasak	191	184	7	-
Bimanese	198	179	16	3
Florenese	76	74	2	-
Savunese	36	35	-	1
Rotinese	59	56	3	-
Timorese	133	128	5	-
Alorese	26	26	-	-
Macassarese	199	172	19	8
Buginese	205	172	19	14
Toraja	101	99	2	-
Ternatans	213	186	21	6
Makianese	30	30	-	-
Galelarese	155	133	19	3
Asmat	163	156	5	2

3.4.3. Phosphoglycerate kinase

Electrophoretic variation of phosphoglycerate kinase (PGK; E.C. 2.7.2.3), a glycolytic enzyme catalysing the reversible conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate, was discovered in samples from New Guinea and Samoa, and family studies showed an X-linkage of the structural locus for this enzyme (Chen *et al.* 1971). The variants, PGK 2-1 and PGK 2 were later found not to be restricted in the region as originally suggested, since PGK 2-1 has also been reported from a European, a Canadian Indian and a native of Kenya (Chen and Giblett, 1972). However, whether or not the variants are structurally identical cannot be stated with certainty. It is of interest that the PGK^2 allele reaches high frequency in Micronesia, while another allele, PGK^4 is polymorphic in New Guinea and West Irian (Omoto and Blake, 1972).

During the present study, no PGK variants have been recorded in Indonesian populations, not even in the Asmat where PGK^4 has been reported previously, though in low frequency (Gajdusek *et al.* 1978).

3.4.4. Phosphoglycolate phosphatase

Since phosphoglycolate phosphatase (PGP; E.C. 3.1.3.18) activity was demonstrated in human erythrocytes by Badwey (1977), electrophoretic variation as well as the occurrence in other tissues has been described by Barker and Hopkinson (1978). Six phenotypes determined by three alleles at an autosomal locus have been described, PGP 1, PGP 2-1, PGP 2, PGP 3-1, PGP 3-2 and PGP 3. All these phenotypes are found in Europeans, but only PGP 1 and PGP 2-1 were present in small population samples of Asiatic Indians and no PGP variants were detected in Black Africans. The gene frequencies for PGP^1, PGP^2 and PGP^3 in Europeans are 82.6%, 12.9% and 4.5% respectively (Barker and Hopkinson, 1978). Slightly lower PGP^2 and PGP^3 gene frequencies have been reported for various

Jewish communities (Golan *et al.* 1981). Polymorphic frequency of $\underline{PGP}^2$ in Asiatic Indians is confirmed by Blake and Hayes (1980) who reported the $\underline{PGP}^2$ gene frequencies of 3.5% to 6.5% in some Indian populations. $\underline{PGP}^2$ gene frequencies in Amerindian populations are among the highest so far reported (Blake and Hayes, 1980).

In the South East Asia and Pacific region, $\underline{PGP}^2$ occurs at polymorphic level only in Guam and only a single example of PGP 2-1 has been found in a Malay from Singapore as well as a perso from Western Samoa (Blake and Hayes, 1980). The absence of PGP variants in Indonesian populations during the present study is, therefore, comparable to the virtual absence of the variants in South East Asia demonstrated previously.

3.4.5. Phosphoserine phosphatase

Following the investigation of the substrate specificity of the genetic variants of human phosphoglycolate phosphatase (Barker and Hopkinson, 1978), phosphoserine phosphatase (PSP; E.C. 3.1.3.3), a phosphohydrolase which catalyses the hydrolysis of D-phosphoserine has been demonstrated in various tissues (Moro-Furlani *et al.* 1980) including the red blood cell.

Two different types of rare electrophoretic variants (PGP 2-1 and PGP 3-1) have been identified in post mortem kidney homogenates. However similar variants failed to be detected in red cell samples (Moro-Furlani *et al.* 1980). The absence of PSP variants in the red blood cell during this study is therefore not surprising.

3.4.6. Superoxide dismutase

Superoxide dismutase (SOD), which catalyses the dismutation of free $O_2^{\cdot -}$ radicals to yield hydrogen peroxide and oxygen, is found in various human tissues. Two isozymes, referred to as A and B are present.

However, only isozyme A is observed in red blood cells (Beckman *et al.* 1973b).

Polymorphism of red cell SOD has been reported to occur in northern Sweden (Beckman, 1973; Beckman *et al.* 1973a) and northern Finland (Beckman and Pakarinen, 1973). Three phenotypes were described, SOD 1, SOD 2-1 and SOD 2, determined by two codominant alleles, $\underline{SOD}^1$ and $\underline{SOD}^2$. Elsewhere variants have been reported only sporadically except in one series from the Caspian Sea area of Iran (Kirk *et al.* 1977).

During this study, no single example of an SOD variant was observed in Indonesian populations, although Teng and Lie-Injo (1977) have reported a variant in one series from a neighbouring population of Indonesia, the Filipino.

SECTION B. SERUM PROTEIN MARKERS

3.5. Introduction

In the Indonesian archipelago, despite its strategic geographical setting between Asia to the north and Australasia to the south and marked ethnic variation, very limited data on serum protein and other blood genetic markers are available. In this study, three serum protein systems were examined; haptoglobin (Hp), transferrin (Tf) and ceruloplasmin (Cp). Variation was observed for haptoglobin and transferrin in almost all populations studied, while no variation was detected in ceruloplasmin.

3.6. Haptoglobin

Haptoglobin (Hp) is an α-2 glycoprotein which binds haemoglobin to form a haptoglobin-haemoglobin complex. It has been suggested that through this complex formation, haptoglobin prevents haemoglobin loss by renal excretion. Its clinical, biochemical, genetical and anthropological importance has led this serum protein to be the subject of many extensive investigations and detailed reviews are provided by Baksi *et al.* (1977), Kirk (1968a) and Mourant *et al.* (1976).

Electrophoretic variation of human haptoglobin was first described by Smithies (1955a, 1955b) and it was shown that this variation was under the control of two allelic autosomal genes, $\underline{Hp}^1$ and $\underline{Hp}^2$, with the resultant phenotypes being Hp 1-1, Hp 2-1 and Hp 2-2 (Smithies and Walker, 1956). Connell *et al.* (1962) reported subtyping for this variation; using acidic starch gels containing urea after reductive cleavage of the Hp molecule it was shown that the Hp 1-1 phenotype could be subtyped into three different phenotypes called 1F-1F, 1F-1S and 1S-1S and Hp 2-1 into either 2-1F or 2-1S under the control of $\underline{Hp}^{1F}$ and $\underline{Hp}^{1S}$ genes.

A number of other variants were reported later, including Hp 1-J (see Kirk, 1968a). Another frequent variant is anhaptoglobinaemia (Hp O), a condition in which the haptoglobin level is too low to be detected by staining after electrophoresis. The absence or near absence of haptoglobin is very common in individuals with increased red cell destruction (haemolytic disease), and the frequency of Hp O is often high in populations where diseases such as malaria, result in excessive red cell breakdown (Kirk, 1968a). In newborn children, haptoglobin also may not be detected, due supposedly to delayed synthesis (Beckman and Grivea, 1964). However, evidence for an $\underline{Hp}^{O}$ allele with no demonstrable gene product has been reported (Cann and van West, 1966; Harris *et al.* 1958), but this strictly genetic basis for anhaptoglobinaemia seems to be rare.

Because the genotype of Hp O persons cannot be inferred, gene frequency calculations have been carried out by omitting the Hp O persons from the total. Since Hp 2-2 persons, who possess lower serum haptoglobin levels than Hp 1-1 or 2-1 (Nyman, 1959) are likely to be classified as Hp O more frequently than the other genotypic classes, some bias will be introduced into the gene frequencies recorded. The extent of the bias will vary with the Hp O frequency observed in each population.

As shown in table 3.14.a., three common phenotypes Hp 1-1, Hp 2-1 and Hp 2-2 are found in all populations studied in the present investigation. One Hp 1-J was observed in Galelarese. In addition to the three common phenotypes, the Hp O phenotype was present in almost all populations except the Savunese and Makianese. Omitting Hp O from the gene frequency calculations, the observed phenotype distributions fit well with Hardy-Weinberg equilibrium, with the exception of the Javanese (rural) and Savunese which show a significant deviation ($P<0.05$).

Apart from the Asmat, with the highest $\underline{Hp}^1$ gene frequency (79.5%) and the Alorese (56.3%) the $\underline{Hp}^1$ gene frequency distribution in other Indonesian populations ranges from 26.4% in the Savunese to 50% in the Makianese.

In the Balinese, the $\underline{Hp}^1$ gene frequency is very low in Ped (14.3%), Banjar Rangkan (14.6%) and Sembiran (19.6%) villages. In other villages it varies from 27.8% in Rendang to 43.7% in Julah (see table 3.14.b). The average $\underline{Hp}^1$ gene frequency for the Balinese as a whole is 30.2%. The phenotype distribution is in agreement with Hardy-Weinberg equilibrium. Breguet *et al.* (1982a) reported the $\underline{Hp}^1$ gene frequency of 25.3% in Balinese from Tenganan Pageringsingan; this falls within the range of the present study.

As shown in table 3.14.c, previous studies on the Toba Batak (McDermid *et al.* 1973), Batak, Minangkabau and Atjehnese from Medan (Lie-Injo *et al.* 1974) show $\underline{Hp}^1$ frequencies close to that in Java. In neighbouring populations of South East Asia, the Proto-Malays and the Kadazans (Kirk and Lai, 1961; Tan *et al.* 1979) have slightly higher $\underline{Hp}^1$ gene frequencies, but they all fall in the range of the Indonesian populations. The high frequency of $\underline{Hp}^1$ in the Asmat is comparable to that found in the previous studies in Irian Jaya (Gajdusek *et al.* 1978 and Kirk *et al.* 1973). Therefore, with the exception of the Savunese with a low $\underline{Hp}^1$ gene frequency, it appears that the $\underline{Hp}^1$ gene frequency distribution in the Indonesian archipelago shows a trend to increased values towards the east, being highest in Irian Jaya (Fig. III. 11). This trend is consistent with the suggestion put forward by Kirk and Lai (1961) that there is a steady increase in $\underline{Hp}^1$ values as one moves from the Asiatic mainland through the Indonesian islands to Micronesia and Polynesia.

Apart from the Asmat with a very high frequency of Hp O, the Hp O frequencies found in the present study ranged from zero

Table 3.14.a.

Distribution of Haptoglobin (Hp) phenotypes and gene frequencies in Indonesian populations

Population	Number Tested	Phenotypes				Gene Frequencies		
		1-1	2-1	2-2	"0"*	Hp^1	Hp^2	$\chi^2_{d.f=1}$
Javanese (urban)	203	11	81	108	3	.2575	.7425	0.6986
Javanese (rural)	204	24	73	105	2	.2995	.7005	3.8885
Javanese (total)	407	35	154	213	5	.2786	.7214	0.8874
Balinese	1010	92	423	491	4	.3017	.6983	0.0043
Sasak	191	32	84	69	6	.4000	.6000	0.5405
Bimanese	198	41	90	61	6	.4479	.5521	0.5235
Florenese	76	13	34	25	4	.4167	.5833	0.0588
Savunese	36	5	9	22	0	.2639	.7361	4.5754
Rotinese	59	11	32	15	1	.4655	.5345	0.6856
Timorese	133	31	63	34	5	.4883	.5117	0.0074
Alorese	26	8	11	5	2	.5625	.4375	0.1135
Macassarese	199	32	86	80	1	.3788	.6212	1.1762

Table 3.14.a. Cont'd

Population	Number Tested	Phenotypes				Gene Frequencies		
		1-1	2-1	2-2	"O"*	Hp^1	Hp^2	$\chi^2_{d.f=1}$
Buginese	205	30	90	84	1	.3676	.6324	0.5340
Toraja	101	22	50	26	3	.4796	.5204	0.0479
Ternatans	213	50	95	58	10	.4803	.5197	0.7951
Makianese	30	6	18	6	0	.5000	.5000	1.2000
Galelarese	155†	22	79	43	10	.4271	.5729	2.1105
Asmat	156	75	44	3	34	.7951	.2049	1.2917

* Hp O is omitted from gene frequency calculation

† Hp1-J is omitted from gene frequency calculation

Table 3.14.b.

Distribution of Haptoglobin (Hp) phenotypes and gene frequencies in Balinese populations

Village	Number Tested	1-1	2-1	2-2	Gene Frequencies* Hp^1	Gene Frequencies* Hp^2	χ^2 d.f=1
Dauh Waru	80	9	32	39	.3125	.6875	0.3819
Penebel	58	7	29	22	.3707	.6293	0.2980
Ayunan	69	5	33	31	.3116	.6884	0.9095
Renon	95†	12	41	40	.3495	.6505	0.0859
Serangan	39	3	16	20	.2821	.7179	0.0066
Banjar Rangkan	48	1	12	35	.1458	.8542	0.0006
Ped	70	1	18	51	.1429	.8571	0.1750
Rendang	124	8	53	63	.2782	.7218	0.5112
Seraya	88	10	32	46	.2955	.7045	1.4093
Songan	116	19	62	35	.4310	.5690	0.9333
Julah	49ϕ	7	28	13	.4375	.5625	1.6461
Sembiran	102	2	36	64	.1961	.8039	1.4569
Beratan	32ϕ	6	11	14	.3710	.6290	1.7810
Wanayu	40	2	20	18	.3000	.7000	1.4512

* Hp 0 is omitted from gene frequency calculation

† including 2 Hp 0

ϕ " 1 Hp 0

Table 3.14.c.

Additional Haptoglobin (Hp) gene frequency data in Indonesia and neighbouring areas

Population	Number Tested	Gene Frequencies* Hp^1	Hp^2	Reference
Indonesia				
Batak (Toba)	168	.2982	.7018	McDermid *et al.* 1973
Batak (Medan)	271	.284	.716	Lie-Injo *et al.* 1974
Minangkabau	67	.216	.784	-ditto-
Atjehnese	24	.292	.708	-ditto-
Balinese	304	.2525	.7475	Breguet *et al.* 1982a
Asmat	119	.797	.203	Gajdusek *et al.* 1978
Awyu	219	.921	.079	-ditto-
Moni	258	.743	.257	-ditto-
Ekagi	23	.826	.174	-ditto-
Lake Plain	170	.661	.339	-ditto-
Dani	199	.8182	.1818	Kirk *et al.* 1973
Malaysia and Philippines				
Malays	236	.23	.77	Kirk and Lai, 1961
Proto Malays	66	.47	.53	-ditto-
Chinese (Malaya)	167	.28	.72	-ditto-
Malayan Aborigines	202	.24	.76	Lie-Injo *et al.* 1967
Kadazans	216	.495	.505	Tan *et al.* 1979
Negrito	128	.2190	.7810	Omoto *et al.* 1978
Tagalog	253	.3426	.6574	-ditto-
Thailand				
Bangkok	92	.2337	.7663	Present study
Australia				
Aborigines				
Elcho Is.	35	.14	.86	Kirk *et al.* 1971
Groote Eylandt	90	.29	.71	-ditto-
Bathurst Is.	104	.41	.59	-ditto-

Table 3.14.c. Cont'd

Population	Number Tested	Gene Frequencies* Hp 1	Hp 2	Reference
Papua New Guinea				
Manus Is.	183	.6544	.3456	Malcolm *et al.* 1972
Motu	753	.5350	.4650	Blake (unpublished)
Western Pacific area				
Solomon Island				
Santa Cruz Is.	276	.7574	.2426	Mazzur and Blake, 1981
Fiji				
Fijians	332	.5274	.4726	Blake (unpublished)

* Hp 0 is omitted from gene frequency calculation.

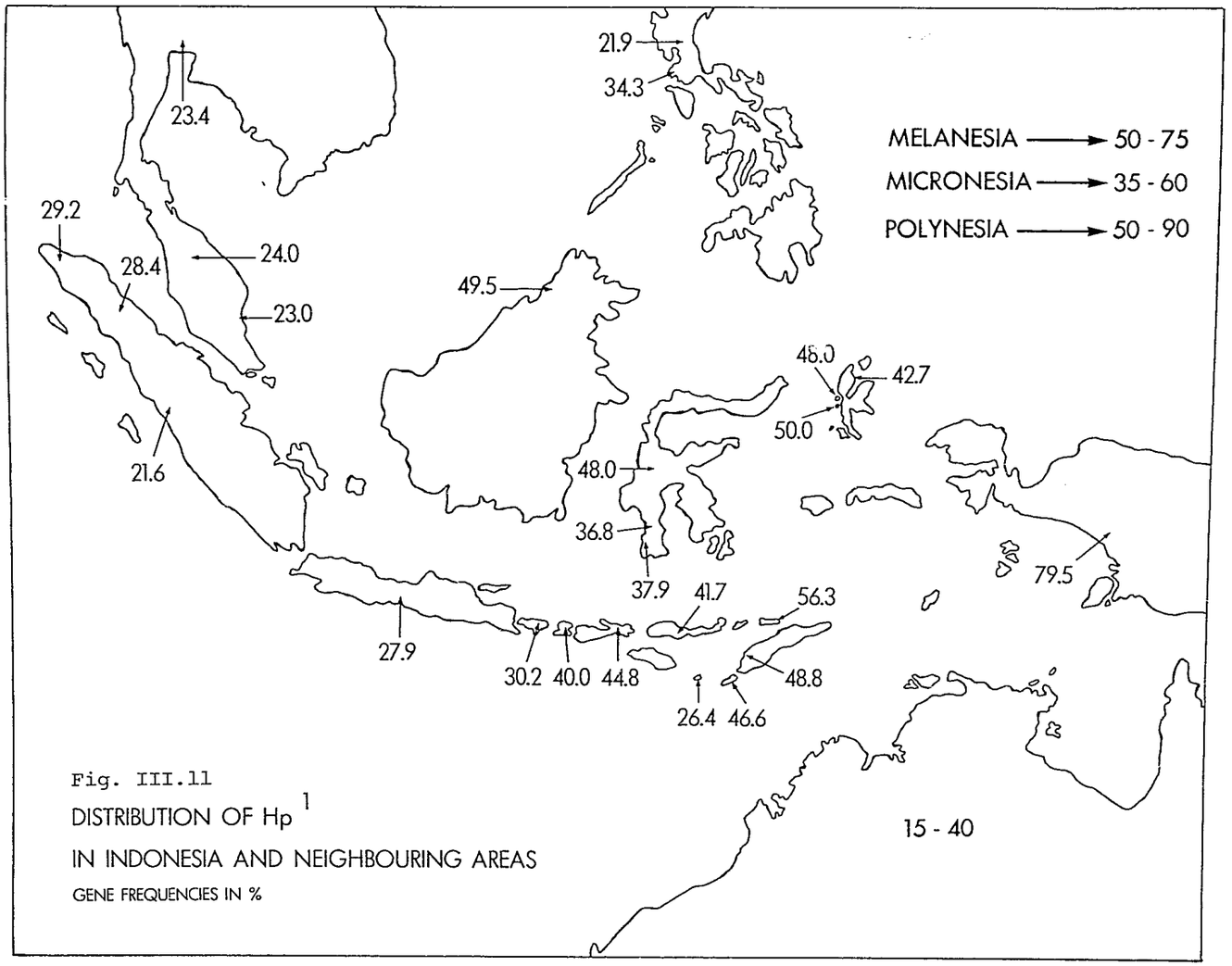


Fig. III.11

DISTRIBUTION OF Hp[1]

IN INDONESIA AND NEIGHBOURING AREAS

GENE FREQUENCIES IN %

to just over 6% in the Galelarese. Among the Balinese villages Hp O was almost absent, being observed in only four persons out of the 1010 persons typed. In Black Americans the Hp O phenotype is frequently associated with the unusual phenotype, Hp 2-1 (modified) (Giblett and Steinberg, 1960), but no Hp 2-1 (modified) phenotypes have been observed during the present study of Indonesian populations. Further, detailed investigations by Constans *et al.* (1981) using a sensitive radio-immuno electrophoretic technique of 313 Hp O samples from Congo pygmies showed that all but 2% of these could be given to Hp genotypes (58% Hp 2-2, 32% Hp 2-1 and 8% Hp 2-1 M).

Studies in a rural African community in Mali also show that not all cases of anhaptoglobinaemia are of genetic aetiology (Rougemont *et al.* 1980). In this population the low level of haptoglobin was thought to be due to malarial haemolysis. The number of subjects with low haptoglobin levels detected three years before, dropped significantly after one year's treatment with chloroquine. Since Indonesia is also a malarial country, it seems most likely that the Hp O individuals in Indonesia are of the same environmentally-induced type. If this is the case, the low frequency of Hp O in Bali suggest a better control of malaria in that island than elsewhere.

3.7. Transferrin

Transferrin (Tf), or siderophilin, is in the β-globulin fraction of serum proteins. Its important role is transporting iron from plasma to the bone marrow or tissue storage compartment. Its electrophoretic variation was first described by Smithies (1957). Since then extensive studies on human serum transferrin have been carried out in various populations.

So far, a large number of variants have been recorded (see Giblett,

1969; Kirk, 1968b; Mourant *et al.* 1976). Two broad categories are recognized: those with bands migrating anodally to the common C band are called B and those cathodal to C are called D variants. Of the alleles controlling D variants, $\underline{Tf}^{D1}$ and $\underline{Tf}^{DChi}$ are found with appreciable frequencies in some populations. $\underline{Tf}^{D1}$ is common among Africans, Black Americans, Australian Aborigines and Melanesians, whereas, $\underline{Tf}^{DChi}$ is more common among East Asians and some American Indian tribes. The amino acid substitution of these variants has been characterized (Wang and Sutton, 1965; Wang *et al.* 1967a). It is interesting that the Tf D_1 variant found in Black Americans has the same amino acid substitution as that found in the Australian Aborigines (Wang *et al.* 1967b) despite the geographical distinctiveness of the two populations.

The application of isoelectric focussing technique makes it possible to characterise more genetic heterogeneity of this protein. By this technique it has been shown that $\underline{Tf}^{C}$ is polymorphic. Three phenotypes of Tf C, i.e. TfC 1, TfC 2-1 and TfC 2 have been reported. The gene frequency for $\underline{Tf}^{C\ 1}$ is 81.9% in the German population (Kuhnl and Spielmann, 1978b) and 81.4% in the Danish population (Thymann, 1978). A similar result also has been reported for the Swedish population (Stibler *et al.* 1979). Even in U.S. Blacks and Whites, the $\underline{Tf}^{C\ 1}$ gene frequency is also similar (Kueppers and Harpel, 1980).

It is certain that more isoelectrofocussing data from other populations will be reported in years to come. However, in the present study only conventional starch gel electrophoresis technique were employed, except for the discrimination between D_1 and D_{Chi}. For this latter purpose, all Tf D samples were rerun on agarose gels using Ashton and Braden (1961) buffer to obtain maximum resolution between the D_1 and D_{Chi} bands.

As shown in table 3.15a, in the present investigation absence of variation of transferrin was found only in the Alorese and Makianese, both with notably small sample sizes. In the other populations the common $\underline{Tf}^{C}$ allele was present in high frequency, ranging from 86.1% in the Savunese to 99.7% in the Galelarese.

An interesting finding was the coexistence of the $\underline{Tf}^{D1}$ and $\underline{Tf}^{D}$Chi alleles in some populations. The presence of $\underline{Tf}^{D1}$ only was observed in the Rotinese, Ternatans and Asmat. Conversely the presence of $\underline{Tf}^{D}$Chi only was seen in the Florenese and Galelarese. From Lombok island towards the west, the $\underline{Tf}^{D}$Chi gene frequencies were higher than those of $\underline{Tf}^{D1}$. An almost equal frequency of both variants was found in the Bimanese and towards the east the $\underline{Tf}^{D1}$ gene frequencies were higher than those of $\underline{Tf}^{D}$Chi (Fig. III.12). In the northern area, $\underline{Tf}^{D}$Chi was found at higher frequencies than those of $\underline{Tf}^{D1}$ in the Macassarese and Buginese, while equal frequencies were observed in the Toraja. In the Moluccas, however, as noted above, only $\underline{Tf}^{D1}$ was found in the Ternatans and only $\underline{Tf}^{D}$Chi was found in the Galelarese.

Among Balinese both Tf D_1 and Tf D_{Chi} variants were found, both variants coexisting in all villages except Renon, Serangan, Seraya and Sembiran (Table 3.15.b). In the other villages, the $\underline{Tf}^{D1}$ gene frequency varied from 0.4% in Songan to 7.1% in Julah. On the other hand, $\underline{Tf}^{D}$Chi was found in all villages with the gene frequency ranging from 1.4% in Ped to 13.3% in Julah. Therefore, in Julah, both $\underline{Tf}^{D1}$ and $\underline{Tf}^{D}$Chi reach the highest frequency. No specific trend of the Tf D variant distribution from one village to another is observed. In general, with the exception of Ped, in which equal proportions of $\underline{Tf}^{D1}$ and $\underline{Tf}^{D}$Chi occurs, the $\underline{Tf}^{D}$Chi gene frequency was found to be higher than that of $\underline{Tf}^{D1}$ in other villages. The average gene frequency for the Balinese as a whole was 91.5%, 1.2% and 7.2% for $\underline{Tf}^{C}$, $\underline{Tf}^{D1}$, and $\underline{Tf}^{D}$Chi respectively. This average figure was

slightly lower for $\underline{Tf}^{C}$ in comparison with the result for Balinese from Tenganan Pageringsingan reported by Breguet *et al.* (1982a).

As shown in table 3.15.c, in a previous study, McDermid *et al.* (1973) found only $\underline{Tf}^{D}$Chi in the Toba Batak. Similarly, Lie-Injo *et al.* (1974) found only $\underline{Tf}^{D}$Chi in the Batak, Minangkabáu and Atjehnese from Medan. In the eastern part of Indonesia, only $\underline{Tf}^{D1}$ was found in Irian Jaya (Gajdusek *et al.* 1978; Kirk *et al.* 1973), which is comparable to the Asmat in the present study. In the neighbouring areas, only $\underline{Tf}^{D}$Chi has been found in the Malaysian populations (Kirk and Lai, 1961; Tan *et al.* 1979). However, $\underline{Tf}^{D1}$ has been found in Thailand (present study) but in New Guinea and the western Pacific islands, no $\underline{Tf}^{D}$Chi has been found (Blake, personal communication; Kirk *et al.* 1971; Malcolm *et al.* 1972; Mazzur and Blake, 1981). Therefore, it is apparent that from mainland South East Asia across the Indonesian archipelago, $\underline{Tf}^{D}$Chi reaches only as far as Timor and Halmahera in the east.

In addition to the Tf D variants, a single CB phenotype was found in both the Javanese (rural) and Timorese series and a single example of BDChi was found in the Balinese. Since reference samples for Tf B variants were not available, the exact nature of these Indonesian B variants could not be ascertained. However, the variants from the Javanese and Timorese are electrophoretically identical, whereas the B variant from the Balinese moves more anodally in electrophoresis.

Another interesting result is one example of a novel CD phenotype found in the Javanese (urban) series. Electrophoretically, the D band is very slow, much slower than any D variants described in the literature. To distinguish the variant from a possible artifact, the individual possessing the variant was resampled and the earlier finding was confirmed (see Fig. III.13). It is proposed to call the variant $CD^{Yogyakarta}$ (CD^{YOG}) after the locality where it is found. However, it

Table 3.15.a.

Distribution of Transferrin (Tf) phenotypes and gene frequencies in Indonesian populations

Population	Number Tested	Phenotypes				Gene Frequencies	
		C	CD_1	CD_{Chi}	Others	Tf^C	$\frac{Tf^{D1}}{Tf^{DChi}}$
Javanese (urban)	203*	186	1	13	2†	.9554	.0025 / .0421
Javanese (rural)	204	184	2	16	1† 1††	.9485	.0041 / .0441
Javanese (total)	407*	370	3	29	3† 1††	.9520	.0037 / .0431
Balinese	1010#	844	25	131	7† 1ϕ	.9147	.0124 / .0724
Sasak	191	174	6	11	-	.9555	.0157 / .0288
Bimanese	198	176	10	12	-	.9444	.0253 / .0303
Florenese	76	72	-	4	-	.9737	.0000 / .0263
Savunese	36	27	7	1	1ϕϕ	.8611	.1250 / .0139
Rotinese	59	53	6	-	-	.9492	.0508 / .0000
Timorese	133	121	8	3	1††	.9549	.0301 / .0113

Table 3.15.a. Cont'd

Population	Number Tested	Phenotypes				Gene Frequencies	
		C	CD_1	CD_{Chi}	Others	Tf^C	$\frac{Tf^{D1}}{Tf^{DChi}}$
Alorese	26	26	-	-	-	1.0000	$\frac{.0000}{.0000}$
Macassarese	199	190	2	7	-	.9774	$\frac{.0050}{.0176}$
Buginese	205	198	3	4	-	.9829	$\frac{.0073}{.0098}$
Toraja	101	99	1	1	-	.9901	$\frac{.0050}{.0050}$
Ternatans	213	206	6	-	$1^{\phi\phi}$	.9812	$\frac{.0188}{.0000}$
Makianese	30	30	-	-	-	1.0000	$\frac{.0000}{.0000}$
Galelarese	155	154	-	1	-	.9968	$\frac{.0000}{.0032}$
Asmat	156	127	25	-	$4^{\phi\phi}$	.8942	$\frac{.1058}{.0000}$

* 1 Tf CD^{YOG} omitted from gene frequency calculation

\# 2 CD are unidentifiable and omitted from gene frequency calculation

† D_{Chi} D_{Chi} †† CB ϕ BD_{Chi} $\phi\phi$ D_1D_1

Table 3.15.b.

Distribution of Transferrin (Tf) phenotypes and gene frequencies in Balinese populations

Village	Number Tested	Phenotypes				Gene Frequencies	
		C	CD_1	CD_{Chi}	Others	Tf^C	$\frac{Tf^{D1}}{Tf^{DChi}}$
Dauh Waru	80	70	2	7	1*	.9313	$\frac{.0125}{.0562}$
Penebel	58	49	2	6	1†	.9138	$\frac{.0172}{.0603}$
Ayunan	69	57	3	9	-	.9130	$\frac{.0217}{.0652}$
Renon	95	82	-	12	1*	.9263	$\frac{.0000}{.0737}$
Serangan	39	33	-	5	1*	.9103	$\frac{.0000}{.0897}$
Banjar Rangkan	48	43	2	2	1*	.9375	$\frac{.0208}{.0417}$
Ped	70	66	2	2	-	.9714	$\frac{.0143}{.0143}$
Rendang	124	98	3	22	1*	.8911	$\frac{.0121}{.0968}$
Seraya	88	74	-	14	-	.9205	$\frac{.0000}{.0795}$
Songan	116	102	1	13	-	.9397	$\frac{.0043}{.0560}$

Table 3.15.b. Cont'd

Village	Number Tested	Phenotypes				Gene Frequencies	
		C	CD_1	CD_{Chi}	Others	Tf^C	$\frac{Tf^{D1}}{Tf^{DCh}}$
Julah	49	30	7	11	1*	.7959	$\frac{.0714}{.1327}$
Sembiran	102#	83	-	16	1*	.9100	$\frac{.0000}{.0900}$
Beratan	32	29	1	2	-	.9531	$\frac{.0156}{.0312}$
Wanayu	40	28	2	10	-	.8500	$\frac{.0250}{.1250}$
Total	1010#	844	25	131	7* 1†	.9147	$\frac{.0124}{.0724}$

* $D_{Chi}\ D_{Chi}$

† BD_{Chi}

\# 2 CD are unidentifiable and omitted from gene frequency calculation.

Table 3.15.c.

Additional Transferrin (Tf) gene frequency data in

Indonesian and neighbouring areas

Population	Number Tested	Gene Frequencies			Reference
		Tf^C	Tf^{D1}	Tf^{DChi}	
Indonesia					
Batak (Toba)	167*	.9461	-	.0449	McDermid *et al.* 1973
Batak (Medan)	262*	.9540	-	.044	Lie Injo *et al.* 1974
Minangkabau	62	.992	-	.008	-ditto-
Atjehnese	22	.977	-	.023	-ditto-
Balinese	304	.8503	.0033	.1464	Breguet *et al.* 1982a
Asmat	118	.966	.034	-	Gajdusek *et al.* 1978
Awyu	216	.896	.104	-	-ditto-
Moni	255	.880	.120	-	-ditto-
Ekagi	23	.956	.044	-	-ditto-
Dani	201	.9303	.0697	-	Kirk *et al.* 1973
Malaysia and Philippines					
Malays	236	.977	-	.023	Kirk and Lai, 1961
Proto Malays	66	.985	-	.015	-ditto-
Malayan Aborigines	202†	.985	-	-	Lie-Injo *et al.* 1967

Table 3.D.c. Cont'd

Population	Number Tested	Gene Frequencies: T^C	T^{D1}	T^{DChi}	Reference
Malaysia and Philippines					
Chinese	103	.956	-	.044	Kirk and Lai, 1961
Kadazans	179	.994	-	.006	Tan *et al.* 1979
Negrito	128[x]	.9961	-	-	Omoto *et al.* 1978
Tagalog	253[x]	.9877	-	-	-ditto-
Thailand					
Bangkok	92*	.9783	.0054	.0109	Present study
Australia					
Aborigines					
Elcho Is.	35	.87	.13	-	Kirk *et al.* 1971
Groote Eylandt	97	.79	.21	-	-ditto-
Bathurst Is.	104	.82	.25	-	-ditto-
Papua New Guinea					
Goras Is.	183	.9836	.0164#	-	Malcolm *et al.* 1972
Wola	113	.9474	.0526	-	Blake (unpublished)

Table 3.15.c. Cont'd

Population	Number Tested	Gene Frequencies			Reference
		Tf^C	$Tf^{D}1$	$Tf^{D}Chi$	
Western Pacific area					
Solomon Islands					
Santa Cruz Is.	276	.9420	.0580	-	Mazzur and Blake, 1981
Fiji					
Fijians	332	.9985	.0015	-	Blake (unpublished)

* 1 Tf CB

† 6 Tf CD resemble $Tf^{D}Chi$ and $Tf^{D}1$

ϕ No Tf^{D} typing

\# $Tf^{D}Manus$

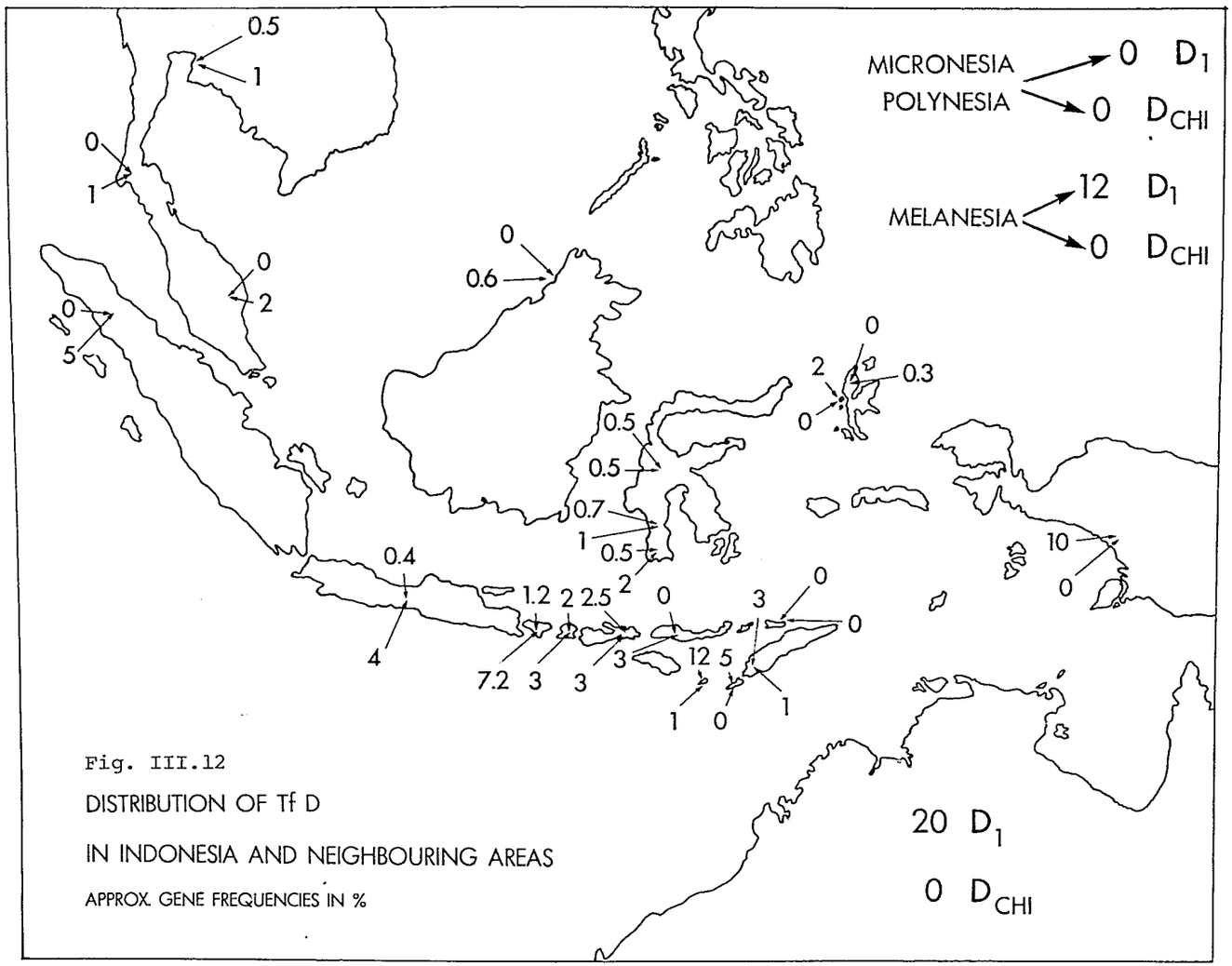


Fig. III.12

DISTRIBUTION OF Tf D

IN INDONESIA AND NEIGHBOURING AREAS

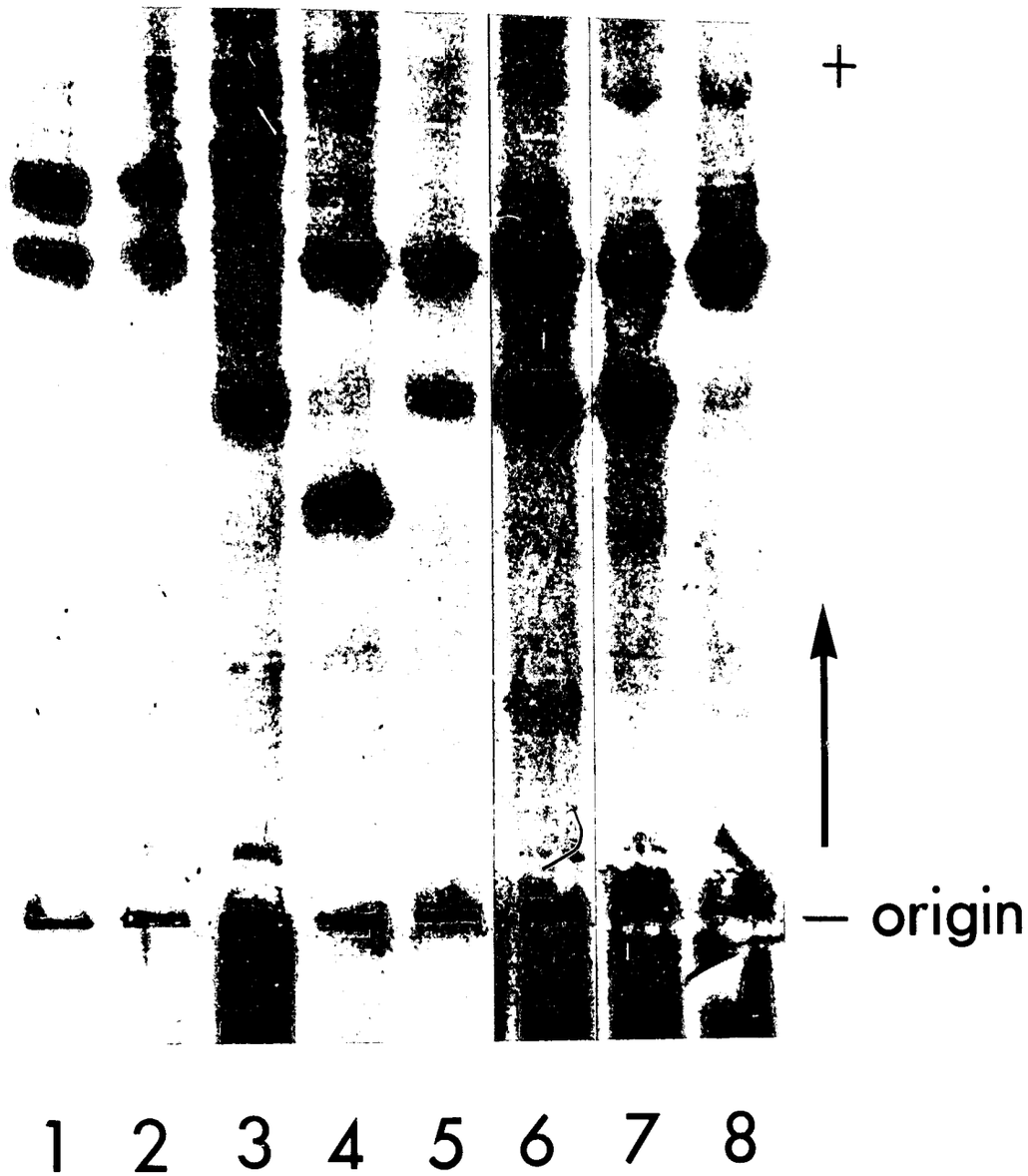
APPROX. GENE FREQUENCIES IN %

Fig. III.13 Variants of transferrin (Tf)

1. Tf CB from Javanese
2. Tf CB from Timorese
3. Tf BD_{Chi} from Balinese
4. Tf $CD^{Yogyakarta}$ (Tf CD^{Yog})

5 and 7. Tf CD_{Chi}

6. Tf CD_1
8. Tf C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was not possible to carry out a family study.

3.8. Ceruloplasmin

Ceruloplasmin (Cp) is a copper-containing protein in the α2-globulin fraction of serum. Its electrophoretic variation was first reported by Shreffler *et al.* (1967) among Black Americans. Five different phenotypes were described, Cp A, Cp B, Cp AB, Cp AC and Cp BC, with the A type being the fastest form, B the common, intermediate form and C the slowest form. Further variants, Cp New Haven (Cp NH) and Cp Bridgeport (Cp Bpt) have also been described (Shokeir and Shreffler, 1970).

Apart from the common Cp B variant, Cp^A is found in appreciable frequencies in some Black African populations (McDermid, 1971; Shokeir and Shreffler, 1970). It is also found in very low frequencies in Australian Aborigines (McDermid, 1971), Europeans (Bajatzadeh and Walter, 1969) as well as in some Indian populations (Walter *et al.* 1972).

During this present study, apart from the Balinese for whom Cp testing could not be carried out, no Cp variants have been detected in any Indonesian populations.

CHAPTER 4

DISEASE-ASSOCIATED GENE MARKERS

4.1. Introduction

In the present chapter three genetic traits will be discussed which, at least under some conditions, are involved in disease processes. All three traits, abnormal haemoglobins (haemoglobin E in particular), glucose-6-phosphate dehydrogenase (G6PD) deficiency and ovalocytosis are widely distributed in Indonesia and neighbouring areas.

4.2. Haemoglobin E

Haemoglobin E (Hb E), an anomaly of the human βchain of haemoglobin, was first discovered by Itano *et al.* (1954) in a child with atypical anaemia. It was later shown that this anomaly was due to the replacement of glutamic acid by lysine in position 26 of the βchain of haemoglobin (Hunt and Ingram, 1961).

Three conditions associated with Hb E have to be considered. Firstly, in the haemoglobin E trait, Hb E occurs in heterozygous form with normal Hb A. Secondly, haemoglobin E disease represents the homozygous form of Hb E. Thirdly, haemoglobin E-thalassaemia disease, which is the double heterozygous form of the Hb^E and thalassaemia genes. In addition, but more rarely, Hb E is found in combination with other abnormal haemoglobins (Askoy and Lehmann, 1957). In the Hb E trait there are neither physical or haematological abnormalities. However, in the latter two conditions, clinical abnormalities may be encountered. Both are associated with mild anaemia and splenomegaly. The patient may remain apparently healthy unless the disease is precipitated by infection or some other stress (Chernoff *et al.* 1956; Lehmann and Huntsman, 1974; Lie-Injo and Oey, 1957).

Since its discovery almost 30 years ago, it has been shown that this abnormal haemoglobin is not uncommon and is widely distributed in South East Asia. The highest frequencies are found in the Province of Surin, Thailand (Flatz, 1967; Livingston, 1967), among the Kachari of Assam, in northeastern India, where the highest frequency of the $\underline{Hb}^{E}$ gene (50.99%) has so far been reported (Das *et al.* 1975). A gene frequency of 8.75% was reported in Burmese (Lehmann *et al.* 1956) and 16.4% in Laos (Baup, 1964). Higher frequencies are found in Thailand, i.e. 10.2%, 24% and 31.2% in the central, north east and Khmer (Surin) provinces respectively (Flatz *et al.* 1965), and Detraverse *et al.* (1959) reported gene frequencies of 1.3% and 4.2% in north-central and south Vietnam respectively. Moving down towards the Malayan peninsula, Lehmann and Singh (1956) reported a gene frequency of 3.8% in Malays, while Vella (1958) reported 2.7% in the same population. A higher frequency (16.7%) was reported in the Malayan aborigines (Lie-Injo and Chin, 1964). Vella and Tavaria (1961) reported the frequency of 2.8% in Murut of Serawak, whereas Coulbourne *et al.* (1958) reported only 0.27% in the Land and Sea Dayaks. In the Philippines; Blackwell *et al.* (1965) reported the frequencies of the $\underline{Hb}^{E}$ gene of 0.7% in western Visayas, but only 0.3% in Luzon.

In Indonesia, studies on Hb E were pioneered by Lie-Injo (1955) and, in a later study, Lie-Injo (1959) reported $\underline{Hb}^{E}$ gene frequencies of 1.0%, 1.1%, 1.5% and 4.1% in Palembang, Minangkabau, Batak and Atjehnese respectively. Distribution in other Indonesian populations was also provided.

The present investigation has shown Hb E to be present in the majority of the populations sampled (Table 4.1.a). Hb E was not present in the Florenese and Alorese of the Lesser Sunda Islands, or among the Ternatans and Makianese in the Moluccas. It was also absent in the Toraja of Sulawesi. Neither was Hb E detected among the

Asmat in Irian Jaya. The overall gene frequencies in the other populations range from 0.2% among the Buginese to 25% in the Savunese.

The absence of Hb E in Flores is surprising, since the island is surrounded by populations with high frequency of $\underline{Hb}^{E}$. However, as mentioned in Chapter 2, samples collected in Kupang, Timor, do not represent any particular ethnic group in Flores. Therefore, it is still possible that eventhough Hb E is not found in these samples, further study with larger series from a particular ethnic group in Flores may reveal the existence there of this abnormal haemoglobin.

Another interesting finding is the very high frequency of Hb E in the Savunese. In addition to the small sample size, it is possible that in an island with a small population, the penetration of the $\underline{Hb}^{E}$ gene would be more complete than on larger islands. A similar situation can be observed in Madura, a small island offshore from east Java, where the $\underline{Hb}^{E}$ gene frequency is higher (7%) than that in east Java (2.3%) (Lie-Injo, 1959).

In the Balinese, the gene frequency ranges from 0.4% in Rendang to 9% in Serangan (see Table 4.1.b). As shown in Fig. IV.2, high frequencies of $\underline{Hb}^{E}$ are found in the coastal areas of north, west and south Bali, being highest in the small island of Serangan. The average $\underline{Hb}^{E}$ gene frequency for the total Balinese is 1.5%, a figure which is comparable to that of 1.1% in Balinese from Tenganan Pageringsingan reported by Breguet *et al.* (1982b).

As shown in Fig. IV.1, the high frequencies of $\underline{Hb}^{E}$ in mainland South East Asia, decline towards the Malaysian peninsula and the Indonesian archipelago. In the archipelago it is clear that the gene frequency is relatively low in the Greater Sunda islands, but its frequency increases again in the Lesser Sunda islands, being highest in the Savunese. To the north Hb E is still to be found as far as Halmahera (present study) and

Table 4.1.a.

Distribution of Haemoglobin (Hb) phenotypes and gene frequencies in Indonesian populations

Population	Number Tested	Phenotypes			Gene Frequencies		χ^{2*} d.f=1
		AA	AE	EE	Hb^A	Hb^E	
Javanese (urban)	203	196	7	-	.9828	.0172	0.0629
Javanese (rural)	204	194	9	1	.9730	.0270	5.0934
Javanese (total)	407	390	16	1	.9779	.0221	3.3712
Balinese	970	941	29	-	.9851	.0149	0.2246
Sasak	191	181	10	-	.9738	.0262	0.1380
Bimanese	198	165	33	-	.9167	.0833	1.6364
Florenese	76	76	-	-	1.0000	.0000	0.0000
Savunese	36	20	14	2	.7500	.2500	0.0494
Rotinese	59	48	11	-	.9068	.0932	0.6235
Timorese	133	119	13	1	.9436	.0564	0.8843
Alorese	26	26	-	-	1.0000	.0000	0.0000
Macassarese	199†	193	5	-	.9850	.0125	0.0337

Table 4.1.a. Cont'd

Population	Number Tested	Phenotypes			Gene Frequencies		χ^{2*} d.f=1
		AA	AE	EE	$\underline{Hb}^A$	$\underline{Hb}^E$	
Buginese	205$^{\phi}$	202	1	-	.9927	.0024	0.0066
Toraja	101$^{\phi}$	99	-	-	.9901	.0000	0.5048
Ternatans	213	213	-	-	1.0000	.0000	0.0000
Makianese	30	30	-	-	1.0000	.0000	0.0000
Galelarese	155$^{\#}$	153	1	-	.9935	.0032	0.0032
Asmat	163	163	-	-	1.0000	.0000	0.0000

* d.f=2 for Macassarese, Buginese and Galelarese

† 1 Hb AO; $\underline{Hb}^O$ = .0025 in Macassarese

ϕ 2 Hb AO; $\underline{Hb}^O$ = .0049 in Buginese and .0099 in Toraja

\# 1 Hb AS; $\underline{Hb}^S$ = .0032

Table 4.1.b.

Distribution of Haemoglobin E in

Balinese populations

Village	Number Tested	Phenotypes			Gene Frequencies		χ^2 d.f=1
		AA	AE	EE	Hb^A	Hb^E	
Dauh Waru	80	75	5	-	.9687	.0312	0.0832
Ayunan	68	67	1	-	.9926	.0074	0.0037
Renon	96	86	10	-	.9479	.0521	0.2898
Serangan	39	32	7	-	.9103	.0897	0.3791
Rendang	124	123	1	-	.9960	.0040	0.0020
Seraya	85	84	1	-	.9941	.0059	0.0030
Beratan	32	28	4	-	.9375	.0625	0.1422
Others	446	446	-	-	1.0000	.0000	0.0000
Total	970	941	29	-	.9851	.0149	0.2246

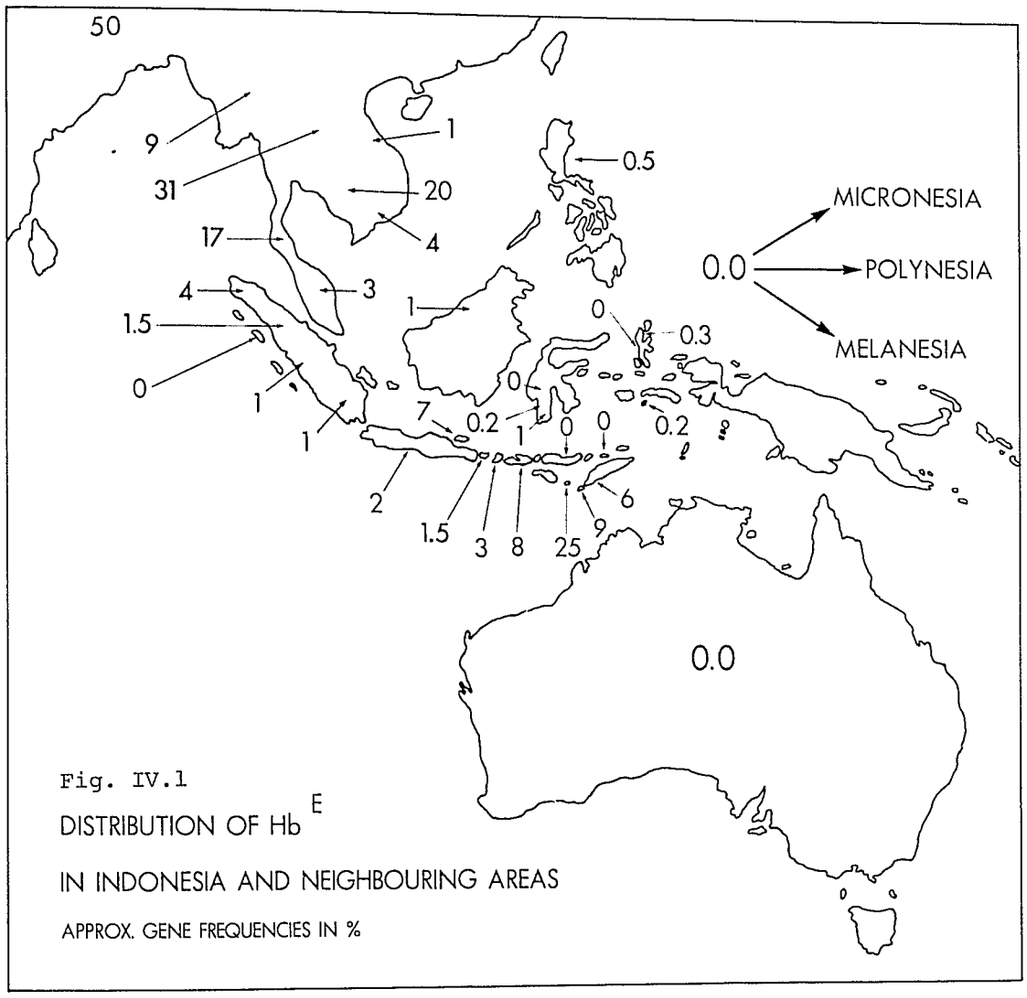


Fig. IV.1

DISTRIBUTION OF Hb^{E}

IN INDONESIA AND NEIGHBOURING AREAS

APPROX. GENE FREQUENCIES IN %

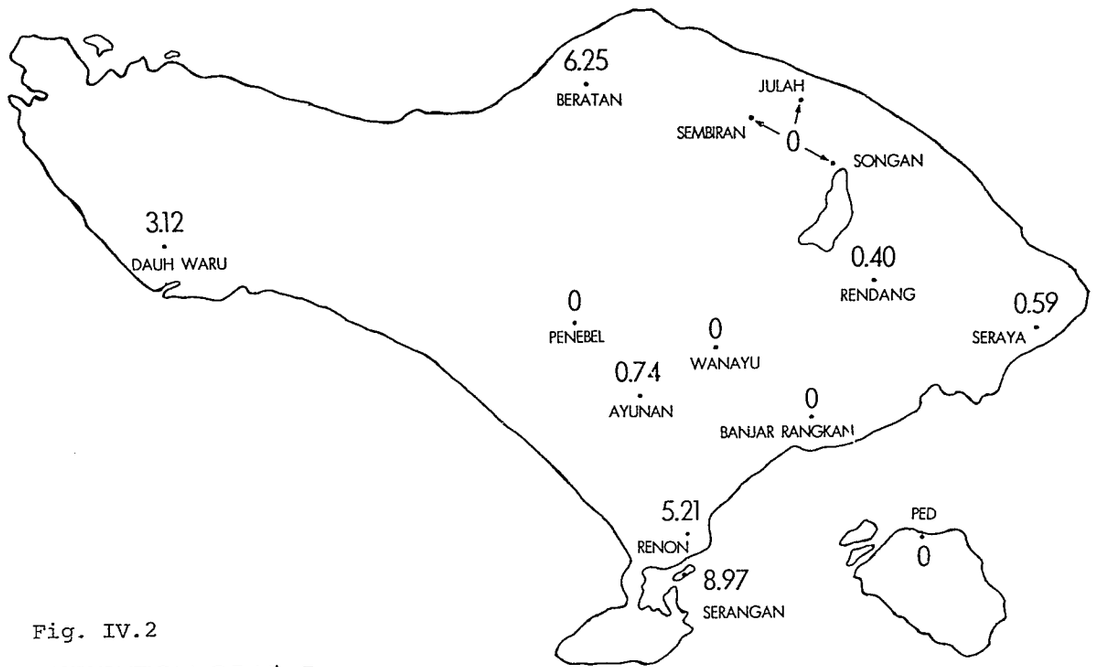


Fig. IV.2

DISTRIBUTION OF Hb E

IN BALINESE

GENE FREQUENCIES IN %

Ambon (Lie-Injo, 1959). Further east and south east, Hb E is not found in Melanesia, Micronesia, Polynesia or Australia (the Aboriginal population). The absence of Hb E in the Asmat as reported here is consistent with previous reports of the absence of Hb E in New Guinea (Kirk, 1965). In addition, sporadic cases of Hb E have been reported in other parts of the world outside South East Asia (Flatz, 1967), and recently a case of Hb E was reported in a Black American family with no known Asian ancestry (El-Shirbiny *et al.* 1980).

Finally, it is worth noting that the distribution of Hb E in South East Asia is thought to be related to endemic malaria in the area. By distribution and parasitaemia studies in Thailand, Flatz (1967) and Kruatrachue *et al.* (1969), have demonstrated that heterozygosity of Hb E confers protection against malaria. More recently, direct tests of the ability of malarial parasites to survive in red cells with different abnormal haemoglobins have shown that homozygous EE individuals could be significantly protected from *P. falciparum* (Nagel *et al.* 1981). However, there is no evidence for the selective pressure of malaria acting on the heterozygote as expected in a classical balanced polymorphism. It has been suggested that the greater fitness of the heterozygote is responsible for the maintenance of sickle cell polymorphism (Allison, 1954). Whether or not it is true for Hb E polymorphism is not clear. However, Deka (1981) found no differences in relative fertility between persons with normal and Hb E.

4.3. Other haemoglobins

In addition to Hb E, several other haemoglobin variants have been reported in Indonesia (Lie-Injo, 1964). During the present study only two other variants were encountered, Hb O and Hb S respectively.

4.3.1. Haemoglobin O

Haemoglobin O, formerly called haemoglobin Buginese X (Lie-Injo and Sadono, 1958), has been found in the populations of Sulawesi such as Buginese, Macassarese, Toraja and Gorontalo, but not in Minahasa. A few years later another haemoglobin O was reported by Ramot *et al.* (1960) in an Arab family. It was shown, subsequently, that these two haemoglobins are structurally different. Haemoglobin O found in Sulawesi (Hb O Indonesia) is an alpha-chain variant with the substitution of a glutamic acid residue in position 116 by lysine (Lehmann and Huntsman, 1974).

The original Hb O Indonesia was found in heterozygous form and no haematological abnormality was encountered in one patient and a mild anaemia in another patient with malarial parasites (Lie-Injo and Sadono, 1958). During the present study another haemoglobin variant was observed, in combination with Hb A, in Sulawesi. From its relative mobility in relation to Hb E and its geographical localization, it appears to be the same as Hb O Indonesia and this designation has been adopted here. Its gene frequency in the Macassarese, Buginese and Toraja was 0.2%, 0.5% and 1.0% respectively. The frequency in the Macassarese and Buginese was slightly lower than that reported by Lie-Injo and Sadono (1958). No Hb O was found in other Indonesian populations. Unlike Hb E which has a wider geographic distribution, Hb O seems to be confined to Sulawesi and therefore indicates a genetic relationship between the various populations of the island. Whether or not this haemoglobin variant offers any selective advantages is uncertain. Its coexistence with low frequencies of Hb E raises the possibility that Hb O offers a selective advantage against malaria, but no direct confirmation of this has been reported so far.

4.3.2. Haemoglobin S

Haemoglobin S (Hb S) was the first abnormal haemoglobin to be characterized by a change in electrophoretic mobility by Pauling *et al.* (1949). It is widely distributed in populations of Africa, the Middle East and parts of India. As for Hb E it is also an anomaly of the β chain due to the substitution of a glutamic acid residue in position 6 by valine (Hunt and Ingram, 1959).

During the present study, a single heterozygous AS person was discovered in the northern tip of Halmahera among 155 Galelarese. The electrophoretic mobility of the abnormal haemoglobin was checked against Hb S obtained from a sickle-cell patient in south India. It was further discriminated from Hb D by electrophoresis in citrate-agar at pH 6.0-6.3. Both tests showed the abnormal Hb to be the same as Hb S. In no other Indonesian populations was this trait found. No family study or other haematological examinations were carried out, so that further explanation regarding this particular case is not available.

4.4. Glucose-6-phosphate dehydrogenase (G6PD) deficiency

Glucose-6-phosphate dehydrogenase (G6PD) catalyses the first step in the pentose phosphate pathway. A large number of genetic variants have been reported, most of them showing enzyme deficiency in erythrocytes (Beutler and Yoshida, 1973; Yoshida and Beutler, 1978). However, some variants with normal erythrocyte enzyme level have also been reported (Chockkalingam *et al.* 1982; Panich *et al.* 1980; Yoshida and Beutler, 1978).

G6PD activity is controlled by a locus on the X-chromosome, but many of the variants reported, either with reduced enzyme activity and/or altered substrate specificity have an electrophoretic mobility identical with the common B type enzyme. Of the variants with altered electrophoretic mobility the most notable is the A type present in Black Africans

or African-derived populations. It is important to note also that in Africa the deficiency state is associated with the A type enzyme, whilst elsewhere it is a deficiency of the B type enzyme. Since the variant genes are allelic, African males are either Gd^B, Gd^A or Gd^{A-}: elsewhere males are either Gd^B or Gd^{B-}.

In the South East Asian region G6PD deficiency has been widely reported (see Mourant *et al.* 1976). In Indonesia in particular, this enzyme deficiency was first reported by Lie-Injo and Poey-Oey (1964). Later, Kirkman and Lie-Injo (1969) gave biochemical characteristics of G6PD variants from Indonesia and more recently Chockkalingam *et al.* (1982) reported biochemical characteristics of two G6PD variants found in the Balinese. These variants were clearly different from other previously reported variants. However, one of these variants, the Bali + variant, was said to have many features in common with a variant reported in Africa by Reys *et al.* (1970) though the similarity needs further examination. The other variant, the Tenganan variant, differs from Gd Indonesia (Kirkman and Lie-Injo, 1969) by its slow electrophoretic mobility, low Km G6P and low heat stability.

In the neighbouring area, a number of new variants have been reported in Papua New Guinea (Chockkalingam and Board, 1980). It is possible that a similar heterogeneous situation may be encountered in the Indonesian part of New Guinea, in Irian Jaya. However, in the present study the fluorescent screening test of Beutler and Mitchell (1968) only was employed to detect deficient individuals, and no attempt was made to further characterize the types of deficiency variants detected. Also, although both males and females were tested, gene frequencies for the deficiency allele is based on the hemizygous males. Detection of heterozygous females is unreliable, particularly when using a simple screening test, and the frequency of homozygous deficient females generally is too small to give reliable gene frequency estimates.

During the present study, among the population groups tested (see Table 4.2), no G6PD deficiency was detected in the Javanese from Yogyakarta. However, towards the east of the present survey area, G6PD deficiency was found in appreciable frequencies (see also Fig. IV.3). In the Lesser Sunda islands the incidence varied between populations. It was not detected in the Alorese, though the sample size was small. In the other populations the incidence ranged from 4% in the Florenese to 18.9% in the Sasak. No result was obtained from the Balinese. In Sulawesi the incidence ranged from 1.1% in the Toraja to 10.3% in the Macassarese. Further towards the east it was not possible to test G6PD deficiency in the Ternatans and Makianese or in the Asmat, but in the Galelarese, from the northern tip of Halmahera, the incidence was 2.7%.

In the previous studies Lie-Injo and Poey-Oey (1964) reported a low incidence (1.1%) in Indonesians mostly from West Java and Untario *et al.* (1978) reported a slightly higher frequency (3.5%) in East Java. Recently Breguet *et al.* (1982b) reported a frequency of 9.6% in Balinese from Tenganan Pageringsingan, which is comparable to the incidence in the other Lesser Sundas' populations. G6PD deficiency was also found in Irian Jaya with an incidence of up to 8%, as well as in Papua New Guinea and Micronesia (see Livingston, 1967; WHO, 1966, 1967). To the west the incidence of G6PD deficiency seems to be very low. Lie-Injo *et al.* (1973a) found only one case of G6PD deficiency in 168 Batak males, one in 46 Minangkabau males and no deficiency among 16 Atjehnese males.

As for the haemoglobinopathies, the geographic distribution of G6PD deficiency is similar to that of falciparum malaria and hence it has been suggested that it owes its distribution to selection by this parasite (Motulsky, 1960). The strongest proof for this malarial selection would be the demonstration of decreased mortality from malaria among trait carriers. However, as Hitzeroth and Bender (1980) noted,

Table 4.2.

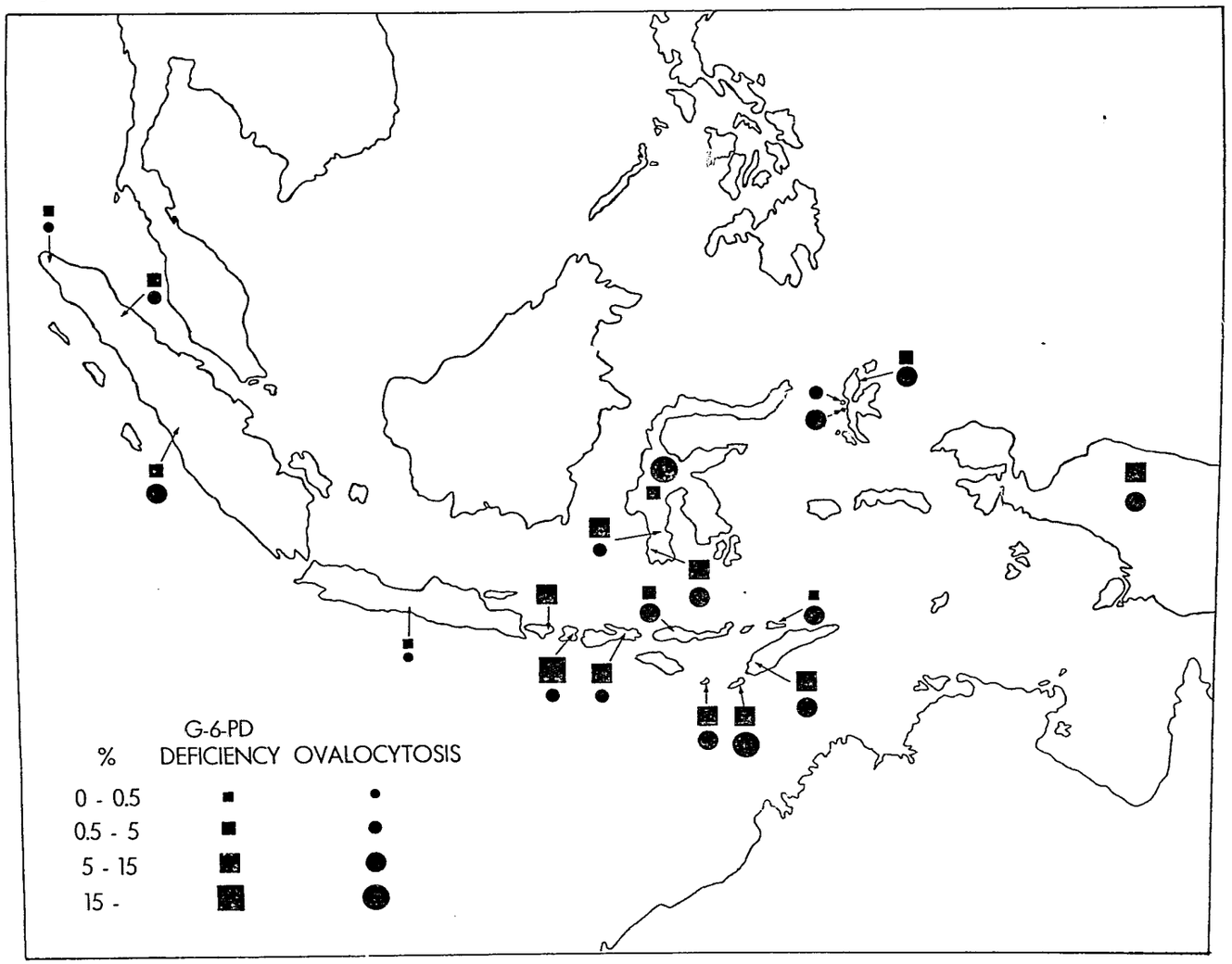
Incidence of G6PD deficiency in

Indonesian populations

Population	Number of males tested	Incidence of G6PD deficiency	
		Number	%
Javanese (total)	295	0	0.0
Balinese	970*	no result	-
Sasak	127	24	18.9
Bimanese	125	15	12.0
Florenese	50	2	4.0
Savunese	17	1	5.9
Rotinese	36	2	5.6
Timorese	58	7	12.1
Alorese	14	0	0.0
Macassarese	116	12	10.3
Buginese	79	7	8.9
Toraja	93	1	1.1
Ternatans	117	no result	-
Makianese	17	no result	-
Galelarese	109	3	2.7
Asmat	110	no result	-

* male and female

Fig. IV.3 Distribution of G6PD deficiency and ovalocytosis in Indonesia



this malarial hypothesis is based mainly on the correlation between the geographic distribution of endemic *P. falciparum* and that of the enzyme deficient alleles, $\underline{Gd}^{A-}$ and $\underline{Gd}^{B-}$ as well as on some comparative parasitological studies. In fact parasitological studies are controversial (Martin *et al.* 1979). However, Luzzatto (1979) pointed out that independent evidence from genetic heterogeneity, from clinical findings and from cellular studies, all support selective advantage of at least some $\underline{Gd}^{+}/\underline{Gd}^{-}$ heterozygotes with respect to *P. falciparum*. Luzzatto *et al.* (1969) reported on a study of female children with acute *P. falciparum* and who were heterozygous for G6PD deficiency. Because of the Lyon effect, the patients have two populations of erythrocytes, one with normal and one with deficient G6PD activity. The study showed the parasite rate was 2-80 times higher in the normal than in the deficient erythrocytes. Therefore, being aware of the controversial reports on G6PD deficiency and malaria, Luzzatto (1979) concluded that the protective mechanism in heterozygotes is associated with the fact that they are physiologic genetic mosaics, harbouring a dual population of normal and deficient red cells in their blood.

More recently, another explanation of the effect of malarial selection on G6PD deficiency has been put forward by Gloria-Bottini *et al.* (1980). Analysing a set of environmental and sociocultural variables in Sardinian populations they suggested that thalassemia may be the primary genetic factor selected by malaria, whereas, G6PD selection may be a secondary adaptation phenomenon strongly dependent on other genetic and environmental variables.

A correlation between the distribution of G6PD deficiency and that of haemoglobin E has also been observed in the South East Asian region. Lie-Injo *et al.* (1964) noted that the G6PD deficiency trait in the Malay groups decreases from north to south and Lie-Injo and Poey-Oey

(1964) pointed out that the frequency of G6PD deficiency showed a correlation with the frequency of Hb E in Jakarta, Singapore, Malaysia and Thailand. Since these two genetic traits are both thought to confer a selective advantage against malarial parasites, this correlation would be expected. However, the distribution of the two traits is not concordant. Hb E appears to have followed population movements from South East Asia down into the Indonesian archipelago but not to have spread further. G6PD deficiency, on the other hand, still occurs, sometimes with high frequency further to the east as, for instance, in New Guinea.

Finally, G6PD deficiency is important clinically by virtue of its association with sensitivity to certain drugs, such as primaquine, and its relationship to some types of neonatal jaundice and haemolytic anaemia (Doxiadis *et al.* 1961; Kirkman, 1971). The latter condition is very important since blood destruction in G6PD deficient subjects sometimes occurs during infection, a condition commonly found in this tropical region. Further screening on other population groups with larger series is necessary to establish the real situation of this genetic trait in the archipelago and such data would be of value in community health programs.

4.5. Ovalocytosis

The discovery of a high frequency of ovalocytosis in the Toraja, by Bonne and Sandground (1939), was probably the first report on ovalocytosis in Indonesia. Since then no other reports from the Indonesian archipelago were available until Lie-Injo *et al.* (1973a) reported their findings in north Sumatera. Subsequently, Sembiring *et al.* (1975) also reported ovalocytosis among the Batak and Minangkabau from north Sumatera.

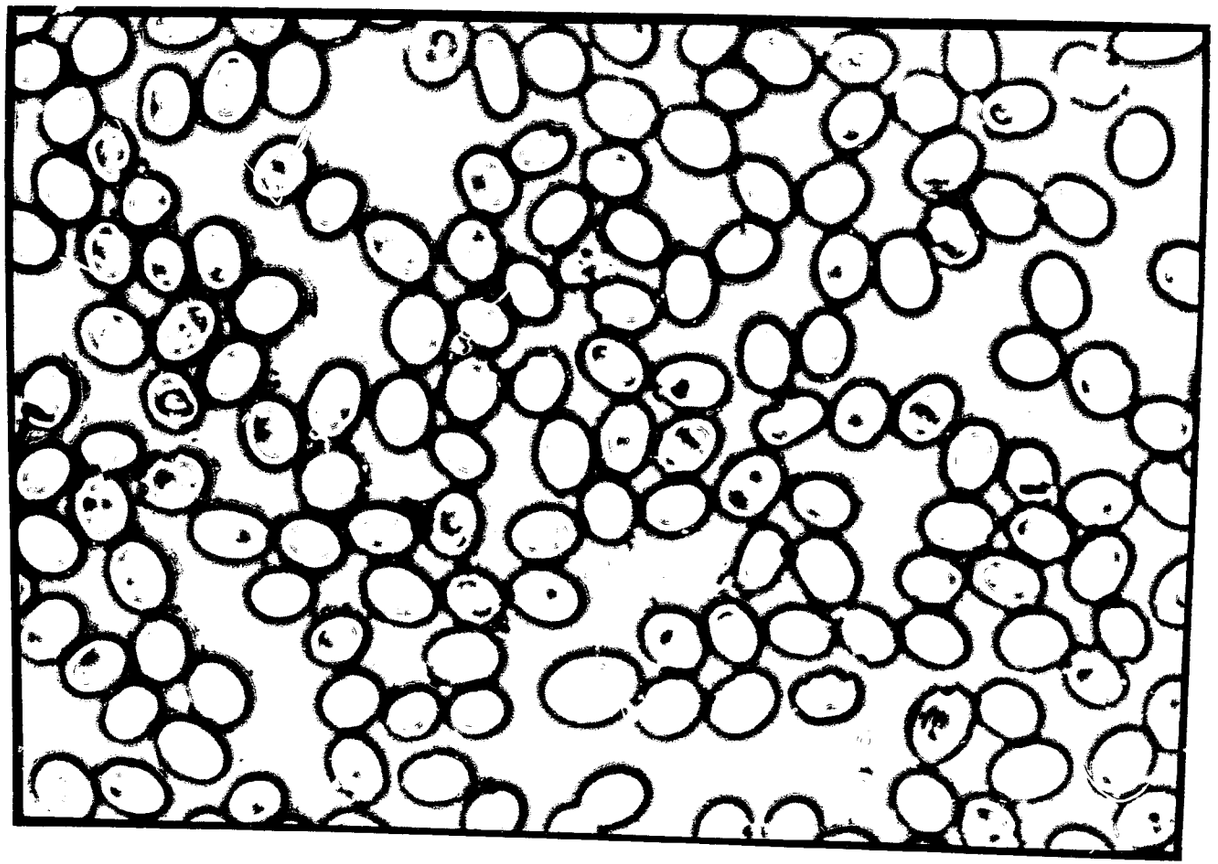
As an abnormality of red cell morphology, the term ovalocytosis

and elliptocytosis have been used interchangeably. The diagnosis of both conditions is established from the morphological features of the blood film. Bannerman and Renwick (1962) suggested that at least 25% of red cells in the stained blood film should be oval, elliptical or rod shaped before diagnosis of elliptocytosis is made.

Amato and Booth (1977) were the first to provide a clear description of differences in both abnormalities. Ovalocytosis, which is found in higher frequencies (2-20%) is considered to be limited to parts of South East Asia and Melanesia, congruent with regions of malarial endemicity and is inherited in an autosomal recessive manner. On the other hand, elliptocytosis, a relatively rare disorder, has no geographic restriction and is inherited in an autosomal dominant manner. Two forms of elliptocytosi have been demonstrated, one linked to the Rh-blood group system and the other unlinked (McKusick, 1978). In elliptocytosis, haemolysis sometimes occurs and selective antigenic depression is not observed, unlike ovalocytosis which shows no haemolysis and is associated with selective antigenic depression (Booth *et al.* 1977).

As suggested by Amato and Booth (1977), diagnosis of ovalocytosis is made when the proportion of oval cells in a blood film is more than 50%. In order to avoid the reading of the symptomatic elliptocytosis/ovalocytosis accompanying secondary anaemias, individuals with apparent anaemia during the physical examination were excluded from the analysis. Based on this criterion, the examination of unstained blood films taken during this study showed ovalocytosis to be present in all populations tested (Table 4.3). A typical blood film is shown in Figure IV. 4. With the exception of the Javanese in whom only one individual with ovalocytosis was found, the incidence in the other populations ranged from 3.2% in the Sasak, to 23.7% in the Rotinese. In Sulawesi the

Fig. IV.4 Appearance of ovalocytosis on blood film
Original magnification x 400



APPEARANCE OF OVALOCYTOSIS ON BLOOD FILM.
ORIGINAL MAGNIFICATION x 400

Table 4.3.

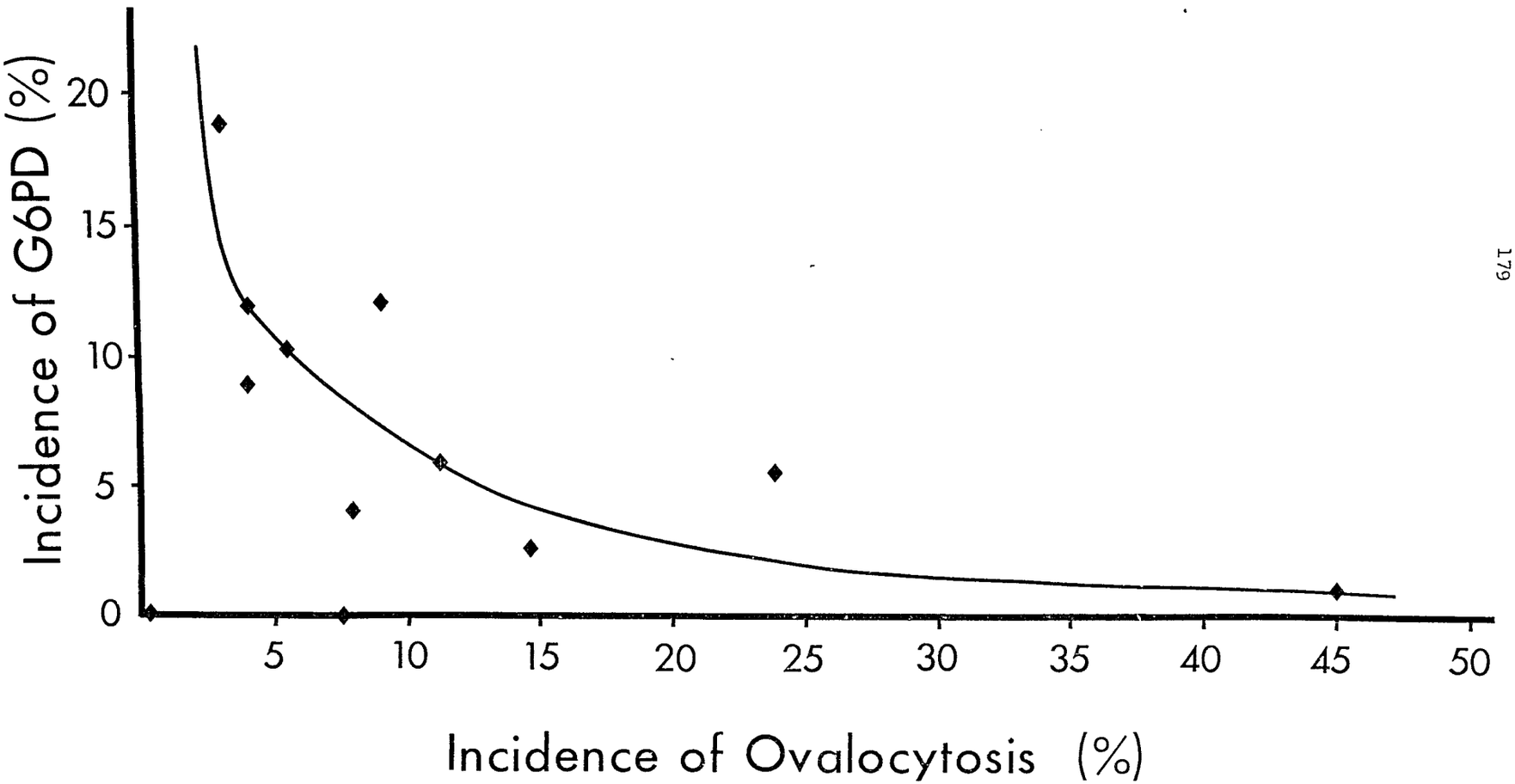
Incidence of ovalocytosis in Indonesian populations

Population	Number tested*		Incidence of ovalocytosis†		
	Male	Female	Male	Female	Total
Javanese (total)	286	107	1(0.3)	0(0.0)	1(0.2)
Balinese	n.t.		-	-	-
Sasak	124	61	2(1.6)	4(6.6)	6(3.2)
Bimanese	124	72	5(4.0)	3(4.2)	8(4.1)
Florenese	50	25	4(8.0)	2(8.0)	6(8.0)
Savunese	17	19	2(11.8)	2(10.5)	4(11.1)
Rotinese	36	23	12(33.3)	2(8.7)	14(23.7)
Timorese	58	73	6(10.3)	6(8.2)	12'9.2)
Alorese	14	12	1(7.1)	1(8.3)	2(7.7)
Macassarese	115	80	5(4.3)	6(7.5)	11(5.6)
Buginese	78	124	1(1.3)	7(5.6)	8(4.0)
Toraja	n.t.		-	-	-
Ternatans	110	87	3(2.7)	6(6.9)	9(4.6)
Makianese	17	12	2(11.8)	1(8.3)	3(10.3)
Galelarese	106	38	18(17.0)	3(7.9)	21(14.6)
Asmat	n.t.		-	-	-

* n.t. = not tested

† figures in bracket denote (%)

Fig. IV. 5 Relation between incidence of G6PD deficiency and ovalocytosis in Indonesia



incidence was 5.6% and 4.0% in the Macassarese and Buginese respectively. The Toraja were not tested but Bonne and Sandground (1939) have reported previously an incidence of ovalocytosis close to 50% in Toraja living near Lake Lindu in Central Sulawesi. An apparently higher incidence in male subjects was observed in the Rotinese, Makianese and Galelarese, whereas, higher incidence in female subjects was observed in the Sasak, Macassarese, Buginese and Ternatans.

Other previous studies in Indonesian populations revealed an incidence of 1.7% in the Batak and 7.2% in the Minangkabau from north Sumatera (Sembiring *et al.* 1975) and Lie-Injo *et al.* (1973a) found one ovalocytic in 89 individuals from Batak. Other studies in areas neighbouring Indonesia have also revealed a high incidence of this red cell abnormality. Among 440 healthy and hospitalized Malayan aborigines, the incidence of ovalocytosis was 12.3% (Lie-Injo, 1965). Another study with larger series of Malayan aborigines (Lie-Injo *et al.* 1972) showed the incidence ranged from 6.6% to 20.9%. Ganesan *et al.* (1975) reported an incidence of 12.7% and 9.0% respectively in the Land Dayaks and Sea Dayaks of Sarawak. Studies have also been carried out in Papua New Guinea, where the incidence ranges from 1 to 16% in populations along the western border of Papua New Guinea (Holt *et al.* 1981). In the same report it was observed that, among 21 individuals from Irian Jaya, three of them showed ovalocytosis. Further studies are needed in South East Asia to map the distribution of the trait in the region in greater detail.

As shown in Fig. IV. 3, the distribution of ovalocytosis is coextensive with the distribution of G6PD deficiency. Coincidently, as shown in Fig. IV. 5, with the exception of the Alorese with a small number of males tested for G6PD deficiency and the Javanese, who showed a low incidence of ovalocytosis and no G6PD deficiency, there

is a trend of inverse proportion for the incidence of G6PD deficiency and ovalocytosis in the other populations. As noted in the previous section (section 4.4), G6PD selection may be a secondary adaptation phenomenon which is dependent on other genetic and environmental variables (Gloria-Bottini *et al.* 1980). Furthermore, thalassemia was suggested as the primary genetic factor selected by malaria. Whether or not in the South East Asian region thalassemia and/or ovalocytosis plays a similar role, needs further study. However, it seems possible that the high incidence of ovalocytosis in these populations is maintained also by selective advantage. In fact, Serjeantson *et al.* (1977) have presented strong evidence that ovalocytosis does confer increased resistance to malaria. In their study of 1616 persons (1416 normals and 199 with ovalocytosis) aged 0 - above 14 years, they showed that ovalocytics were significantly less frequently parasitized by *P. vivax* and *P. malariae*. More recently Kidson *et al.* (1981) also have shown, by direct assay of invasion in culture, that ovalocytic erythrocytes are resistant to infection by *P. falciparum*.

CHAPTER 5

GENETIC DISTANCE ANALYSIS

5.1. Introduction

In chapter 3 detailed information was given on the distribution of phenotypes and of gene frequencies for serum protein and red cell enzyme systems in Indonesian populations from various parts of the country. Comparisons were made, system by system, between these populations and also with populations in countries neighbouring Indonesia. In this chapter use will be made of multivariate procedures which enable the information provided by each of the separate blood genetic systems to be combined into a single measure of genetic divergence between populations.

Many of these measures of genetic divergence are related to Wright's F statistics (Wright, 1931, 1943, 1946). They have been developed further to examine particular aspects of population structure based on gene frequency estimates, such as Harpending and Jenkins (1973) R statistics applied to populations assumed to have been derived from a common ancestor. An example of the application of R statistics is that of Roberts *et al.* (1981) to an analysis of populations in northwest England.

Other measures of genetic divergence, generally called genetic distance measures, have been devised and reviews of the theory underlying genetic distance measurement have been provided for instance by Canning and Cavalli-Sforza (1973), Chakraborty (1976), Jorde (1980) and Nei (1975). However, application of different indices of genetic distance to particular data sets has shown that the distance values derived by the various methods are highly correlated. For example,

using data from seven tribal groups of Gujarat and 15 world populations, Sanghvi and Balakrishnan (1972) showed that Edwards and Cavalli-Sforza's index (E) was very highly correlated with their own indices (B and G). Employing data for 15 Western Pacific and New Guinea and 12 Australian Aboriginal populations, Kirk (1977) also stressed the essentially similar result from Morton's hybridity index, Cavalli-Sforza's (1969) and Nei's (1972) indices. These three measures were also shown to give essentially similar results when used for analysis of genetic data from Irian Jaya populations (Gajdusek *et al.* 1978).

Since the various genetic distance measures give essentially similar rankings to the distances between populations, the analysis presented here will be restricted to the genetic distance index proposed by Nei (1972). This was developed from an earlier method by Nei (1971) used for measuring the mean number of codon differences and estimating the evolutionary divergence time between species. However, although this earlier method is suitable for measuring macroevolutionary processes, it is not useful for studying within-species differences where few, if any, codon changes have gone to fixation in the population. Accordingly Nei (1972) developed a new index which estimates the codon differences from averaging the data provided from the polymorphic genetic systems studied in the populations. Nei's index has two advantages, firstly that Nei and Roychoudhury (1974a) provided estimates of variances for the genetic distance values obtained and, secondly, since his measure is given in terms of average codon differences, it can be used directly to estimate times of divergence between populations. This has been done, for example, by Nei and Roychoudhury (1974b) for the divergence times between the major races of man.

The Nei genetic distance values given in the present chapter were obtained using the computer program written by Dr Bronya Keats for the

Department of Human Biology at the ANU, using the University's UNIVAC 1100 computer.

5.2. Results

Previously, multivariate statistical procedures have been applied by Glinka (1978, 1981) to the analysis of anthropometric data for Indonesian populations and the results of this analysis were discussed in Chapter 1. However, the extension of this approach to the measurement of genetic distances derived from blood genetic markers in Indonesia has been extremely limited. Lie-Injo (1976) included data from Javanese in a genetic distance analysis of South East Asian populations, using available blood group data. However, for other genetic markers only the Batak from Sumatera were included in her analysis. In the eastern part of Indonesia, where more extensive genetic data was available, Gajdusek *et al.* (1978) carried out a genetic distance analysis between populations of Irian Jaya.

Here, genetic distances will be discussed under three headings. Firstly, for Balinese villages, looking at distances in relation to geographical separation and some historical factors which might have influenced local population structure. Secondly, the genetic differentiation between each of the Indonesian populations and, thirdly, examining the distance relationships between Indonesian populations and their neighbours, particularly focussing on the information this might provide about the role of Indonesia as a base for the movement of populations further out into the Pacific.

5.2.1. Genetic distance between Balinese populations

For the purpose of the genetic distance analysis only Balinese populations consisting of more than 40 samples were included. There are 10 populations which fulfill this requirement, i.e. those from Dauh

Waru, Penebel, Ayunan, Renon, Banjar Rangkan, Rendang, Seraya, Julah and Sembiran (see Table 2.2 and Fig. II.2).

Gene frequency data for 13 loci showing variation were employed for the analysis (10 red cell enzyme systems, two serum protein systems and haemoglobin) i.e. ACP, ADA, AK, ESD, GLO I, GPT, 6-PGD, PGM_1, PGM_2, CA_1, Hp, Tf and Hb_β chain. Genetic distances generated from these gene frequency distributions are shown in table 5.1.a. For the villages it is clear that the closest distance is observed between Rendang and Seraya followed by Renon and Seraya. The farthest distance is seen between Banjar Rangkan and Julah followed by Banjar Rangkan and Songan, though the distance values for these are just less than twice their standard error, which is the level usually accepted for significance. The next greatest distance, which is significant, is between Dauh Waru and Julah (see also Table 5.1.b).

These distances can be visualized in the dendrogram constructed from the distance matrix (Fig. V.1). As shown in this dendrogram, two clusters are distinguishable; the small Songan-Julah cluster consisting of villages geographically located in the northeastern part of Bali and another big cluster consisting of villages located on the southeastern and southwestern part of Bali. In this big cluster, Banjar Rangkan seems to be very distinctive from the others and accounts for the earliest split, followed by Dauh Waru. It is worth noting that in this cluster the distances between Banjar Rangkan and Dauh Waru, Banjar Rangkan and Ayunan, Banjar Rangkan and Sembiran and Dauh Waru and Julah are more than twice their standard error. It is also interesting that Sembiran, which is geographically close to Julah, is genetically different and falls within this cluster.

The distance matrix can be used also in a principle component analysis to give a visual display in a two dimensional diagram constructed

Table 5.1.a.

Nei's genetic distances (Dx100) between Balinese populations based on 13 loci showing variation

	Dauh Waru	Penebel	Ayunan	Renon	Banjar Rangkan	Rendang	Seraya	Songan	Julah
Penebel	.29								
Ayunan	.71	.25							
Renon	.39	.28	.43						
Banjar Rangkan	.68	1.03	1.09	.62					
Rendang	.31	.21	.21	.17	.47				
Seraya	.32	.28	.28	.14	.44	.07			
Songan	1.29	1.00	.78	.77	1.93	.90	.69		
Julah	1.45	.70	.54	.87	2.02	.70	.87	.99	
Sembiran	.73	.68	.55	.30	.42	.24	.29	1.13	1.25

Table 5.1.b.

Standard errors for distances shown in Table 5.1.a.

	Dauh Waru	Penebel	Ayunan	Renon	Banjar Rangkan	Rendang	Seraya	Songan	Julah
Penebel	.15								
Ayunan	.37	.12							
Renon	.27	.15	.20						
Banjar Rangkan	.32	.59	.49	.39					
Rendang	.17	.09	.09	.06	.25				
Seraya	.18	.12	.14	.05	.23	.03			
Songan	.97	.68	.31	.58	1.12	.54	.41		
Julah	.64	.26	.20	.38	1.07	.31	.37	.43	
Sembiran	.44	.33	.27	.22	.19	.10	.14	.70	.64

Fig. V.1 Dendrogram based on Nei's genetic distance for Balinese populations using 13 loci showing variation

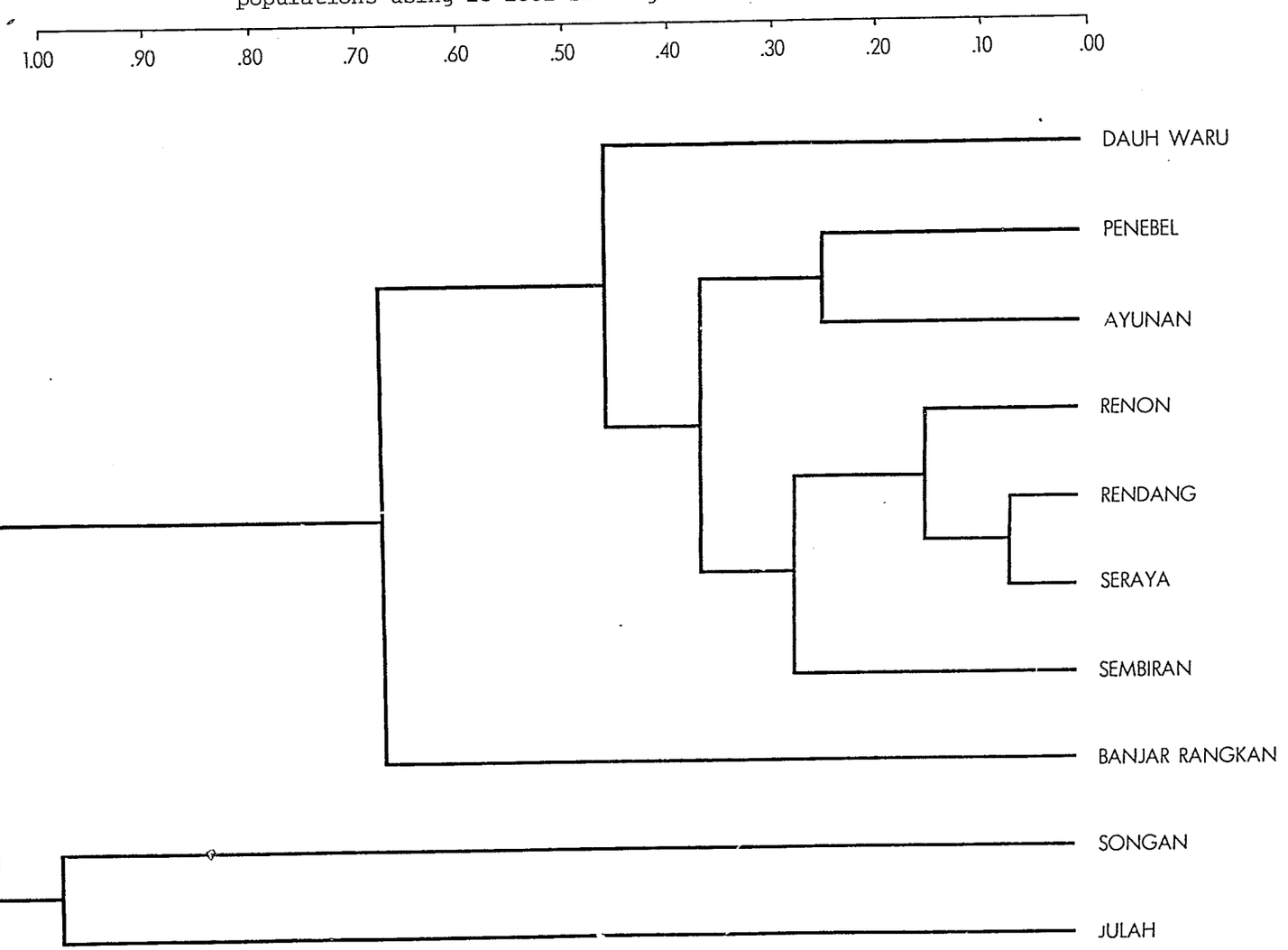
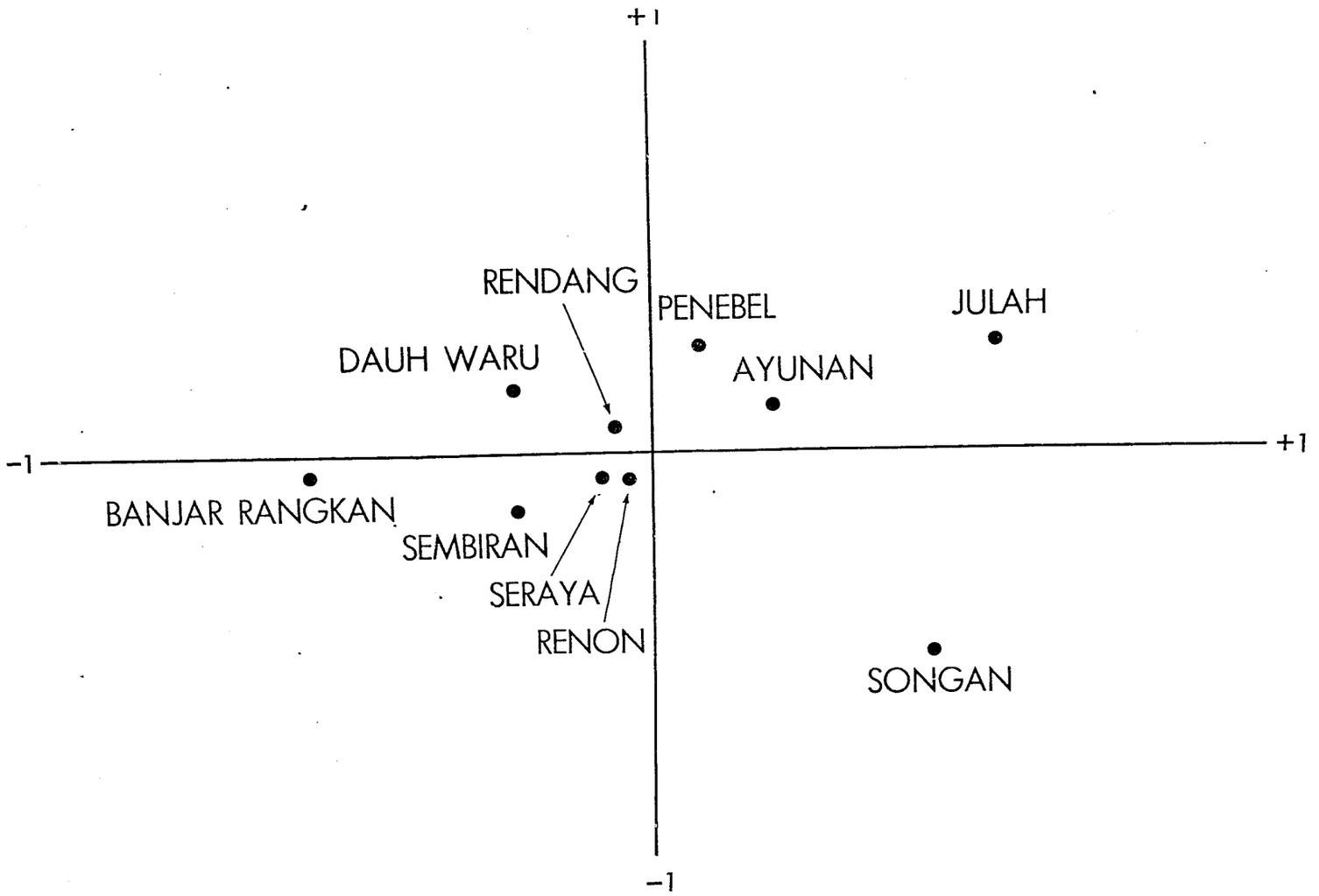


Fig. V.2 Eigenvector diagram based on Nei's genetic distance for Balinese populations using 13 loci showing variation



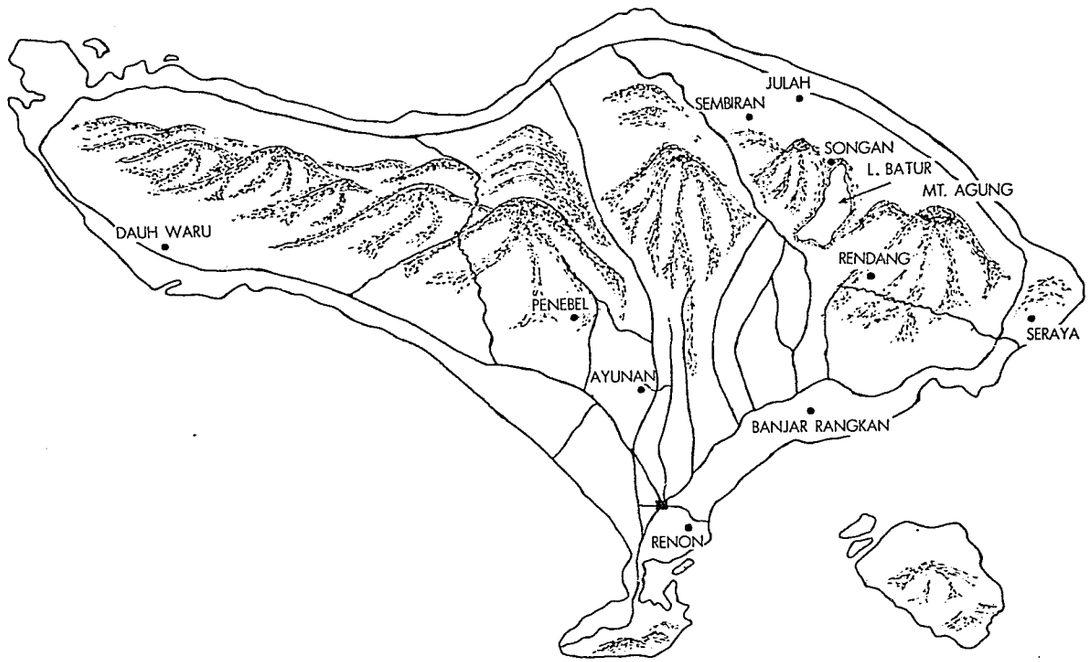


Fig. V.3

Map of Bali showing topography of ten villages included in the genetic distance analysis

using the first and second eigenvectors (Fig. V.2). In this diagram it is clearer that Julah and Songan, despite their apparent geographical closeness, are both quite distant from one another and from the other villages. Banjar Rangkan is again quite distinctive from the other south Balinese villages, whereas Sembiran from the northern part of the island is closer to the other south Balinese populations.

The overall pattern of genetic heterogeneity of these populations is interesting despite their religious and cultural similarities. Various factors must have been responsible which make up the difference in the genetic structure of each village. As shown in Fig. V.3, the island is topographically divided into southern and northern regions. The southern region, which slopes from the inland high mountain ranges down towards the south, is more fertile and densely populated in contrast to the northern region where the mountains run down close to the sea. Since the earlier historical periods, the southern slopes have been the centre of population activities and experienced various influences from outside the island, from Java in particular. The difference in the genetic structure between some of the northern slope populations and those of the southern one is a consequence of this topographical difference since the mountain ranges may have acted as a potential geographic barrier to gene flow in the old days.

Among eight regencies in Bali, seven of them are located in the southern slopes region, where interrelationship of the populations was known to occur. Close genetic distances between populations in this region may, therefore, be expected and is, in fact, shown by close distances between Renon, Rendang, Seraya, Penebel and Ayunan. However, in former times, the westernmost regency, Jembrana, was isolated from the rest of the Balinese populations. This isolation is supported by the fact that it did not contribute to the cost of maintaining Bali's

chief temple, Pura Besakih (Goris, 1937). Only recently has this isolation been brought to an end with the construction of a road, which has been followed by population migration from other parts of Bali. The genetic distinctiveness of Dauh Waru, which belongs to this regency, therefore, is compatible with this explanation.

Although there is a common interrelationship among all Balinese populations, some degree of isolation still can be observed in various Balinese populations. Among populations in the southern region, Tenganan Pageringsingan is well known as a village completely different from other Balinese villages as shown by its typical village appearance and other cultural differences, such as a strictly endogamic mating system (Korn, 1960; Raka Dherana, 1976). The genetic structure of the population of this village has been studied by Breguet *et al.* (1982a and b). The genetic distinctiveness of such a village from the other villages, is understandable. It is possible that the same situation applies to Banjar Rangkan which is genetically distinct from other southern Balinese villages despite its geographical closeness. Further studies with a larger series (Banjar Rangkan is the smallest included in the genetic distance analysis) may be necessary, as well as more detailed study of the structure of its population to determine if there is a reason for its distinctiveness.

From the northern part of the island, Songan is a typical mountain village where pre-Hindu traditions have been preserved to a large extent. Together with other villages, such as Kedisan, Abang and Terunyan, they represent what is termed the Lake Batur-type village (Grader, 1960). The difference in organization between these villages and other villages seems to be followed by differences in other aspects, including their genetic structure as shown by the distinctiveness of Songan from Julah or Sembiran as well as from the rest of the Balinese villages studied here. Geographically, these villages are located in the *Bali-aga*

or "original Balinese" territory, which extends from Kubutambahan eastwards (Grader, 1960 and Swellengrebel, 1960). This is an area where the village organization was not influenced by the ruler of the kingdoms. Whether or not the lack of the ruler influence on such villages lead to a complete isolation is still open to discussion. However, the close genetic relationship between Sembiran and southern slope populations indicates that, at least in this case, the isolation was not so great as it was for Julah and Songan. Once again, topographical features may provide an explanation. Sembiran lies close to one of the two main routes which pass through the mountains from north to south. This route may well have made possible gene flow which brought Sembiran closer to Rendang which, in turn, is closely related to Seraya and Renon.

It has not been possible in the present investigation to develop detailed historical or genealogical information for each of the Balinese villages. But it is clear from the brief discussion above that Bali offers a valuable opportunity for further exploration of the way in which genetic relationships between populations have been shaped by geographical and cultural factors.

5.2.2. Genetic distance between Indonesian populations

In the present section all Indonesian populations, for which genetic data are available, have been included in the analysis, even though in cases such as the Savunese, Alorese and Makianese, the population sample sizes were small. Attention will be drawn to this where appropriate.

Information for the Batak was obtained mainly from McDermid *et al.* (1973), Blake (1976, 1978a) and Ghosh (1977a). For the Niasans information was from unpublished data (Blake, personal communication), and for the Balinese, previous publications by Breguet *et al.* (1982a, b) were employed. Altogether, genetic data from 17 populations was analysed. Because of its distinctiveness in gene frequency distributions the Asmat were not

included in the analysis of genetic distance between Indonesian populations, but were included in the analysis of genetic distance between Indonesian and neighbouring populations.

Data for 18 loci showing variation were used, i.e. ACP, AK, ADA, ESD, GLO I, GPT, 6-PGD, PGM_1, PGM_2, CA_1, CA_2, MDH, PHI, PEP B, Tf, Hp and the Hb α and β chains. Distances between populations generated from the gene frequencies are shown in table 5.2.a, and the standard errors for these distances are given in table 5.2.b.

The Alorese and Savunese are the most distinctive from the rest of the Indonesian populations. However, the Alorese are somewhat closer to populations of the eastern Lesser Sunda Islands and Moluccas, whereas the Savunese are somewhat closer to Bimanese and Javanese than to the other populations. Furthermore, the largest distance is between the Alorese and Savunese despite their relative geographical closeness.

The smaller distance between the Savunese and Javanese and/or Bimanese than between the Savunese and other Lesser Sundas' populations is of interest. Geographically Savu, which together with Raijua makes up the Savu group, Sumba, Ndao, Roti, Semau and Timor, constitute the outer arc of the Lesser Sundas. Linguistically this arc is divided into two groups. The peoples of Sumba, Raijua, Savu and Ndao speak related languages which belong to the Sumba-Bima group, whereas, the peoples of Roti, Semau and most of Timor speak languages belonging to the Timor-Ambon group (Fox, 1977). Therefore, the smaller distance between the Savunese and Bimanese is compatible with this linguistic grouping. Furthermore, there is a local Savunese legend about an early folk-ancestor known as *Madja-Pahi*. It is sometimes regarded by them as a link with the fifteenth century kingdom on Java, known as Majapahit (Lebar, 1972). If one accepts the validity of this legend, the relationship between the Savunese and Javanese as expressed by somewhat smaller genetic distance is understandable. However, further studies with larger series is necessary to obtain the

Table 5.2.a.

Nei's genetic distances (Dx100) between Indonesian populations based on 18 loci showing variation

	Sasak	Bimanese	Timorese	Florenese	Rotinese	Savunese	Alorese	Javanese	Ternatans	Galelarese	Makianese	Balinese	Batak	Niasans	Macassarese	Buginese
Bimanese	.10															
Timorese	.19	.16														
Florenese	.11	.15	.11													
Rotinese	.35	.32	.17	.22												
Savunese	.77	.57	.99	1.00	.92											
Alorese	.75	.80	.33	.41	.51	2.29										
Javanese	.17	.28	.59	.37	.57	.56	1.44									
Ternatans	.35	.35	.27	.14	.40	1.30	.41	.79								
Galelarese	.27	.30	.15	.15	.09	1.00	.52	.45	.44							
Makianese	.17	.25	.12	.11	.30	1.34	.26	.59	.23	.27						
Balinese	.22	.43	.58	.45	.67	.69	1.44	.15	.91	.51	.66					
Batak	.13	.19	.29	.21	.52	.62	1.04	.22	.50	.35	.43	.18				
Niasans	.21	.30	.30	.23	.47	.66	.91	.32	.48	.32	.38	.25	.10			
Macassarese	.06	.08	.20	.14	.40	.61	.87	.22	.35	.29	.26	.26	.05	.12		
Buginese	.03	.13	.25	.18	.38	.68	.92	.12	.50	.25	.25	.17	.11	.16	.05	
Toraja	.20	.15	.26	.33	.46	.73	1.08	.36	.63	.33	.41	.43	.23	.37	.12	.15

Table 5.2.b.

Standard errors for distances shown in Table 5.2.a.

	Sasak	Bimanese	Timorese	Florenese	Rotinese	Savunese	Alorese	Javanese	Ternatans	Galelarese	Makianese	Balinese	Batak	Niasans	Macassarese	Buginese
Bimanese	.06															
Timorese	.10	.11														
Florenese	.07	.08	.05													
Rotinese	.15	.17	.12	.08												
Savunese	.34	.24	.39	.45	.35											
Alorese	.42	.45	.21	.19	.23	1.02										
Javanese	.09	.15	.30	.23	.26	.28	.81									
Ternatans	.25	.27	.15	.07	.19	.74	.19	.52								
Galelarese	.14	.17	.08	.08	.03	.38	.30	.26	.23							
Makianese	.08	.10	.04	.06	.12	.52	.16	.31	.09	.11						
Balinese	.11	.19	.28	.25	.28	.37	.79	.07	.54	.21	.35					
Batak	.07	.11	.17	.12	.27	.31	.54	.15	.32	.20	.22	.08				
Niasans	.10	.16	.21	.11	.22	.32	.50	.17	.28	.15	.23	.09	.04			
Macassarese	.03	.03	.09	.09	.20	.29	.47	.12	.29	.16	.11	.11	.03	.06		
Buginese	.02	.05	.11	.14	.14	.33	.58	.05	.39	.11	.15	.09	.06	.06	.03	
Toraja	.10	.08	.19	.29	.25	.36	.79	.21	.60	.19	.27	.25	.16	.22	.07	.07

Fig. V.4 Dendrogram based on Nei's genetic distance for Indonesian populations using 18 loci showing variation

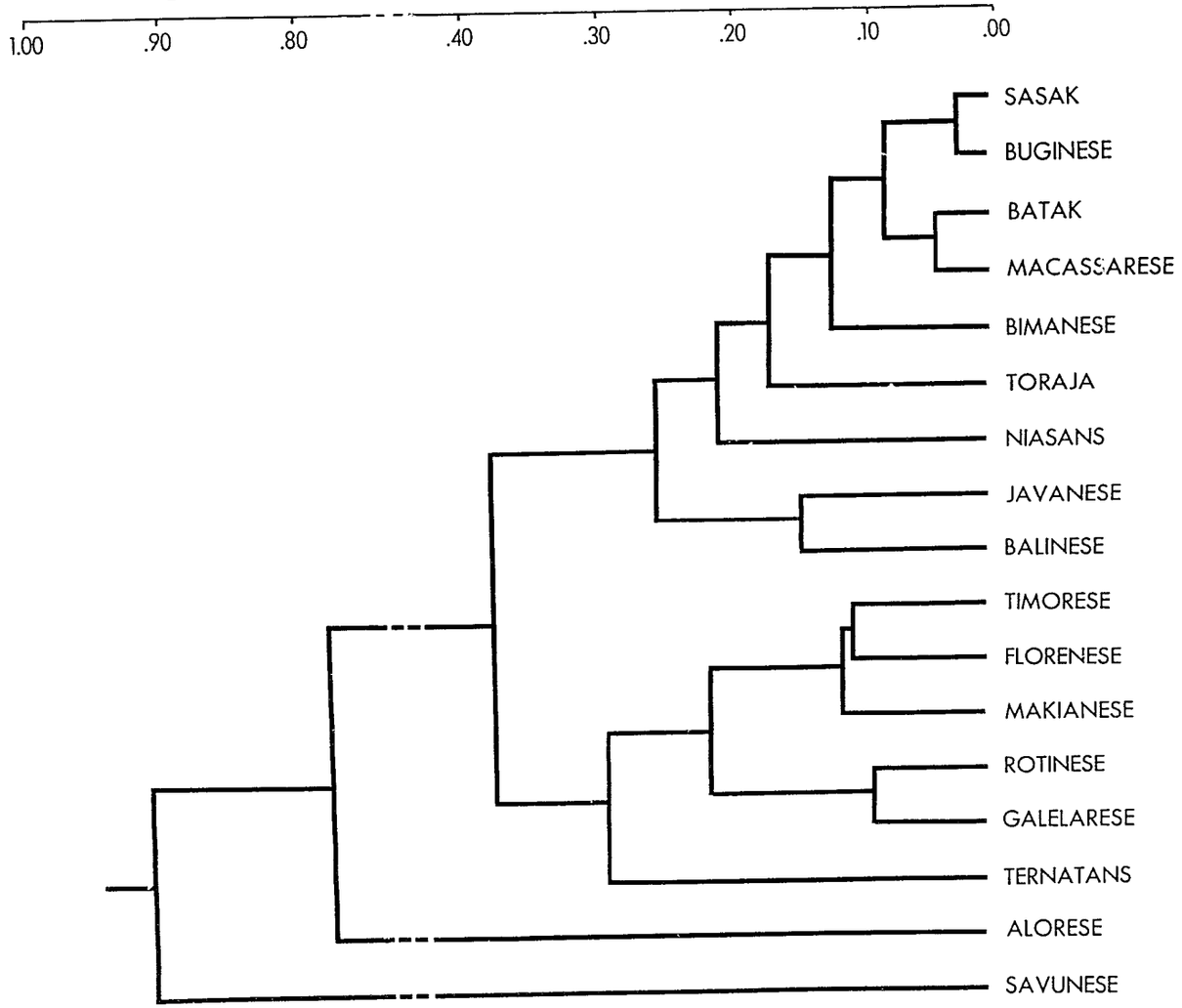
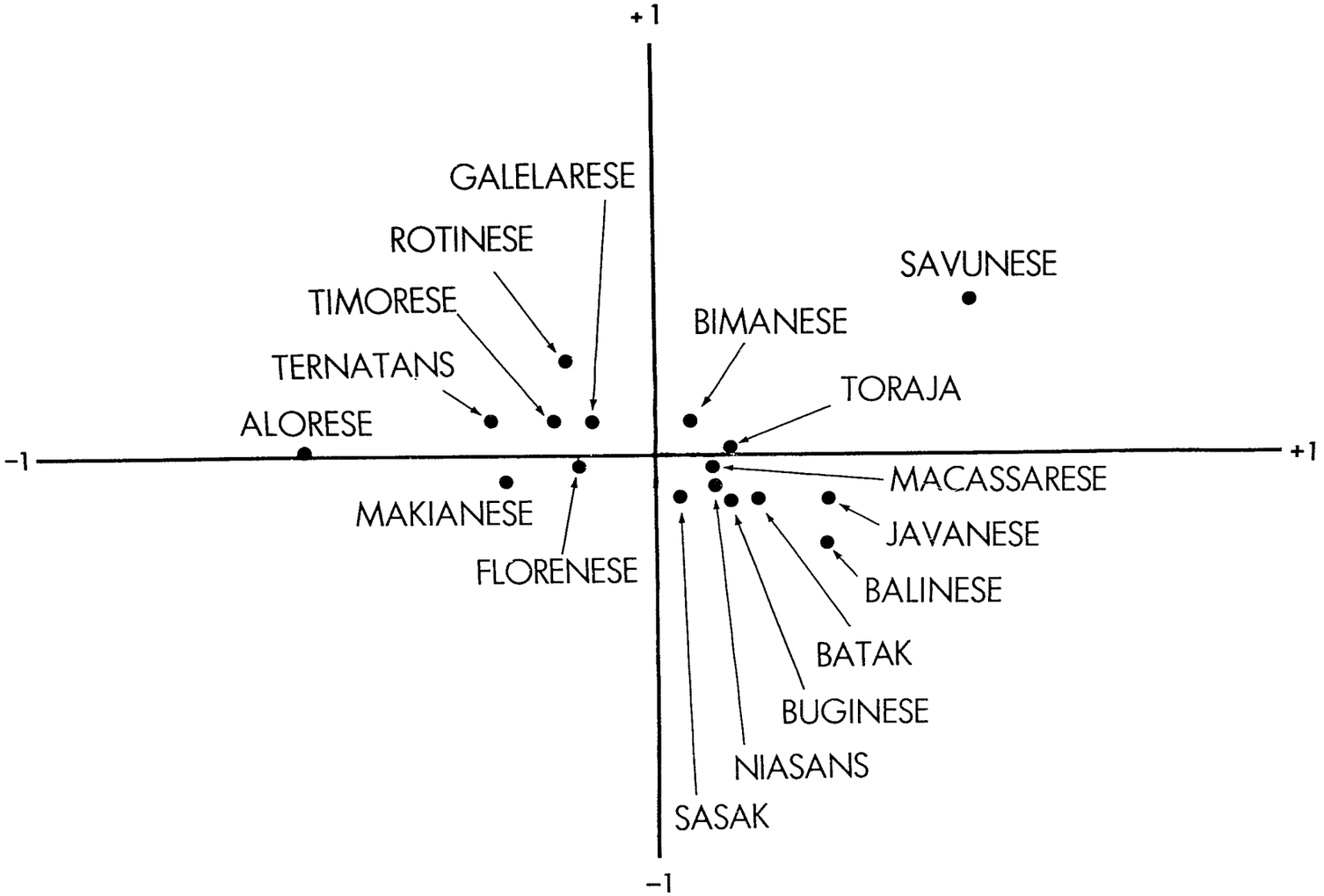


Fig. V.5 Eigenvector diagram based on Nei's genetic distance for Indonesian populations using 18 loci showing variation.



actual position of the Savunese compared to other Lesser Sunda populations and Indonesian populations in general.

The smallest distance is between the Sasak and Buginese followed by that between Macassarese and Buginese and Macassarese and Batak (though these distances are not quite twice of their standard error and therefore fall below the normal acceptable level of significance) and between Macassarese and Sasak (see Table 5.2.b).

As shown in the dendrogram (Fig. V.4) the Savunese and Alorese are very distinctive and are the earliest to split. The other populations fall into two clusters. The first cluster is comprised of the Timorese, Florenese, Makianese, Rotinese, Galelarese and Ternatans, which may be designated as the eastern Indonesian cluster. The second cluster consists of nine other populations which may be designated as the western Indonesian cluster. It appears that all populations of the Greater Sunda Islands and some populations of the Lesser Sunda Islands, from the Bimanese westwards, constitute the western Indonesian cluster. Within this cluster the small distances between the Macassarese and Batak, as well as between the Macassarese and Sasak and the Buginese and Sasak, is of interest since geographically they are quite distant and separated by sea barriers. However, it has long been known that the Buginese and Macassarese are seafarers contributing their influence in many coastal areas of the Indonesian archipelago. Therefore, despite separation by sea, for seafaring people it confers a convenient route of contact rather than a barrier.

The two dimensional eigenvector diagram (Fig. V.5) also shows the Savunese and Alorese as very distinctive. Again, the other populations can be distinguished as two clusters, the western Indonesian and the eastern Indonesian clusters respectively. It is clear that, even though the Savunese are very distinctive, they are still somewhat closer to

the western Indonesian cluster than to the eastern one. On the other hand the Alorese, who are also distinctive, are somewhat closer to the eastern Indonesian cluster than to the western cluster. It is also of interest that the Ternatans and Galelarese, who are non-Austronesian speakers are, in fact, grouped into the eastern Indonesian cluster together with other populations from the Lesser Sunda Islands east of Sumbawa.

The western Indonesian cluster may be further split into the Javanese-Balinese sub-cluster and another sub-cluster comprised of the other seven populations. With the exception of the Batak and Niasans from Sumatera, this sub-division seems to coincide with the geographical relationships as one moves from the west to the east of the Indonesian archipelago. Considering population movements from mainland South East Asia into the Indonesian archipelago, the Javanese-Balinese sub-cluster may be accounted for by these populations absorbing more genes from the mainland migrants. Towards the east, the eastern Indonesian cluster may have absorbed the least genes. A transitional cluster in between these extremes may have absorbed some genes from the mainland but less than the Javanese-Balinese cluster and more than the eastern Indonesian cluster.

5.2.3. Genetic distance between Indonesian and neighbouring populations

For the final distance analysis six populations from the South East Asian and Western Pacific regions were contrasted with fourteen of the Indonesian populations. The latter were represented by five populations of the Greater Sunda Islands (Batak, Javanese, Macassarese, Buginese and Toraja), six populations of the Lesser Sunda Islands (Balinese, Sasak, Bimanese, Florenese, Rotinese and Timorese), two populations from the Moluccas (Ternatans and Galelarese) and also the Asmat of Irian Jaya. Populations from the neighbouring areas were Malays,

Kadazans of Sabah, Tagalog of the Philippines, representing the northern and northwestern neighbours, in addition to a Motu-speaking population from Port Moresby in Papua New Guinea, Fijians of Fiji and an Aboriginal population from Angurugu in North Australia.

As mentioned in the previous section, information for Balinese was obtained from publications by Breguet *et al.* (1982a, b) and for the Batak was obtained mainly from McDermid *et al.* (1973) in addition to other sources. For the neighbouring populations genetic data were taken from various sources mentioned elsewhere in chapters 3 and 4. The data for Malays mainly comes from Blake *et al.* (1973a) in addition to serum protein data reported by Kirk and Lai (1961). Information for the Kadazans was obtained from Tan *et al.* (1979) and for the Tagalog is based on the publication by Omoto *et al.* (1978). Information for Motu-speakers and Fijians were from unpublished data (Blake, personal communication) and, finally, Aboriginal data was from publications by Blake (1979) and Kirk *et al.* (1971).

Eighteen loci showing variation were used for this distance analysis, i.e. ACP, AK, ADA, ESD, GLO I, GPT, 6-PGD, PGM_1, PGM_2, CA_1, CA_2, MDH, PHI, PEP A, Tf, Hp, Hb α and β.

Several striking features emerge from the distance values given in Table 5.3.a (standard errors are given in table 5.3.b), and in the dendrogram derived from the distance matrix (Figure V.6). For Indonesian populations alone the Asmat population of Irian Jaya now becomes the most distinctive, a position which is interesting since Asmat speak a non-Austronesian, or Papuan language which is more closely related to other Papuan languages located mainly in highland areas of Papua New Guinea and Irian Jaya.

The Asmat population first splits in the dendrogram with the Aborigines from north Australia. Although in terms of genetic distance

Table 5.3.a.

Nei's genetic distances (Dx100) between Indonesian and neighbouring populations based on 18 loci showing variation

	Sasak	Bimanese	Timorese	Florenese	Rotinese	Javanese	Ternatans	Asmat	Galelarese	Balinese	Batak	Motu	Angurugu	Kadazans	Malays	Tagalog	Fijians	Macassarese	Buginese
Bimanese	.10																		
Timorese	.19	.16																	
Florenese	.11	.15	.11																
Rotinese	.35	.32	.18	.22															
Javanese	.17	.28	.60	.37	.57														
Ternatans	.35	.35	.28	.14	.40	.79													
Asmat	2.71	2.48	1.66	1.97	1.70	3.79	1.66												
Galelarese	.27	.30	.15	.15	.09	.45	.43	1.96											
Balinese	.22	.43	.59	.45	.67	.15	.91	3.87	.51										
Batak	.13	.19	.30	.21	.52	.22	.50	3.12	.35	.18									
Motu	.48	.24	.32	.45	.67	.84	.50	2.21	.61	.96	.44								
Angurugu	1.62	1.57	1.25	1.32	1.76	2.02	1.54	3.10	1.49	1.68	1.05	1.41							
Kadazans	.64	.54	1.07	1.01	1.06	.63	1.20	4.43	1.19	.94	.97	1.09	3.53						
Malays	.19	.31	.58	.38	.66	.09	.72	3.86	.51	.15	.12	.75	1.60	.73					
Tagalog	.11	.23	.38	.32	.57	.20	.60	3.41	.43	.18	.1[illegible]	.50	1.63	.67	.09				
Fijians	.40	.48	.31	.30	.72	1.04	.24	1.69	.70	1.06	.67	.63	1.68	1.41	.94	.68			
Macassarese	.06	.08	.20	.14	.40	.21	.35	2.79	.29	.26	.05	.29	1.35	.71	.15	.06	.48		
Buginese	.03	.13	.25	.18	.38	.12	.50	2.99	.25	.17	.11	.49	[illegible].63	.68	.12	.06	.58	.05	
Toraja	.20	.15	.27	.34	.46	.36	.64	2.[illegible]9	.34	.43	.23	.25	[illegible].78	.69	.38	.18	.77	.13	.15

Table 5.[illegible].b.

Standard errors for distances shown in Table 5.3.a.

	Sasak	Bimanese	Timorese	Florenese	Rotinese	Javanese	Ternatans	Asmat	Galelarese	Balinese	Batak	Motu	Angurugu	Kadazans	Malays	Tagalog	Fijians	Macassarese	Buginese
Bimanese	.06																		
Timorese	.10	.11																	
Florenese	.07	.08	.05																
Rotinese	.15	.17	.12	.08															
Javanese	.09	.15	.30	.23	.26														
Ternatans	.25	.27	.15	.07	.19	.52													
Asmat	1.24	1.18	.78	.88	.83	1.91	.66												
Galelarese	.14	.17	.08	.08	.03	.26	.22	1.00											
Balinese	.11	.19	.29	.25	.28	.07	.55	1.94	.21										
Batak	.07	.11	.18	.12	.27	.15	.32	1.54	.20	.08									
Motu	.27	.12	.15	.22	.46	.45	.30	1.11	.38	.48	.27								
Angurugu	.66	.68	.60	.6[illegible]	.96	.97	.68	1.94	.83	.71	.41	.72							
Kadazans	.29	.23	.55	.49	.51	.32	.72	2.01	.62	.44	.42	.43	1.42						
Malays	.13	.22	.34	.22	.29	.04	.48	1.93	.23	.07	.05	45	.69	.40					
Tagalog	.05	.09	.17	.20	.22	.10	.47	1.64	.17	.09	.05	.25	.61	.35	.06				
Fijians	.23	.2	.13	.13	.30	.54	.12	.65	.28	.56	.36	.39	.73	.69	.53	.44			
Macassarese	.03	.03	.09	.09	.20	.12	.28	1.31	.16	.11	.03	.15	.52	.32	.10	.03	.27		
Buginese	.02	.05	.12	.14	.14	.05	.39	1.47	.11	.09	.06	.27	.66	.34	.08	.04	.36	.03	
Toraja	.10	.08	.19	.30	.25	.21	.62	1.64	.19	.25	.16	.11	.78	.37	.28	.10	.57	.08	.07

Fig. V.6 Dendrogram based on Nei's genetic distance for Indonesian and neighbouring populations using 18 loci showing variation

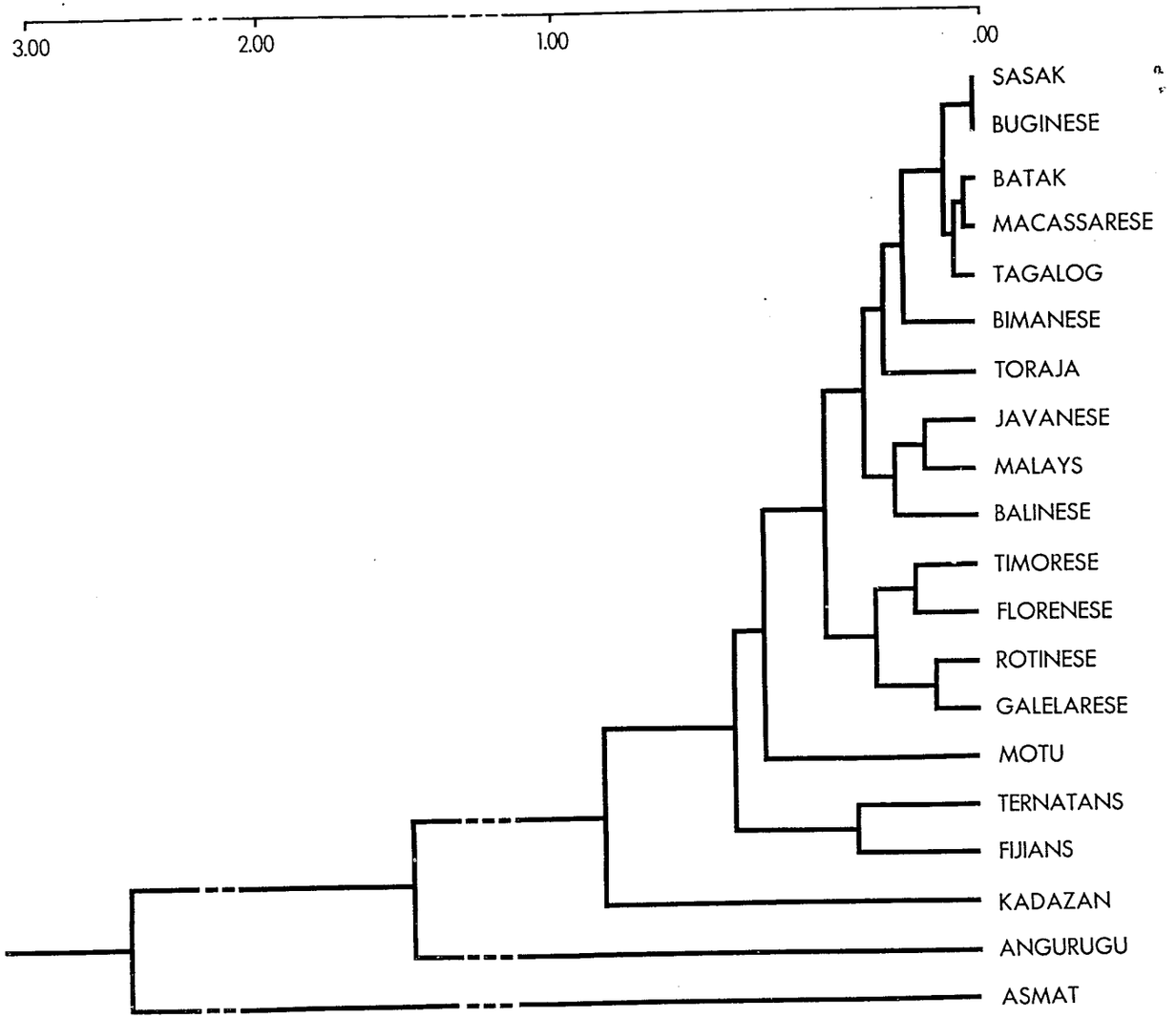
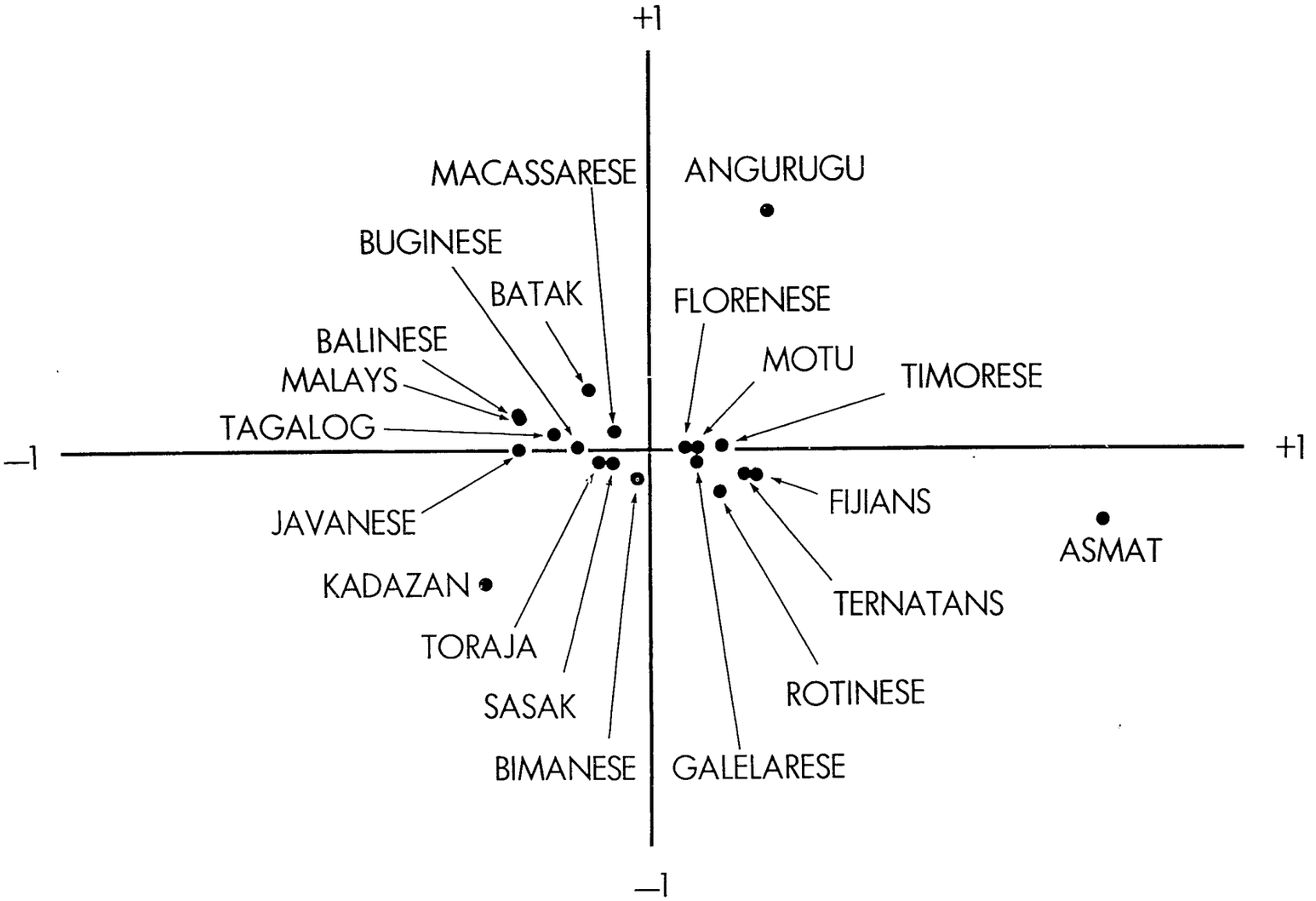


Fig. V.7 Eigenvector diagram based on Nei's genetic distance for Indonesian and neighbouring populations using 18 loci showing variation



the Aborigines and the Asmat population are not particularly close, previous studies (Keats, 1977) also have shown that Australian Aborigines have some affinities with Papuan-speaking populations in New Guinea. The other Indonesian populations, with the exception of Ternatans again constitute two clusters. The first cluster consists of the Timorese, Florenese, Rotinese and Galelarese and the second cluster, which is bigger than the first, consists of ten populations including the Tagalog and Malays. This cluster can be divided further into the Javanese-Malays-Balinese sub-cluster and the other seven populations sub-cluster. The close distance between the Javanese and Malays is consistent with observations by Lie-Injo (1976) using the available blood group data. It is interesting that the Ternatans come together with the Fijians.

The genetic distance relationships become even clearer when the distance matrix is used for the principal component analysis shown in Figure V.7, using the first two eigenvectors. Close to the centre of the diagram there are two clusters. One is formed by Fijians and Motu-speakers from Port Moresby, together with Indonesian populations from the Moluccas, and the Lesser Sunda Islands east of Sumbawa. The other comprises Indonesian populations from Sumbawa to the west and including the Malays and Tagalog-speakers from the Philippines. The Aborigines from north Australia and Asmat from Irian Jaya are most remote on one side, whilst Kadazans stand apart on the other side. The Aboriginese are somewhat closer to Timorese and Florenese than are the Asmat, a point of interest since one of the postulated routes of entry for Aboriginese into Australia was via Timor (Birdsell, 1977).

One of the surprising results of the genetic distance analysis, both in this section and in that dealing solely with Indonesian populations, is the aberrant position of the Kadazans from Sabah. Ethnograph-

ically Kadazans might be expected to fall in the main western population cluster, rather than distinctly removed from this. Perhaps variation of results between laboratories could explain this aberrant situation, since the data for Kadazans were the only ones used from the publications of Tan *et al.* (1979). A further study of Kadazans could help to resolve this problem.

The importance of the present analysis is the emphasis it gives to the closer relationship between Indonesian populations and others in neighbouring regions speaking Austronesian languages. There is a cline from the western Indonesian cluster, which includes both Malays and Philippine Tagalog, through the eastern Indonesian cluster which includes the Motu-speakers of Port Moresby and the Fijians at the limit of this cluster. The north Australian Aborigines remain distinctive, as was demonstrated also by Keats (1977) and the Papuan-speaking New Guineans, the Asmat, also remain distinctive.

By contrast, however, the two populations included in the present survey who speak non-Austronesian languages, i.e. Ternatans and Galelarese, are in the eastern Indonesian cluster, still genetically very remote from Asmat, which is the other non-Austronesian speaking population included in the present analysis. This suggests that both the Ternatans and Galelarese are examples of people who have maintained a particular cultural trait, in this case language, over a long period of time whilst incorporating genes from neighbouring populations.

CHAPTER 6

DISCUSSION AND CONCLUSION

6.1. Introduction

As outlined in Chapter 1, Indonesia has long been recognized as a region characterized by population mixture, being subject to movements of people from the dominant Asian landmass to the north. Such population movements from mainland South East Asia through the Indonesian archipelago and eventually towards the east and southeast may be divided broadly into two different periods; the Pleistocene epoch during the greater part of which Sunda land and Sahul land were in existence, and the Holocene period when these lands had broken up.

During the first period, Howells (1973) suggests that Indonesia was the home of "old Melanesians" who, at that time, crossed the sea into the adjacent Sahul land, which later became the present New Guinea, Australia and Tasmania. To the north the "old Melanesians" reached as far as the northern end of Luzon in the Philippines. During the existence of these Pleistocene landbridges, Glinka (1978) also suggests the movement of "Proto-Malays" in a south east direction to the Lesser Sunda Islands. They were followed according to Glinka by the "Deutero-Malay" populations who spread into Indonesia after the disappearance of the landbridges, but did not reach New Guinea and Australia.

A similar theme is followed by Bellwood (1978) who discusses the various migrations which have made up the present Indonesian population, running as a cline from Mongoloid populations in the west to populations of Melanesian type in the east.

Despite variations in their physical characteristics and local customs, linguistically the vast majority of the Indonesian populations

at present speak Austronesian (AN) languages, a group of languages which also extend into the Pacific region. The other major linguistic division comprises Non-Austronesian (NAN) languages which are spoken by some peoples in northern Halmahera, Ternate, Alor and Timor (Howells, 1973; Lebar, 1972) and in all Irian Jaya populations except AN speakers in some coastal areas (Voorhoeve, 1975). It is possible that NAN languages were formerly the common languages of the Indonesian region but have been replaced by pressure from AN - speaking migrants. This replacement could have been of two types. Firstly, a colonization by people who either physically displaced the earlier NAN speakers, or who mixed with them extensively and enforced a language change or, secondly, through contact with a dominant culture leading to adoption of a language likely to be important in trade or social interchange.

If the speakers of the two types of languages, AN and NAN, represented distinct genetically different populations, then the alternatives in language replacement will have resulted in different genetic consequences. The complete replacement model will lead to a more uniform distribution of genes representing the AN-speakers. The cultural absorption model will lead to the retention of a significant proportion of genes representing NAN-speakers.

Previous studies in New Guinea have shown a close relationship between linguistic and genetic differentiation and, particularly, between NAN and AN-speakers (Keats, 1977). However, other factors, some of which may be ecological, can result in modifications of the close relationship between language and genes (Kirk, 1982).

The results of the investigations of Indonesian populations recorded in the previous chapters of this thesis will be examined below to see what support they give to the views of physical anthropologists on migrations of peoples from South East Asia into Indonesia and beyond out

into the Pacific, and also to examine broadly the way in which these results can be interpreted to support one or other view of language replacement in the Indonesian region.

6.1.1. Blood genetic markers: clinal variations

Alleles at several of the polymorphic loci studied show marked clinal variation across the archipelago. For the red cell enzyme systems these include $\underline{GLO}^1$, $\underline{GPT}^1$ and $\underline{PGD}^C$. The $\underline{GLO}^1$ gene frequency shows a trend decreasing towards the east, whereas the $\underline{GPT}^1$ and $\underline{PGD}^C$ gene frequencies are increasing towards the east. However, the clinal pattern for these alleles changes sharply when approaching Irian Jaya where the Asmat possess some extreme values compared to the other Indonesian populations.

The serum protein locus, Hp, also reveals a clinal variation, with a trend of increasing $\underline{Hp}^1$ frequency towards the east. As well, there is a general clinal variation for the transferrin alleles $\underline{Tf}^{D\text{Chi}}$ and $\underline{Tf}^{D1}$, although, because of low frequencies, the distribution is patchy.

In the Asia-Pacific region, only $\underline{Tf}^{D\text{Chi}}$ is commonly found in mainland South East Asia. On the other hand $\underline{Tf}^{D1}$ is widely distributed in Australian Aboriginal and Melanesian populations (see Kirk, 1968b) but is also rarely found in Asia. During the present study, however, the $\underline{Tf}^{D\text{Chi}}$ and $\underline{Tf}^{D1}$ alleles are found to coexist in Java and to the east. The $\underline{Tf}^{D\text{Chi}}$ allele reaches as far as Halmahera and Timor in the east and is completely absent and replaced by a high frequency of $\underline{Tf}^{D1}$ in the Asmat. There seems to be a continuous pattern from Java to the Lesser Sunda Islands. The gene frequency for $\underline{Tf}^{D1}$ and $\underline{Tf}^{D\text{Chi}}$ in the Bimanese of Sumbawa is approximately equal. From Lombok to the west, the $\underline{Tf}^{D\text{Chi}}$ gene frequencies are higher than that for $\underline{Tf}^{D1}$, while to the east a reciprocal situation is observed.

Since Tf D_1 is found widespread in Melanesian and Australian

Aboriginal populations we can argue that it was present also in older populations of Indonesia (Howells' "old Melanesians"; Glinka's "Proto Malays"?), so that its patchy distribution in modern populations represents remnants of the genes of these earlier groups. Similarly since Tf D_{Chi} is an east Asian population marker, the degree of its penetration is an indication of the extent of gene flow from east Asia into Indonesia. The extent of this penetration reaches an eastern limit of Halmahera in the north and Timor in the south. The absence of Tf D_{Chi} further east, and the presence of Tf D_1 in these more easterly areas is strong genetic evidence in support of metrical and anthroposcopic data indicating the survival of a significant proportion of Melanesian or Australoid traits in these populations.

A similar distribution to that of Tf D_{Chi} is shown by another South East Asian marker, Hb E. Though this allele distribution may have been affected by malarial selection, it also reaches its eastern limit as far as Halmahera in the north and Timor in the south. This again seems to delimit the extent of later gene flow from the Asian mainland ("Deutero-Malay"?).

6.1.2. The distribution of rare markers

Although transferrin variants and Hb E in many populations are relatively rare, their overall distribution in the region puts them into the polymorphic class. However, there are other specific marker genes which are relatively rare in most populations, but nevertheless are spread over a wide geographic area. Frequently these rare alleles are important in demonstrating gene exchange between populations, but because of their low frequency they are not useful in quantifying the amount of gene interchange.

One of these rare alleles is $\underline{PGM}_2^9$, which is widespread in New Guinea (Blake and Omoto, 1975). The present study has shown that this

allele occurs in some other Indonesian populations apart from the Asmat. To the west, this allele is found in Java and its distribution is similar to the distribution of the $\underline{Tf}^{D1}$ allele in the archipelago. As the $\underline{Tf}^{D1}$ allele provides evidence of a genetic relationship between Indonesian populations and populations of New Guinea, Australia and other parts of the western Pacific, the $\underline{PGM}_2^9$ allele provides evidence of a genetic relationship between Indonesian populations and the population of New Guinea in particular. Two other rare alleles which are present in New Guinea populations, $\underline{MDH}_1^3$ and $\underline{PEP\ B}^2$, are also found sporadically in some Indonesian populations. This further strengthens the argument that there are "genetic remnants" in present Indonesian populations remaining either from a population which provided the early migrants into Sahul land or, alternatively, represent a backward gene flow from New Guinea westwards.

This latter hypothesis would be supported by the presence of an allele such as $\underline{MDH}^3$, which is widespread in New Guinea and occurs sporadically in Indonesia, but is completely absent in Australian Aborigines.

Further evidence of gene flow from Asia is provided by rare alleles such as $\underline{PGM}_1^7$. At the PGM_1 locus, the occurrence of $\underline{PGM}_1^7$ has been reported sporadically in Chinese, Indonesians, Thais, West Malayan Aborigines and two Asiatic Indian populations, but achieves a high value of 8% in the Western Caroline Islands (Blake and Omoto, 1975). It is completely absent in New Guinea and Australia. The occurrence of this allele in the Javanese during the present study, therefore, provides evidence for a genetic link between Indonesian populations, the Javanese in particular, and the populations of South East Asia and some populations in the Western Pacific other than New Guinea. As well, the occurrence of the rare allele, $\underline{GPT}^6$ in some Indonesian

populations, with a particularly high frequency in the Toraja and its distribution in neighbouring populations with high frequencies in the Ifugao of the Philippines and in Guam, also indicates genetic relationship between Indonesian populations and these other neighbours.

Another rare allele which is widely distributed in the Western Pacific region is the $\underline{CA}_1^3$ allele, originally reported as CA Ic Guam (Tashian *et al.* 1963). The distribution of this allele appeared to be rare and sporadic until Omoto (1979) reported a high frequency of $\underline{CA}_1^{3N}$ in the Mamanwa Negritos of Mindanao, the Philippines. Since $\underline{CA}_1^{3N}$ has been found to be identical with that of $\underline{CA}_1^3$ (Omoto *et al.* 1981) its distribution in the western Pacific area suggests a gene diffusion from some population groups of South East Asia who possibly were the ancestral Negrito or Proto-Malay populations (Omoto, 1980).

In the Indonesian archipelago the $\underline{CA}_1^3$ allele is also found in some populations although in low frequencies. $\underline{CA}_1^3$, however, is not present in New Guinea or in Australia. This suggests that, after the migration of the "old Melanesians" to Sahul land, a new mutation occurred which has been carried by sea routes to various islands in the central western Pacific and Indonesia as well as to the Malaysian peninsula. But, although $\underline{CA}_1^3$ reaches its highest frequency in the Mamanwa Negritos, this allele is absent in the Aeta Negritos. Omoto (1980) suggests independent origins for these two Negrito populations, not only because of the discrepancy in the distribution of $\underline{CA}_1^3$ but also because of the converse situation in which the $\underline{AK}^2$ allele has a high frequency in the Aetas, but is absent in the Mamanwas. However, an alternative explanation seems more likely. A rare gene introduced into a small, inbred group, can be lost again in some cases whilst, in other cases, by chance it may increase in frequency quite rapidly. All that can be inferred from the distribution of $\underline{CA}_1^3$ at present is that there has been gene

exchange over a relatively wide area from Guam westwards, with a suggested focus of the dispersal in the Luzon area and that this dispersal is relatively recent, post-dating the entry of people into the New Guinea area and probably post-dating the later migrations of the early Austronesian-speakers out past New Guinea into the Western Pacific.

The distribution of the $\underline{AK}^2$ allele in the Asia-Pacific region is also of interest. It is completely absent in the Western Pacific area. However, in the Indonesian archipelago, low frequencies are found in some populations. This may also indicate a genetic link between Indonesian populations and the Negrito population of the Philippines. But since $\underline{AK}^2$ is regarded also as a Caucasian marker (Tills *et al.* 1970a) and high frequencies are found in the Indian subcontinent, the introduction of the $\underline{AK}^2$ allele by Europeans or Indians into Indonesia is another possibility, which probably must have taken place more recently than its introduction from a Negrito source. It is of interest that Teng and Tan (1979) suggest that some or all of the $\underline{AK}^2$ genes in Malay populations of Malaysia and Singapore may derive from racial admixture with Indians.

Elements of a Negrito phenotype still survive in some places in Indonesia (Keers, 1948) but such populations were not included in the present study. It will be important in future investigations to pay special attention to such groups to clarify further the relations between the various Negrito groups in Malaysia and the Philippines and their original wide dispersal in parts of Indonesia.

6.2. Evidence from genetic distance analyses

Just as genetic markers which have been discussed in the previous section can be used to study the genetic variability within and between populations, so also genetic distance computed from the gene frequency distributions can provide a valuable means for interpreting the genetic

divergence between the populations under consideration.

The analysis of data for the Balinese village populations has shown the heterogeneity of these populations despite their religious and cultural similarities. Some local populations are genetically distinctive such as those from Dauh Waru, Julah and Songan and this is consistent with their geographical distances and history. However, larger geographical distance does not necessarily mean greater genetic isolation, as shown for example by the genetic closeness between Sembiran in the northern part of the island and other populations from the south, a relationship due probably to the fact that Sembiran is located close to the main pass between mountain ranges connecting the northern and southern regions. Conversely a closer geographical distance may not always lead to closer genetic resemblance as shown by the relatively large genetic distance between Sembiran and Songan, a fairly isolated mountain village close to Lake Batur.

Despite the genetic heterogeneity within Bali itself, when other Indonesian populations are included in the analysis, even the "isolated" Balinese from Tenganan Pageringsingan are very close to their neighbours, the Javanese. Although it has been claimed by Breguet *et al.* (1982b) that there is direct influence from India by the finding in Bali of LDH CAL-1, a specific "Indian" LDH variant, the Indianization or, more properly, the Hinduization of Bali was exercised mostly by nearby Java, which itself had been the subject of earlier Hinduization (Hanna, 1976). Its separation by sea from Java does not seem to have prevented the continuous gene flow from Java, leading to the genetic similarity between these populations.

When one looks at the general genetic distance analysis between the other Indonesian populations, two populations stand out as being very distinct, the Savunese and Alorese. The small size of the series in both these cases may explain the distinctive genetic posi-

tion. However, another small series, the Makianese, clusters as expected together with other eastern Indonesian populations. Clearly, further studies with a larger number of persons tested is needed to determine the actual position of these populations relative to the other Indonesian populations. If the outcome is similar to that already observed then some other factors must have been involved to make them different from the other adjacent populations. For the Savunese in particular a somewhat smaller genetic distance from the Bimanese is compatible with the linguistic grouping whilst the smaller distance of the Savunese from the Javanese is compatible with the local legend about the relationship of the Savunese to a former Hindu Kingdom in Java.

Interestingly, the genetic distance analysis showed the Indonesian populations can be grouped into two clusters, eastern and western. However, despite this, the general distribution indicates no clear-cut genetic differences between geographically closely related populations. Instead, there is a gradual pattern running across the archipelago in the west-east direction.

It is also worth noting the possible influence of the Macassarese and/or Buginese on the other populations. In the archipelago, separation by sea could have played an important role as a geographical barrier between populations. However, it seems that this "geographical barrier" may, in fact, have provided a better route for population migration and contact in the archipelago. Smaller distances between the Buginese and/or Macassarese and other Indonesian populations is most likely due to the sea-faring expertise of these peoples.

Finally, in the genetic distance analysis which included the Asmat from Irian Jaya and Aborigines from northern Australia as well as other populations in the western Pacific there is a clear-cut separation of the Asmat and the Australian Aborigines from the other Indonesian popu-

lations. Genetic isolation must have taken place in these two populations following the rise of sea level during the late Pleistocene epoch. This isolation in different niches would lead progressively to the alteration of their gene pool, becoming eventually much more distinct from each other. On the other hand the Fijians and also the Motu-speakers from the Port Moresby area of Papua New Guinea are grouped into the eastern Indonesian cluster. This is consistent with their linguistic affiliations, except for the Ternatans and Galelarese from the Moluccas. These latter two populations are NAN speakers, although genetically they are close to AN speakers from the eastern part of Indonesia. It is very likely that the gene flow from the west (mainland South East Asia) continuously penetrated their gene pool without altering their language, a cultural trait which may have been present for a long period of time before the spread of the AN language. The eastern limit of this gene flow from the west is indicated, as discussed above, by the specific genetic markers, Tf D_{Chi} and Hb E. Beyond this limit AN languages are still found in parts of New Guinea and the Pacific but without these genes indicating South East Asian gene flow into Indonesia.

The fact that populations like the Motu-speakers from Port Moresby or the even more distant Fijians cluster with Indonesian populations supports the view put forward by Bellwood (1978) that the Austronesian migrations which swept out into the Pacific, carrying their AN languages with them, originated in Indonesia. Whether this migration originated in Halmahera which, from its geographical location, makes it a likely contender, is unclear. The genetic distance analysis carried out here does not allow one to choose Halmahera as more or less likely than other possible ancestral homes of these Pacific voyagers. However, it must be concluded that if the Austronesians came from Halmahera or similar locations, there are certain specific markers such as Hb E or Tf D_{Chi} which they either did not take with them, or lost at a later

stage. But evidence discussed in the next section suggests that these markers may have been a fairly recent intrusion into the Indonesian area.

6.3. Blood genetic traits associated with malaria

More than a decade ago Lie-Injo (1969) discussed the distribution of Hb E, thalassaemia and G6PD deficiency in South East Asia, including Indonesia, in relation to malarial endemicity in the region. Among these traits, thalassaemia distribution was less well documented due to the lack of a specific test to diagnose the condition. The probable selective advantage of these traits in malarious areas has been recognized and the evidence for this is discussed in Chapter 4 but, more recently, the selective advantage of another trait, ovalocytosis was pointed out (Baer *et al.* 1976; Kidson *et al.* 1981; Serjeantson *et al.* 1977).

Lie-Injo (1969) noted that, in general, the distribution of Hb E and G6PD deficiency are roughly parallel in mainland South East Asia and Indonesia. The frequencies were high in Thailand, but diverged in the Philippines and in the north-east islands of Indonesia. Here G6PD deficiency was relatively frequent and Hb E infrequent. Additional information obtained during the present study is compatible with that observation.

In the Indonesian archipelago, high frequencies of Hb E were observed in the Lesser Sunda Islands, but lower frequencies occurred in other localities. As well as a possible selective advantage maintaining the frequency of this trait, population movements also can play an important role in causing gene frequency variation from one population to another. For example, compared to G6PD deficiency and ovalocytosis, which are found in a wider distribution, Hb E has not been observed further east than Halmahera. It is important that this also coincides with the eastern limit of the Tf D_{Chi} distribution in the archipelago. Therefore it is most likely that Hb E was introduced by

population movements into the archipelago more recently than the other two traits.

Leaving aside the heterogeneity of G6PD deficiency variants in Indonesia (Chockkalingam *et al.* 1982; Kirkman and Lie-Injo, 1969), the distribution of G6PD deficiency is more or less coextensive with ovalocytosis. The present study has shown further than an interaction mechanism seems to exist between these two traits. There is an inverse relationship between them such that as the frequency of G6PD deficiency increases, the frequency of ovalocytosis decreases. This holds beyond Java to the east, except in the very small series of Alorese with no G6PD deficient subject, but Java occupies an exceptional position, having both very low frequencies of G6PD deficiency and ovalocytosis. If this general observation is true, the inconsistency in Java requires explanation.

Finally, from a public health point of view it is important to understand the possible clinical implications related to each of these malaria-related traits.

For ovalocytosis there is no evidence that it causes any deleterious condition. This may, in fact, explain why, in some populations such as the Toraja, frequencies for ovalocytosis reach values close to 50%. The high frequencies achieved sometimes for the $\underline{Hb}^{E}$ allele also suggest that there is, in general, no great disadvantage to the person carrying it either in heterozygous or homozygous combination. In the Hb E heterozygote no clinical abnormalities have been observed, but in the homozygote state even though it is not a severe condition, there may be a slight anaemia, particularly in the presence of infections (Lie-Injo, 1969) or due to a combination of mild haemolysis and a lack of erythropoetic response (Flatz, 1967). Furthermore, combination of this abnormal haemoglobin and thalassaemias lead to a more severe

anaemia. Both α and β-thalassaemias are relatively frequent in Thailand (Lie-Injo, 1969), but very little information on their distribution in Indonesia is available. Further studies, therefore, should be undertaken, particularly into the distribution of the thalassaemias in Indonesia.

G6PD deficiency, which has high frequencies in some Indonesian populations, presents another type of problem which is of potential importance. It is known that G6PD deficient subjects have an increased tendency to neonatal hyperbilirubinaemia or haemolytic anaemia due to infectious diseases (Doxiadis *et al.* 1961; Kirkman, 1971). A haemolytic reaction is also induced by ingestions of certain drugs (WHO, 1967). Of the more common of these used in malarial areas is 8-aminoquinoline (Primaquine). Since the recent spread of chloroquine-resistant strains of malarial parasites, drugs such as Primaquine have been reintroduced, creating a real hazard when used in large doses to treat G6PD deficient persons. This underlines the need for more extensive G6PD screening of the population in all parts of the country where malaria is still present, as well as improving the awareness of local medical practitioners of the dangers associated with the use of drugs likely to induce haemolytic episodes in G6PD deficient persons.

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CHEMICAL AND ENZYMOLOGICAL CHARACTERIZATION OF AN INDONESIAN VARIANT OF HUMAN ERYTHROCYTE CARBONIC ANHYDRASE II, $CAII^{Jogjakarta}$ (17 Lys→Glu)

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ABSTRACT A new variant of human erythrocyte carbonic anhydrase II (CAII) was discovered in a single heterozygous individual during routine screening of blood samples from the island of Java in Indonesia. The normal and variant components of the heterozygous CAII mixture were resolved by isoelectric focussing following purification by a specific affinity matrix. Specific esterase activities and Michaelis-Menten constants were identical. Only very small differences were noted with respect to inhibition by acetazolamide and chloride. Double diffusion analysis showed the immunological identity of the normal and variant enzymes.

The variant CAII was considerably less heat stable than the normal enzyme. The variant was slightly more stable than the normal enzyme upon dialysis against the zinc chelator dipicolinic acid (PDCA) indicating a tighter binding of zinc than the normal enzyme.

Analysis of tryptic peptides from the normal and variant enzymes indicated that, in the variant, lysine at position 17 from the N-terminus had changed to glutamic acid. The differences in physiochemical properties observed for the normal and variant enzyme are discussed in relation to the possible effects of this substitution on the structure of the CAII molecule.

KEY WORDS: Human erythrocyte carbonic anhydrase II, Amino acid substitution, Indonesian variant.

INTRODUCTION:

Carbonic anhydrase activity is now known to be expressed in humans and other placental mammals as the product of three separate genetic loci giving rise to the isozymes carbonic anhydrases I, II and III (CAI, CAII and CAIII) (Tashian, 1977; Carter et al., 1978).

Many placental mammals including man express both CAI and CAII activity in erythrocytes. Even those placental mammals like the cow and the cat which do not express CAI activity in erythrocytes do exhibit such activity elsewhere (in sections of the gastrointestinal mucosa particularly). CAIII activity is found in skeletal muscle. The close linkage of the genes for the CAI and CAII isozymes in mammals (Carter, 1972; De Simone, et al., 1973; Eicher et al., 1976) coupled with the widespread homologies in amino-acid sequence (Andersson et al., 1972; Lin and Deutsch, 1973, 1974; Hendersson, et al., 1976) and three dimensional structures (Notstrand et al., 1975) for the two human erythrocyte isozymes CAI and CAII, has served to fuel speculation that CAI and CAII activities are the product of evolutionary divergence following a gene duplication of a single ancestral form. The recent demonstration of two forms of activity in erythrocytes of the turtle (Hall and Schraer 1979) shows that this suggested duplication took place at least 300 million years ago (Tashian, et al., 1980a).

There is, as yet, no plausible rationale for the existence of three specialized isozymes of carbonic anhydrase in human tissues. One fruitful avenue towards the examination of enzyme structure/function relationships is to consider the effects of naturally occurring aminoacid substitutions. To date, about 20 electrophoretic variants of human CAI and seven variants of human CAII have been reported after

screening of haemolysates from many different human populations (Blake, 1978; Tashian et al., 1980b; Tashian and Carter, 1976). Of these, the amino-acid substitutions have been determined for nine of the CAI variants (Tashian et al., 1980b; Jones and Shaw, 1982) and one of the CAII variants (Lin and Deutsch, 1972).

Here we report the amino-acid substitution and detailed comparative properties of a new variant of CAII discovered in an Indonesian soldier from Jogjakarta during routine electrophoretic scanning. This variant is designated CAII$^{Jogjakarta-1}$ and abbreviated CAII^{Jog-1}. It is apparent that the main effects of the substitution 17 Lys→Glu lie in the markedly greater heat lability and the slightly tighter binding of zinc in the variant. It should be noted that position 17 in CAII is homologous to position 18 in CAI.

MATERIALS AND METHODS

Chemicals

The sources of the chemicals used were the same as listed previously (Jones and Shaw, 1982). In addition fluoroscein diacetate was from Sigma Chemical Co. and Staphyllococcus aureus V8 protease from Miles Laboratories.

Enzyme and Protein Assay

The esterase activity towards p-nitrophenyl acetate was measured according to the method of Osborne and Tashian (1974) as previously described (Jones and Shaw, 1982).

Protein was determined by measuring the absorbance of solutions at 280 nm. For pure samples of carbonic anhydrase II the A_{280} could be converted to mg of protein using the reported $A^{1\%}_{280}$ of 18.7 (Funakoshi and Deutsch, 1969).

Starch Gel Electrophoresis

Gel electrophoresis was performed as previously described (Jones and Shaw, 1982). After electrophoresis the gels were stained for carbonic anhydrase II activity using the specific fluorogenic substrate fluoroscein diacetate (Hopkinson et al., 1974) or for protein using 0.1% amido black.

Sodium Dodecylsulphate Polyacrylamide Electrophoresis

Sodium dodecylsulphate denatured samples of crude haemolysates, purified CAII samples and various standard proteins of known molecular weight were subjected to vertical sodium dodecylsulphate polyacrylamide electrophoresis in thin layer slabs using a 4% stacking gel and a 10% running gel, after the method originally described by Laemmli (1970).

Purification of Carbonic Anhydrase II

The normal and variant CAII isozymes were obtained from 250 ml of citrated whole blood from a single Indonesian soldier who had been identified as heterozygous for the $CAII^{Jog-1}$ variant in a previous survey. The packed cells (150 ml) were washed twice with saline and haemolysed with two volumes of distilled water. Purification was affected by affinity chromatography on an azo-sulphonamide CM-Sephadex resin (CM-Prontosil) according to the method of Osborne and Tashian (1975). CAII was also purified in a similar fashion from 160 ml of packed red blood cells from a single Caucasian blood donor of the common $CAII_1^1$ phenotype.

Column Isoelectric focussing - Separation of the normal and variant components

Twenty mg of the CAII mixture was subjected to isoelectric focussing in a sucrose stabilized gradient in an LKB 8101 double jacketed column using narrow range ampholine of pH 5-8. Focussing was allowed to proceed for 48 hours at 1200 volts and 6°C. The column was then emptied by pumping distilled water into the top using a peristaltic pump to minimize disruption of focussed zones (Righetti and Drysdale, 1976). Small fractions were collected and analysed for esterase activity, pH and absorbance at 280 nm. Peak fractions were pooled, dialysed against 0.02 M Tris H_2SO_4 pH 8.0 with two changes and concentrated to about 1.2 mg/ml. Solutions were stable for several months when kept at 4°C.

Thermostability of enzyme and Lability of Zinc Ion

These studies were performed as previously described (Jones and Shaw, 1982) using an enzyme concentration of 1.2 mg/ml.

Immunological Studies

Antibodies specific to carbonic anhydrase II were raised in rabbits using the purified Caucasian enzyme.

Double diffusion analysis (Ouchterlony, 1953) was performed in wells cut in 1% agar gels made up with phosphate buffered saline (pH 7.4)..

Peptide Mapping and Amino Acid Analysis

The treatment of the protein, the preparation of peptide maps and analysis of peptides were very similar to that reported previously (Jones and Shaw, 1982). For CAII the tryptic hydrolysate was centrifuged but there was no appreciable "core" in any case.

The purified main tryptic difference peptide from both normal and variant components was dissolved in 0.5 ml of 0.5% ammonium bicarbonate pH 8.0 and treated with 50 µg of S. aureus V8 protease at 37°C for 4 hours. Each sample was then lyophilized and the peptide fragments mapped using electrophoresis at pH 6.5 (pyridine/acetic acid/water; 100:5:895 by volume) followed by chromatography overnight in n-butanol/acetic acid/water/ pyridine (15:3:12:10 by volume).

RESULTS

Purification and Separation of Variant and Normal Carbonic Anhydrase II

Figure 1 shows the elution profile of protein from CN-

prontosil affinity matrix. The first arrow indicates the application point for the 0.4M KI buffer. A large peak of protein shown to be CAI was eluted. The second arrow shows the application point of the KCN buffer. A smaller peak of protein containing a mixture of the normal and variant CAII was eluted by this buffer. Peak fractions 77 to 87 were pooled, dialysed against 20mM Tris. H_2SO_4 pH 8.0 with two changes and concentrated in an Amicon cell to about 2mg/ml. A total of 20.6 mg of CAII protein was eluted from the affinity matrix.

Column Isoelectric Focussing

Twenty mg of the mixture of normal and variant CAII were subjected to narrow range iso-electric focussing (pH 5-8). Figure 2 shows the resolution of the variant(pI 6.8)and normal (pI 7.6) components. The small peak at pI 6.2 probably represents the minor component of the variant CAII since it does not appear in isoelectric profiles of normal CAII. The minor component of the normal CAII is masked by the much larger peak of variant protein (See Fig. 3). The protein (A_{280}) and activity profiles are quite consonant suggesting that the resolved normal and variant enzymes are indeed pure. Peak fractions for the normal and variant protein were separately pooled and dialysed with two changes against 20mM Tris H_2SO_4 buffer at pH 8.0 and concentrated to about 1.2 mg/ml. A total of 8.6 mg of variant CAII and 8.5 mg of normal CAII were recovered from the isoelectric focussing column.

Starch Electrophoresis of Normal and Variant CAII

Figure 3 shows the electrophoretic separation of the normal and variant CAII activity. Slot 1 shows the unresolved, purified mixture of CAII from the blood of the Indonesian heterozygote. Both components can be clearly seen; the normal enzyme migrating cathodally and the variant anodally. The minor component of the normal enzyme is also clearly visible in between the major normal and variant zones of activity. The minor component of the variant zone, while not clear in the photograph, may be seen in a corresponding position to the anodal side of the variant component. Slot 2 shows the normal CAII purified from a single homozygous Caucasian blood donor. The major and the minor zones of activity both correspond with the normal component of the heterozygote mixture. Slot 3 shows the normal component of the heterozygote mixture (Slot 1) after resolution by column isoelectric focussing (pI 7.6, Fig. 1) while Slot 4 shows the variant component (pI 6.8, Fig. 1). As mentioned before, the variant is slightly contaminated with the minor component of the normal zone of activity.

Sodium Dodecyl-Sulphate Polyacrylamide Gel Electrophoresis Molecular Weights under Denaturing Conditions

Figure 4 shows the electrophoresis of SDS denatured purified human normal and variant carbonic anhydrase. It is clear that the CAII samples (lanes 1-4) all have a slightly greater mobility than the human CAI sample (lane 5). The Caucasian CAII (lane 1) and the unresolved mixture from the variant heterozygote, may be very slightly contaminated with CAI protein although both isoelectrically focussed resolved components (lanes 3 and 4) appear to be quite pure even at this very high sample loading. Both normal and variant

components have the same apparent molecular weight of 28,000 dalton (Table 1) as extrapolated from a standard curve of Rf Vs. log molecular weight for the proteins bovine serum albumin, ovalbumin, human erythrocyte carbonic anhydrase I and trypsinogen.

Michaelis-Menten Kinetics

The double reciprocal plot of the variation of enzyme activity with substrate concentration for the hydrolysis of p-nitrophenyl acetate is shown in Fig 5. Clearly the normal and variant proteins behave in an identical fashion. The values for Km and Vmax derived from this plot are presented in Table 1.

Acetazoloamide Inhibition

Figure 6 shows the inhibition of both normal and variant enzymes by different concentrations of the specific carbonic anhydrase inhibitor acetazoloamide. The variant enzyme may be slightly more susceptible to inhibition than the normal component although this difference is indeed minimal. I_{50} values derived from this plot are presented in Table 1.

Chloride Inhibition

Figure 7 shows the effect of increasing concentrations of sodium chloride on the activity of the normal and variant CAII. Both enzymes are equally resistant to chloride inhibition. I_{50} values derived from this plot are presented in Table 1.

Heat Inactivation

Figure 8 shows that the variant enzyme is considerably less stable at 55°C than the normal enzyme. Half life values derived from this plot are presented in Table 1.

Lability of Zinc Ion

When normal and variant enzymes are dialysed against a solution of the potent zinc chelator, pyridine 2,6 dicarboxylic acid it is apparent that the variant enzyme is more resistant to loss of activity (Fig. 9). The results of Fig. 9 were replotted on a log/log scale and the half life values obtained by extrapolation of the linearized plots are presented in Table 1. The results are the average of three experiments and suggest that the zinc ion is bound slightly more tightly in the variant enzyme. Activity could be restored in both cases by addition of zinc or cobalt ions (Zn^{++} and Co^{++}).

Double Immunodiffusion

Figure 10 demonstrates that the variant and normal components are probably immunologically identical although at later times a faint spur was evident at the junction of the precipitin lines for the variant and Caucasian CAII. Interestingly enough the precipitin zone for the Caucasian enzyme (Well 3) has split into two components. Close examination shows that, although not clearly resolved, both components exist in the precipitin zones for the normal (Well 1) and variant (Wells 2,4) CAII components of the heterozygote.

Comparative Peptide Analysis

Figure 11 compares the tryptic peptide patterns of the CAII from a single Caucasian blood donor (a); the normal component of the Indonesian heterozygote (b) and the variant component (c). These maps were first sprayed with Fluram to visualize the peptides and the main difference peptide 1 cut from the maps as a rectangle before staining with ninhydrin for photographic purposes. A smaller "ghost" peptide (labelled α) was also cut from the maps and by amino acid analysis was shown in both normal and variant to be derived from the main difference peptide 1 possibly by partial Trp degradation. Another peptide

labelled 2 in Fig 11(b) did not seem to be present in the pattern from the variant (Fig. 11(c)). Accordingly, it was later cut from the map and analysed for amino acid content. These were the only obvious tryptic difference peptides for the normal and variant CAII.

The main tryptic difference peptide 1 was sewn into a fresh sheet of paper and remapped using electrophoresis at pH 1.9 to remove any possible contaminants. The purified difference peptides were analysed for amino acid content (Table 2). It is clear that the peptide 1 in the normal enzyme is shorter than the peptide 1 in the variant enzyme. Examination of the published amino acid sequence of human erythrocyte CAII (Henderson et al, 1976) shows that the normal tryptic peptide 1, which is missing in the variant enzyme, is the sequence from 9 His to 17 Lys (See Fig. 12). Analysis of the longer variant peptide which is not present in the normal enzyme is consistent with the composition of the sequence from 9 His to 23 Lys assuming that a lysine has changed to a glutamine or a glutamic acid (Fig. 12). Again the analyses of the variant and normal peptide 1 are perfectly consistent with the substrate specificity of trypsin assuming that 17 Lys has changed to a glutamine or a glutamic acid in the variant. Although tryptic peptide 2, which appeared in the map of the normal enzyme but not the variant, was contaminated with other peptides, amino acid analysis showed that it was the sequence from 18 Asp to 23 Lys (Fig. 12). Its absence in the variant map is again consistent with a Lys→Glx substitution at position 17.

In order to distinguish between glutamine and glutamic acid at position 17, the longer tryptic peptide 1 from the variant map was digested with the V8 protease from S. aureus at pH 8.0. This protease cleaves after glutamic acid residues at pH 8.0 (Houmard and Drapeau, 1972). The products of the digestion were lyophilized and the cleavage fragments mapped using electrophoresis at pH 6.5

and chromatography as described in the methods section. Table 3 shows an amino acid analysis of the two main cleavage products identified by fluram staining of the peptide map. V8 protease peptide 1 (G-VAR-1 in Fig. 12) gives the same composition as the tryptic peptide 2 from the normal enzyme. It is clearly the sequence from 18 Asp to 23 Lys (Fig. 12). Given the specificity of the V8 protease, lysine at position 17 in the variant enzyme must have changed to glutamic acid. The analysis of the V8 protease peptide 2 (G-VAR2 in Fig. 12) shows that the protease has also cleaved after 13 Glu in the variant tryptic peptide 1. The third fragment (Fig. 12) was not detected. Treatment of the normal tryptic peptide 1 did not result in the expected cleavage at 13 Glu, possibly because the shorter peptide was more resistant to hydrolysis.

DISCUSSION

Although seven variants of human erythrocyte CAII have so been reported after screening of haemolysates from different human populations (Blake, 1978; Tashian et al., 1980), only one of these variants has so far been chemically characterized. Lin and Deutsch (1972) found that, in the human CAII designated CAH and later $CAII_2$, which is polymorphic in many negroid populations, the asparagine residue at position 252 from the N-terminus (252 Asn) has been replaced by an aspartic acid residue. The variant enzyme had an isoelectric point which was about 0.5 units lower than the normal enzyme.

In this communication we provide the second full chemical and enzymological account of a variant human CAII - the eighth such variant described in human populations. The larger difference in isoelectric points for the normal and variant enzyme (0.8 units) may be accounted for by the amino acid

substitution identified as 17 Lys→Glu. This would result in a -2 charge change for the variant enzyme, compared with a -1 charge change in the $CAII_2$ variant (Lin and Deutsch, 1972). The substitution 17 Lys→Glu in $CAII^{Jogjakarta-1}$ is consistent with a single base change in the genetic code ($AA^{A}_{G} \rightarrow GA^{A}_{G}$). Our value for the isoelectric point of the normal component of human CAII (7.6) is slightly higher than the value of 7.36 reported by Lin and Deutsch (1972) but identical with the values we obtained for the normal Caucasian homozygous CAII and the normal component of an Australian Aboriginal CAII heterozygous mixture.

The Indonesian variant $CAII^{Jogjakarta-1}$ is clearly indistinguishable from the normal CAII with respect to its specific activity and kinetic constants. Likewise no differences were observed in the apparent molecular weight of the enzyme or its inhibition by chloride ion (Table 1). The small decrease in susceptibility towards acetazolamide inhibition in the variant is probably not significant. The main differences, seen between the normal and the variant enzyme, were the decreased heat stability (Fig. 8, Table 1) and tighter zinc binding (Fig. 9, Table 1) of the variant.

Double diffusion analysis of the variant and normal CAII against antibodies raised against normal CAII from a Caucasian blood donor showed apparent immunological identity (Fig. 10) although, at later times, when the plate was allowed to overdevelop, a faint spur appeared at the junction of the precipitin zones for the variant and normal Caucasian enzyme, suggesting the possibility of a different immunological determinant in the variant.

Since the complete amino-acid sequence (Lin and Deutsch, 1974; Henderson et al., 1976) as well as the detailed 3-dimensional structure at 2A° resolution (Lijas et al., 1972) for human carbonic anhydrase II is well known, it is possible to attempt to explain the properties of the Indonesian variant by considering the effects of a 17 Lys→Glu substitution on the structure and function of the CAII molecule.

In the normal human CAII the residue 17 Lys is hydrogen bonded to both 15 Trp and 19 Phe (all amino acids are numbered from the N-terminus of the normal CAII isozyme). Now 15 Trp and 19 Phe are both closely associated with 63 His in aromatic cluster I (Lindskog et al., 1972; Notstrand et al., 1975) which is important in the overall thermal stability of the enzyme. In addition, both 6 Tyr and 63 His are quite close to the active site and it is to be expected that pertubation in the environment of either of these residues could have appreciable effects on the function of the molecule. In particular, 63 His is very close to 95 His which, through its 3' imidazole nitrogen is one of the three histidines acting as ligands to the active centre zinc ion. It is not clear exactly how 63 His is bonded to 95 His but it is reasonable to expect that changes in the environment of 63 His could, through the interaction with 95 His, have effects on the binding of the zinc ion.

Clearly the change from a positively changed lysine at position 17 to a negatively charged glutamic acid would have quite substantial effects on the packing of aromatic cluster 1 through simple electrostatic effects and also through interference with the hydrogen bonding with 15 Trp and 19 Phe. The decreased thermal stability of the variant enzyme (Fig. 8, Table 1) is consistent with this destabilization

of aromatic cluster 1. It is also apparent that this destabilization of aromatic cluster 1 caused by the 17 Lys→Glu substitution causes the active site zinc ion to become slightly more tightly bound. It is possible that a charge transfer relay from the carboxyl of the new glutamic acid at position 17 through 63 His to 95 His could so increase the electronegativity of the 3' nitrogen of the imidazole group of 95 His as to tighten the binding of the zinc ion.

It is of interest to note that the homologous residue to 17 Lys in normal human CAII at position 18 in CAI is also lysine in all CAI isozymes sequenced to date; however, it is glutamic acid in bovine CAIII (Tashian et al., 1980a; Tashian, 1977.)

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LEGEND

FIGURE 1: Elution profile of protein from CM-Prontosil affinity column. The first vertical arrow shows the point of application of 0.4 M potassium iodide buffer. The second vertical arrow at fraction No. 60 shows the point of application of the 0.2 M potassium cyanide buffer (Osborne and Tashian, 1975). The A_{280} of material being eluted from the affinity column was continually monitored with an ISCO model UA5 Absorbance monitor.

FIGURE 2: Elution profile of protein and p-nitrophenyl acetate esterase activity of carbonic anhydrase II purified from the packed red blood cells of a single individual heterozygous for the Jogjakarta variant (20 mg protein applied).

———————— A_{280}

..... p-Nitrophenyl acetate Esterase Activity

● ● ● ● ● ● ● ● ● pH

FIGURE 3: Starch gel electrophoresis of various purified human carbonic anhydrase II fractions. Stained for activity using fluoroscein diacetate as fluorogenic substrate (Hopkinson et al., 1974).

1. Purified unresolved enzyme mixture from heterozygote.
2. Purified enzyme from single Caucasian blood donor.
3. Purified normal component from isoelectric focussing of Indonesian heterozygote (pI 7.6).
4. Purified variant component from isoelectric focussing of Indonesian heterozygote (pI 6.8).

FIGURE 4: Sodium dodecyl sulphate polyacrylamide gel electrophoresis of purified human carbonic anhydrase II. The horizontal arrow indicates the interface between the 4% stacking gel and the 10% running gel.

1. Purified Caucasian enzyme.
2. Purified Indonesian heterozygous mixture before resolution of normal and variant components.
3. Purified variant component from isoelectric focussing (pI 6.8).
4. Purified normal component from isoelectric focussing (pI 7.6).
5. Purified human erythrocyte CAI.

FIGURE 5: Double reciprocal plot for purified normal and variant carbonic anhydrase II from Indonesian hetrozygote (Substrate p-nitrophenyl acetate). Enzyme concentration 2.15×10^{-10}M in both cases.

o——o Normal component

Δ——Δ Variant component

FIGURE 6: Inhibition of the normal and variant carbonic anhydrase II by acetazolamide. Enzyme concentration: 2.15×10^{-10} Molar in both cases

o——o Normal component

Δ——Δ Variant component

FIGURE 7: Inhibition of the normal and variant carbonic anhydrase II by sodium chloride. Enzyme concentration 2.15×10^{-10} Molar in both cases.

o——o Normal component

Δ------Δ Variant component

FIGURE 8: Heat inactivation of the normal and variant carbonic anhydrase II at $55 \pm 0.1°C$. Enzyme concentration: 4.3×10^{-8} Molar in both cases.

o——o Normal component

Δ——Δ Variant component

FIGURE 9: Inhibition of the normal and variant carbonic anhydrase II by dialysis against pyridine, 2,6 dicarboxylic acid - (a potent zinc chelator). Enzyme concentration 4.3×10^{-8} Molar in both cases.

o——o Normal component of carbonic anhydrase II

Δ——Δ Variant component of carbonic anhydrase II

FIGURE 10: Double - Immunodiffusion analysis of the normal and variant carbonic anhydrase II.

Ab - Antibody raised in rabbits against the purified Caucasian carbonic anhydrase II.

1. Normal component
2. Variant component
3. Caucasian CAII (original antigen)
4. Variant component

FIGURE 11: Two dimensional separation of the tryptic peptides of human erythrocyte carbonic anhydrase II.

(a) From the erythrocytes of a single Caucasian blood donor.

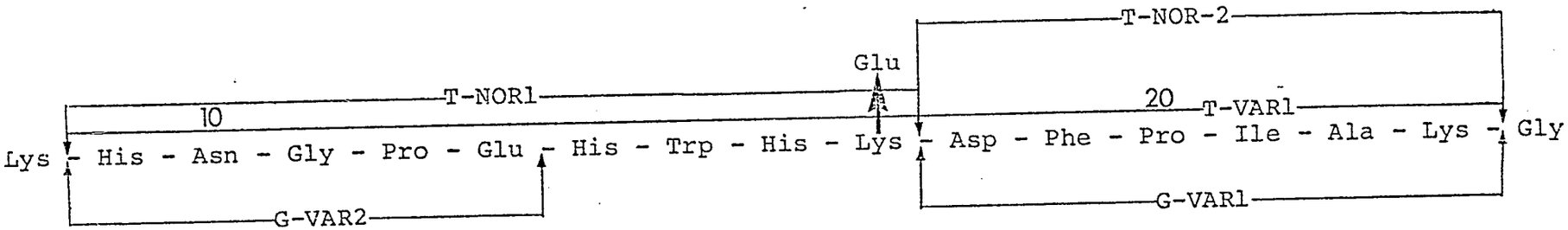
(b) The normal component from the erythrocytes of a single Indonesian heterozygote.

(c) The variant component from the erythrocytes of a single Indonesian heterozygote.

Electrophoresis was performed in pyridine/acetic acid/water (25:25:950 v/v) at pH 4.7 for 90 min. at 40 volts/cm. Ascending chromatography was performed in n-butanol/acetic acid/water/pyridine (15:3:12:10 v/v) for 16 hours. The difference peptides 1 were cut out for purification and subsequent quantitative elution prior to staining with ninhydrin.

FIGURE 12: Relevant segment of human carbonic anhydrase II sequence (Henderson et al., 1976) showing the location of the tryptic peptide fragments from the normal and variant proteins and the S. aureus V8 protease cleavage fragments of the longer variant tryptic peptide 1. The peptides were located by their amino acid analysis and a consideration of the specificities of the protease employed. The variant substitution 17 Lys→Glu was unambiguously identified by analysis of the main tryptic difference peptide 1 of both proteins (Table 2) combined with analysis of the S. aureus cleavage fragment of the longer variant tryptic peptide 1 (Table 3). The amino acids are numbered from the N-terminus of human CAII.

FIGURE 12.


T-NOR-2
Glu
T-NOR1
20
T-VAR1
10
Lys - His - Asn - Gly - Pro - Glu - His - Trp - His - Lys - Asp - Phe - Pro - Ile - Ala - Lys - Gly
G-VAR2
G-VAR1

COMPARATIVE PROPERTIES OF NORMAL AND VARIANT ERYTHROCYTE CARBONIC ANHYDRASE II

PURIFIED FROM HETEROZYGOTE

ENZYME	Specific Esterase Activity (Units/mg)	Km p-nitrophenyl acetate (mM)	Vmax p-nitrophenyl acetate (Units/mg)	I_{50} Acetazolamide Inhibition (M)	I_{50} Chloride Inhibition (mM)	Half Life $t_{\frac{1}{2}}$ at 55°C (min)	Half Life $t_{\frac{1}{2}}$ For dialysis Against PDCA (min)	Isoelectric Point pI	Apparent Molecular Weight
NORMAL	1.68	3.95	8.33	1.22×10^{-7}	310	12	10.8	7.6	28,000
VARIANT	1.78	3.95	8.33	1.04×10^{-7}	310	4.5	17.5	6.8	28,000

p-nitrophenyl acetate esterase activities were measured by the method of Osborne and Tashian (1974). Kinetic constants Km and Vmax were obtained by extrapolation of the double reciprocal plot (Fig. 5). I_{50} values were obtained for acetazolamide and chloride by extrapolation of Figs. 6 and 7 respectively. Isoelectric points were read from the profile in Fig. 2. Half life values at 55°C were extrapolated from Fig. 8. Half life values for dialysis against PDCA were obtained by extrapolation from a log/log plot of the data in Fig. 9. Apparent molecular weights were obtained by extrapolation from a graph of Rf vs log M Wt. after sodium dodecyl sulphate electrophoresis of standard proteins of known molecular weight.

TABLE 2. ANALYSIS OF MAIN TRYPTIC DIFFERENCE PEPTIDE(1) FROM VARIANT AND NORMAL CARBONIC ANHYDRASE II

Amino Acid	Normal	Variant
Aspartic Acid	1.02 (1)	2.12 (2)
Glutamic Acid	1.11 (1)	2.17 (2)
Proline	1.14 (1)	2.40 (2)
Glycine	1.20 (1)	1.17 (1)
Alanine		1.08 (1)
Isoleucine		1.02 (1)
Phenyl alanine		1.00 (1)
Histidine	2.86 (3)	2.99 (3)
Lysine	1.00 (1)	1.14 (1)
Tryptophan	+ve (1)	+ve (1)

The main tryptic difference peptides 1 were cut from the maps as shown in Fig. 11 re-electrophoresed at pH 1.9 and eluted by passage of 0.1M NH_3 as described in the methods section. Aliquots of the purified tryptic difference peptides 1 were then subjected to amino acid analysis. The results are expressed as molar ratios with the assumed integral values in parenthesis.

TABLE 3.

ANALYSIS OF STAPHYLOCOCCUS AUREUS V8 PROTEASE CLEAVAGE FRAGMENTS OF VARIANT TRYPTIC PEPTIDE-1

Amino Acid	Peptide 1	Peptide 2
Aspartic Acid	0.92 (1)	0.74 (1)
Glutamic Acid		1.00 (1)
Proline	0.89 (1)	+ve (1)[a]
Glycine		0.72 (1)
Alanine	1.01 (1)	
Isoleucine	0.93 (1)	
Phenyl Alanine	1.00 (1)	
Histidine		1.17 (1)
Lysine	1.01 (1)	

An aliquot of the purified tryptic difference peptide 1 from the variant enzyme was treated with the Staphylococcus aureus V8 protease (50:1, 37°C, 4hrs) and the cleavage fragments mapped using chromatography at pH 6.5 followed by electrophoresis as indicated in the methods section. The two main fragments 1(G-VAR-1 in Fig 12) and 2 (G-VAR-2 in Fig. 12) were subjected to amino acid analysis and the results expressed as molar ratios. Values in parenthesis are the assumed integral values.

[a] This analysis was performed at the 3 n.mole level and, although proline was clearly present, the analyser failed to give an integrated value.