

Towards understanding the origin and dispersal of Austronesians in the Solomon Sea: HLA class II polymorphism in eight distinct populations of Asia-Oceania

H. Zimdahl,* W. Schiefenhövel,† M. Kayser,‡ L. Roewer§ & M. Nagy§

Summary

HLA class II nucleotide sequence polymorphisms were examined in eight ethnic groups of Asia-Oceania using DNA typing methods. Allele frequencies and characteristic DR/DQ haplotypes were determined and compared with those of other populations of Asia-Oceania. Genetic distances were measured to show the genetic relationship within the studied populations as well as between the studied populations and previously published populations. Phylogenetic trees were constructed based on HLA allele frequencies using the neighbour-joining method. The populations, mainly Trobriand Islanders, Roro, Tolai, Western Samoans and Taiwanese Aborigines, are characterized by a reduced diversity at the HLA loci examined, especially for DPB1. The high frequency of the 'Asian'-specific DPB1*0501 allele in Trobrianders and Roro, but also in Western Samoans and Taiwanese Aborigines, was the most striking result. The prevalence of DPB1*0501 and the short genetic distance from Trobriander and Roro to Taiwanese Aborigines provide evidence that the origin of the Austronesian odyssey is south-east Asia, and Taiwan could be an important part of it. The relatedness of Trobrianders to the Polynesian population from Western Samoa indicates a probable recent common ancestor. The observed lack of diversity may reflect bottleneck(s) and/or limited diversity of the founding population. Analysis of HLA class I antigens, together with mt-DNA and Y-chromosomal studies, will give us further information about the settlement of the Trobriand and other islands during the colonization of the Pacific.

Introduction

The colonization of Oceania has been the subject of a large number of studies in recent years, with linguistic, archaeo-

logical and anthropological evidence being accumulated, and has lately attracted the attention of genetics.

It has been assumed that the human settlement of this vast area of the world occurred in two major migratory stages. The first began \approx 50 000 years ago, with the expansion of human populations resulting in the occupation of Australia and New Guinea, which together formed a landmass called Sahul in that period. A more recent wave of human migration began \approx 5000 years ago: groups of skilful sailors with advanced voyaging technology left southern China and Taiwan and migrated via the Philippines and Indonesia to Melanesia. This scenario is primarily based on linguistic studies and archaeological evidence (Bellwood, 1989). The populations living in this area have been divided into two distinct linguistic groups: non-Austronesian language speakers (NAN) and Austronesian language speakers (AN). In Melanesia, AN and NAN (Papuan speakers) groups are found side by side. The Papuan speakers (NAN) are thought to represent an old stratum of population, mainly because of the extraordinary diversity of the Papuan languages (Wurm, 1983), and to have spread throughout most of New Guinea, with the exception of some northern and south-eastern coastal patches. Austronesian language speakers in Melanesia live mainly on certain islands in the north and east of New Guinea and the above-mentioned coastal patches, and these languages are also spoken by people living as far away as Taiwan, Easter Islands, New Zealand and Madagascar, and in many densely populated areas such as Malaysia, Indonesia and the Philippines (Tryon, 1995). There is linguistic, archaeological, cultural and genetic evidence available suggesting that the origin of Austronesians is in south-east Asia. Some of these studies have indicated a rapid eastward dispersal of Austronesian-speaking migrants who then became the ancestors of modern Polynesians (Diamond, 1988; Serjeantson, 1989; Melton *et al.*, 1995; Redd *et al.*, 1995).

Studies on HLA serology and also at the DNA level have been carried out in different populations of Oceania (Gao & Serjeantson, 1992; Gao *et al.*, 1992a; Imanishi *et al.*, 1992a,b; Cavalli-Sforza *et al.*, 1994; Yoshida *et al.*, 1995). In the present work, we investigated class II antigens at the DNA level in eight well-defined unrelated ethnic groups of Asia-Oceania, in particular Austronesian groups in Melanesia, to trace back their origin and migratory routes.

*Max-Planck-Institut für Molekulare Genetik, Ihnestr. 73, 14195 Berlin, Germany, †Forschungsstelle für Humanethnologie, Max-Planck-Gesellschaft, Erling-Andechs, Germany, ‡Department of Anthropology, The Pennsylvania State University, USA, and §Institut für Rechtsmedizin, Humboldt Universität, 10115 Berlin, Hannoversche Str.6, Germany.

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Correspondence: Marion Nagy, Institut für Rechtsmedizin, Humboldt Universität, 10115 Berlin, Hannoversche Str.6, Germany.

We were particularly interested in the genetic relationship of three distinct cultural and linguistic groups within the Austronesian populations.

- 1 The Trobriand Islanders live on certain islands in the Solomon Sea east of Papua New Guinea, and represent a typical Austronesian population with matrilinear descent rules, powerful male chiefs and an exchange system of ceremonial valuables involving open sea voyaging.
- 2 The Tolai are inhabitants of northernmost New Britain around the town of Rabaul, and have a moiety system: everybody belongs to one of the two matrilinear 'halves' of the community which are mutually exogamous. Trobrianders and Tolai belong to the same linguistic subgroup.
- 3 The Roro are one of the distinct ethnic groups in the Central Province of the south-western New Guinea lowland and on Yule Island, belonging linguistically to a second subgroup with patrilinear descent, like the Papuan populations, with a moiety system, powerful chiefs and pottery tradition.

To compare the different population groups in Melanesia — Austronesians and Papuans — we analysed one population from the Papua New Guinean Highlands (PNGH). We also included Austronesian-speaking populations living outside Melanesia, taking samples from Western Samoa and Taiwan and two Malayo-Polynesian populations from Java and south Borneo, in order to compare these populations with AN-speaking Melanesians.

The HLA system is one of the most polymorphic genetic systems. In particular, the class II region consists of a family of highly polymorphic genes. The continual discovery of new HLA alleles using DNA technology increases the power of HLA to distinguish individuals; for example, the HLA-DRB1 locus, with 184 alleles identified by 1996, occurs on all haplotypes (Kappes & Strominger, 1988; Bodmer *et al.*, 1997). The HLA class II genes are strongly associated with each other. Particular combinations of neighbouring alleles (haplotypes) have characteristic frequencies in certain ethnic groups. Thus, HLA variability at the level of DNA is useful in unravelling the evolutionary relationships between populations and in investigating the evolutionary forces which shaped the genetic profiles of contemporary populations (Imanishi *et al.*, 1992b).

Materials and methods

Population samples

A total of 333 samples from east Asia-Oceania were examined for the distribution of HLA class II alleles: 81 samples from the Trobriand Islands (AN); 26 from Roro, on the south coast of Papua New Guinea (AN); 48 from Tolai (AN) in east New Britain; 22 from Western Samoa (AN); 28 from the Papua New Guinea Highlands (NAN); 59 from Java, from a rural area near Jakarta (AN); 21 from south Borneo, from the town of Bandjarmasin (AN), and 48 from Taiwan, from aboriginals from four tribes:

Ami, Atayal, Bunun and Paiwan (AN). The geographical locations of the populations studied are shown in Fig. 1.

DNA preparation

Genomic DNA was prepared from blood samples using the routine method of phenol/chloroform extraction after digestion with proteinase K (Sambrook *et al.*, 1989).

HLA DNA typing

The SSO-PCR technique was used to type the HLA class II loci DRB1, DQA1, DQB1 and DPB1 according to the 12th International Histocompatibility Workshop protocol (Kimura & Sasazuki, 1992). The exon 2 amplification of DRB1, DQA1, DQB1 and DPB1 was carried out in a PTC100™ Thermocycler (MJ Research, Inc., USA) using Taq-Polymerase (Perkin Elmer, USA) for 30 cycles after 4 min denaturation at 94 °C. The cycle conditions were as follows: 94 °C for 30 s, 55 °C (DRB generic)/57 °C (DRB1 group specific and DQB1)/54 °C (DQA1)/58 °C (DPB1) for 30 s and 72 °C for 1 min 30 s, and a final elongation step of 72 °C for 10 min. The DRB primers used for the generic amplification were 2DRBAMP-A for the 5' region and DRB-AMPB for the 3' region. In the group-specific amplification, the 2DRBAMP-A primer was replaced by 2DRBAMP-2, 2DRBAMP-3 or 2DRBAMP-4. Primers 2DQAAMP-A and 2DQAAMP-B were used for the exon 2 region of DQA1 and primers 2DQBAMP-A and 2DQBAMP-B were used to amplify the polymorphic region of exon 2 of DQB1, and 2DPBAMP-A and 2DPBAMP-B for the DPB1 amplification. Dot-blot labelling hybridization using DIG ddUTP labelling and TMAC hybridization was performed according to the 12th International Histocompatibility Workshop protocols. The DIG-labelled hybrids were detected by the enzyme-linked immunoassay following the protocol of the supplier (Boehringer) and visualized by colour development for alkaline phosphatase. Well typed DNA samples were used as controls. All amplification primers and oligonucleotide probes were synthesized according to the 11th/12th IHW technical handbook produced by TIB MOL-BIOL, Berlin, Germany.

We also used the reverse dot-blot technique, PCR-SSP (ARMS-PCR) and sequence-based typing (SBT) in order to confirm the typing results, especially of rare alleles.

For the reverse dot-blot, amplified biotinylated denatured DNA samples were hybridized with specific oligonucleotide probes immobilized as parallel lines on membrane-based strips following the protocol of the INNO-LiPA HLA typing kit (Buyse *et al.*, 1993) (Innogenetics, Zwijndrecht, Belgium). The HLA class II alleles were interpreted using the software of the INNO-LiPA expert program (V4.0)

The PCR-SSP typing was carried out to determine, in some cases, HLA DRB1*04, 15/16, 08/11/14 subtypes according to the protocol of the DYNAL SSP typing kit (DYNAL, Oslo, Norway).

For direct sequencing analysis of HLA class II alleles,

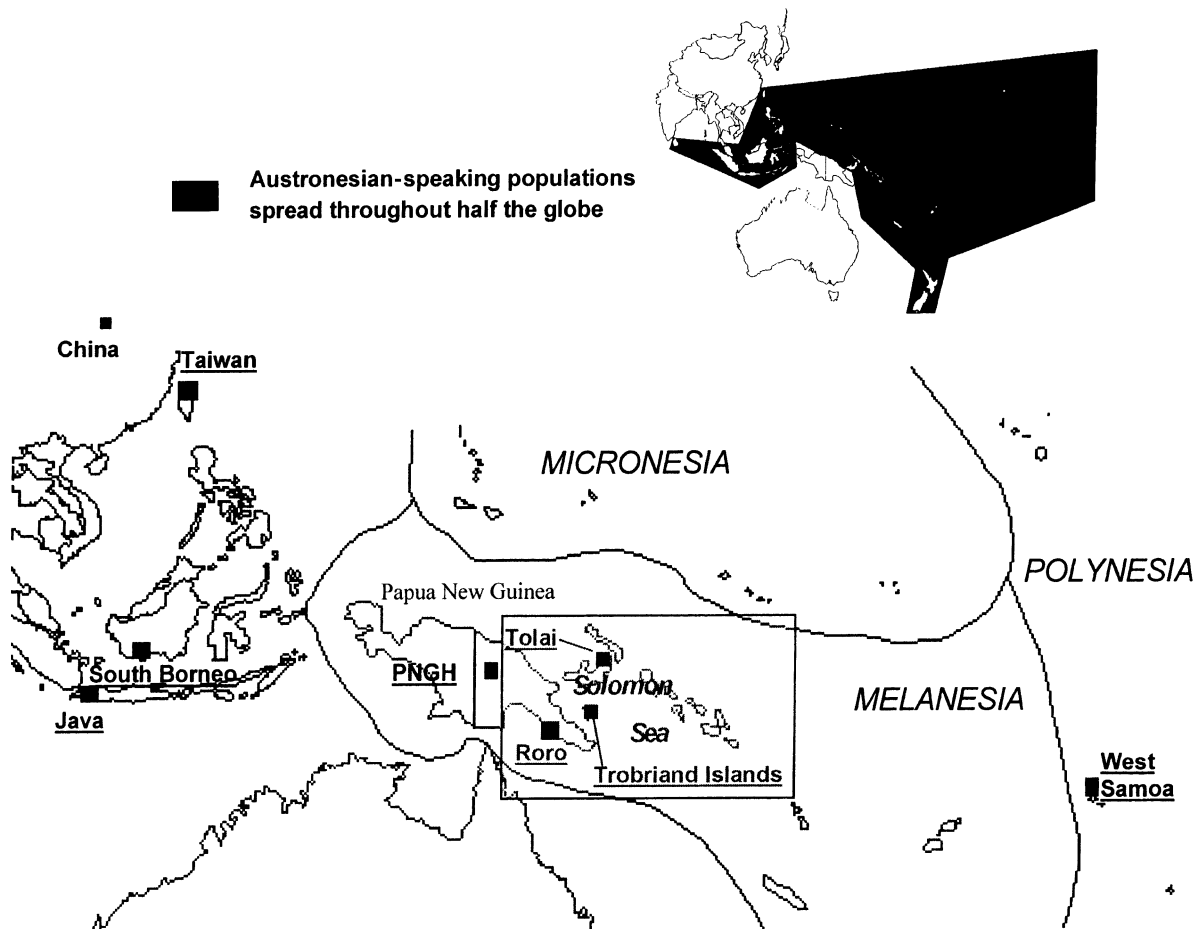


Figure 1. Geographical localization of populations studied (underlined).

the generic and group-specific amplification of the DRB1 gene and the amplification of the DQB1 and DPB1 region were performed as described for PCR-SSO typing. The purification of PCR products by spin-column chromatography was performed according to the user manual (MicroSpin™ S-400 HR Column, Pharmacia, Sweden). The purified double-stranded PCR product was sequenced by cycle sequencing using the SequiTherm™ cycle sequencing kit (Epicentre Technologies, Madison, USA). All sequencing primers were Cy5-labelled primers, synthesized by TIB MOL-BIOL (Berlin, Germany). All group-specific DRB1 PCR products were sequenced with the same reverse primer AMPC-Cy5 (5′Cy5-AAC CCC GTA GTT GTG TCT GCA-3′). In addition, the generic DRB PCR products were analysed with the following relevant group-specific forward sequencing primers: 1002Cy5 (5′Cy5-GGC AGC CTA AGA GGG AGT G-3′), 1003Cy5 (5′Cy5-GTA CTC TAC GTC TGA GTG TCA-3′), 8/12/14Cy5 (5′Cy5-GAG TAC TCT ACG GGT GAG TG-3′) and 1004Cy5 (5′Cy5-GAG CAG GTT AAA CAT GAG TGT-3′). Cycle sequencing of DQB1 alleles was performed using the DQB14-Cy5 forward primer (5′Cy5-TGT GCT ACT TCA CCA ACG-3′) and for the

DPB1 SBT the DPB13-Cy5 forward primer (5′Cy5-CAG GAA TGC TAC GCG TTT AAT G-3′) was used. The cycle sequencing comprised 35 cycles: 94 °C for 30 s, 57 °C for 30 s (for the DQB primer: 50 °C for 30 s), 72 °C for 60 s and a final elongation step at 72 °C for 10 min. The reaction samples were electrophoretically separated using the A.L.F. express™ Automated DNA Sequencer (Pharmacia, Sweden) and fluorescence signals were processed by the program 'A.L.F. Manager (AM V 3.02)'.

Statistics

Allele frequencies f were obtained by direct counting. Unbiased estimates of expected heterozygote frequencies (H) given in Tables 1 and 2 were computed as $n[1 - \sum_{i=1}^k (n_i/n)^2]/(n-1)$, where n_1 , n_2 and n_k are the number of k alleles at a locus in a sample of n genes drawn from the population (Edwards *et al.*, 1992). Standard errors for allele frequencies and heterozygosity given in Tables 1 and 2 were computed as the square root of the variance of a binomial distribution $f(1-f)/2N$ and $f(1-f)/N$, where f represents the frequency and N the number of typed individuals (Edwards *et al.*, 1992). The Hardy-Weinberg

Table 1. HLA-DRB1 allele frequencies, heterozygosities (*H*) and standard error (%) in studied populations of Asia-Oceania

DRB1 allele	Trob (<i>N</i> = 81)	Roro (<i>N</i> = 26)	Tolai (<i>N</i> = 48)	W. Samoa (<i>N</i> = 22)	S. Borneo (<i>N</i> = 21)	Java (<i>N</i> = 59)	PNGH (<i>N</i> = 28)	Taiwan (<i>N</i> = 48)
0101	0.0	0.0	0.0	0.0	0.0	0.8 ± 0.8	0.0	0.0
1501	0.0	0.0	1.1 ± 1.1	2.3 ± 2.2	16.7 ± 5.7	1.7 ± 1.2	1.8 ± 1.7	6.2 ± 2.4
15021	8.0 ± 2.1	23.1 ± 5.8	12.0 ± 3.4	2.3 ± 2.2	14.3 ± 5.4	13.7 ± 3.1	14.3 ± 4.7	9.4 ± 2.9
1601	0.0	0.0	0.0	0.0	4.7 ± 3.3	0.0	1.8 ± 1.7	0.0
1602	3.7 ± 1.5	23.1 ± 5.8	4.3 ± 2.1	0.0	7.1 ± 3.9	1.7 ± 1.2	1.8 ± 1.7	1.0 ± 1.0
0301	0.0	0.0	0.0	0.0	2.4 ± 2.4	2.5 ± 1.4	0.0	0.0
0401	1.2 ± 0.8	1.9 ± 1.9	0.0	2.3 ± 2.2	0.0	0.0	0.0	0.0
0403	12.4 ± 2.6	9.6 ± 4.1	2.2 ± 1.5	11.3 ± 4.8	0.0	0.8 ± 0.8	0.0	9.4 ± 2.9
0404	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.5 ± 3.4
0405	5.0 ± 1.7	3.9 ± 2.6	6.5 ± 2.6	0.0	2.4 ± 2.4	2.5 ± 1.4	16.1 ± 4.9	6.2 ± 2.4
0406	0.0	0.0	0.0	0.0	0.0	1.7 ± 1.2	0.0	0.0
0408	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0 ± 1.0
0410	0.0	0.0	0.0	0.0	0.0	0.0	3.5 ± 2.4	0.0
0411	0.0	1.9 ± 1.9	0.0	0.0	0.0	0.0	0.0	0.0
1101	6.2 ± 1.9	1.9 ± 1.9	54.3 ± 5.2	9.1 ± 4.3	0.0	1.7 ± 1.2	0.0	10.4 ± 3.1
1106	0.0	0.0	0.0	0.0	0.0	0.8 ± 0.8	0.0	0.0
1201	0.6 ± 0.6	3.9 ± 2.6	0.0	2.3 ± 2.2	4.7 ± 3.3	0.0	14.3 ± 4.7	3.1 ± 1.8
1202	1.8 ± 1.0	0.0	0.0	0.0	38.1 ± 7.5	53.4 ± 4.6	0.0	12.5 ± 3.4
13	0.0	0.0	0.0	2.3 ± 2.2	2.4 ± 2.4	1.7 ± 1.2	0.0	0.0
1401	7.4 ± 2.0	3.9 ± 2.6	15.2 ± 3.7	0.0	0.0	0.0	3.5 ± 2.4	10.4 ± 3.1
1404	1.8 ± 1.0	0.0	0.0	0.0	0.0	3.4 ± 1.7	0.0	0.0
1405	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1 ± 1.8
1407	0.6 ± 0.6	3.9 ± 2.6	1.1 ± 1.1	0.0	0.0	0.0	5.4 ± 3.0	3.1 ± 1.8
1408	0.6 ± 0.6	0.0	0.0	9.1 ± 4.3	0.0	0.0	26.8 ± 5.9	0.0
0701	0.0	0.0	0.0	2.3 ± 2.2	2.4 ± 2.4	8.5 ± 2.5	0.0	0.0
08032	32.8 ± 3.7	9.6 ± 4.1	3.3 ± 1.8	11.3 ± 4.8	2.4 ± 2.4	1.7 ± 1.2	10.7 ± 4.1	7.3 ± 2.6
0901	17.9 ± 3.0	13.5 ± 4.7	0.0	45.4 ± 7.5	0.0	1.7 ± 1.2	0.0	4.2 ± 2.0
1001	0.0	0.0	0.0	0.0	2.4 ± 2.4	1.7 ± 1.2	0.0	0.0
<i>H</i> ^{observed}	0.84	0.88	0.69	0.73	0.62	0.66	0.76	0.71
<i>H</i> ^{expected}	0.83 ± 0.04	0.86 ± 0.07	0.66 ± 0.07	0.76 ± 0.09	0.81 ± 0.09	0.69 ± 0.06	0.86 ± 0.06	0.92 ± 0.04

N = number of typed unrelated individuals.

H = Heterozygosity rate (Edwards *et al.*, 1992).

Trob = Trobriand Islanders; Roro = Papua New Guinean Lowlanders; Tolai = population from East New Britain; W. Samoa = population from Western Samoa; PNGH = Papua New Guinean Highlander; Java = Javanese; S. Borneo = population from south Borneo; Taiwan = Taiwanese; in bold: the most frequent allele.

analysis was performed for the observed genotypes to assess the inbreeding status of a population. The chi-square test was used to determine whether there was a significant deviation from Hardy–Weinberg equilibrium. The haplotype frequencies and linkage disequilibrium values for two-locus haplotypes (HLA DRB1 – HLA DQB1) were computed using 2 × 2 contingency tables according to the formulae of Mattiuz *et al.* (1970).

The minimum sample size requirement to represent all alleles in a sample for a *k*-allelic codominant locus with a given level of confidence 1 – α was tested according to Chakraborty (1992). The following inequality was used: $n \geq \ln[1 - (1 - \alpha)^{1/r}] / 2\ln(1 - p)$, with *n* as the minimum number of individuals needed to have *r* alleles with frequency *p* or above represented in the sample.

Allele frequencies were used to measure differences between populations and to compare the results with other populations. Nei's genetic distance was computed between each pair of populations Nei (1972). Phylogenetic trees (dendrograms) were constructed from genetic distances by using the neighbour-joining (Saitou & Nei,

1987)/UPGMA method (Sokal & Michener, 1958). Bootstrapping was performed using 1000 replicates to assess the reliability of individual branches. Genetic distance calculations, the construction of phylogenetic trees and bootstrapping were carried out using the program PHYLIP version 3.57c (Felsenstein, 1989).

Results

In this study we investigated the polymorphism of HLA class II loci in eight ethnic groups of Asia-Oceania (333 samples). All populations were analysed for HLA-DRB1 and DPB1. In addition, depending on the DNA quantity, five populations (247 samples) were studied for HLA-DQB1, DRB1-DQB1 haplotypes and linkage disequilibria and only one population (Trobriand Islanders) was analysed for DQA1. No gross discordance of genotype frequencies from their Hardy–Weinberg expectations could be detected, with the exception of the HLA-DRB1 data from the three samples from south Borneo, Papua New Guinea Highlands and Taiwan.

Table 2. HLA-DPB1 allele frequencies, heterozygosities (*H*) and standard error (%) in studied populations of Asia-Oceania

DPB1 allele	Trob (<i>N</i> = 81)	Roro (<i>N</i> = 26)	Tolai (<i>N</i> = 48)	W. Samoa (<i>N</i> = 22)	S. Borneo (<i>N</i> = 21)	Java (<i>N</i> = 59)	PNGH (<i>N</i> = 28)	Taiwan (<i>N</i> = 48)
0101	0.6 ± 0.6	1.9 ± 1.9	1.1 ± 1.1	0.0	2.4 ± 2.4	2.6 ± 1.4	1.8 ± 1.8	8.3 ± 2.8
0201	0.6 ± 0.6	9.8 ± 4.1	56.5 ± 5.2	2.3 ± 2.3	2.4 ± 2.4	2.6 ± 1.4	26.8 ± 5.9	5.2 ± 2.2
0202	0.0	0.0	1.1 ± 1.1	0.0	0.0	7.6 ± 2.4	1.8 ± 1.8	0.0
0301	0.0	0.0	0.0	2.3 ± 2.3	2.4 ± 2.4	5.1 ± 2.0	7.1 ± 3.4	2.1 ± 1.4
0401	1.2 ± 0.8	11.7 ± 4.4	15.2 ± 3.7	20.4 ± 6.1	16.7 ± 5.7	21.2 ± 3.7	39.3 ± 6.5	0.0
0402	0.0	0.0	0.0	0.0	4.7 ± 3.2	4.2 ± 1.8	0.0	0.0
0501	97.6 ± 1.2	72.1 ± 6.3	27.2 ± 4.6	70.4 ± 6.9	14.3 ± 5.4	16.1 ± 3.4	12.5 ± 4.4	69.8 ± 4.7
0901	0.0	0.0	0.0	0.0	0.0	1.7 ± 1.2	0.0	0.0
1301	0.0	0.0	0.0	0.0	42.9 ± 7.6	19.5 ± 3.6	0.0	2.1 ± 1.4
1401	0.0	1.9 ± 1.9	0.0	0.0	0.0	1.7 ± 1.2	5.3 ± 3.0	11.5 ± 3.2
1501	0.0	0.0	0.0	0.0	0.0	0.8 ± 0.8	0.0	0.0
1701	0.0	0.0	0.0	0.0	0.0	0.8 ± 0.8	1.8 ± 1.8	0.0
1901	0.0	0.0	0.0	0.0	0.0	0.8 ± 0.8	0.0	0.0
2201	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0 ± 1.0
2401	0.0	0.0	0.0	0.0	0.0	0.0	1.8 ± 1.8	0.0
2501	0.0	0.0	0.0	0.0	0.0	0.0	1.8 ± 1.8	0.0
26012	0.0	0.0	0.0	0.0	2.4 ± 2.4	1.7 ± 1.2	0.0	0.0
2801	0.0	0.0	0.0	0.0	7.1 ± 3.9	11.0 ± 2.9	0.0	0.0
3101	0.0	1.9 ± 1.9	0.0	0.0	0.0	2.6 ± 1.4	0.0	0.0
4801	0.0	0.0	0.0	2.3 ± 2.3	0.0	0.0	0.0	0.0
4901	0.0	0.0	0.0	0.0	4.7 ± 3.2	0.0	0.0	0.0
5101	0.0	0.0	0.0	2.3 ± 2.3	0.0	0.0	0.0	0.0
<i>H</i> ^{observed}	0.04	0.44	0.68	0.33	0.81	0.83	0.72	0.44
<i>H</i> ^{expected}	0.04 ± 0.02	0.46 ± 0.09	0.60 ± 0.07	0.42 ± 0.14	0.78 ± 0.09	0.87 ± 0.04	0.77 ± 0.08	0.49 ± 0.07

N = number of typed unrelated individuals.

H = Heterozygosity rate (Edwards *et al.*, 1992).

Trob = Trobriand Islanders; Roro = Papua New Guinean Lowlanders; Tolai = population from East New Britain;

W. Samoa = population from Western Samoa; PNGH = Papua New Guinean Highlander; Java = Javanese; S. Borneo = population from south Borneo; Taiwan = Taiwanese; in bold: the most frequent allele.

Distribution of HLA-DRB1 alleles

The populations examined showed relatively low DRB1 diversity, with heterozygosity rates of between 0.62 and 0.88 (in comparison, German population 0.91), shown in Table 1.

Marked differences between AN populations from Melanesia and Polynesia and the Indonesian populations, including the Taiwanese population, are indicated by the remarkably high frequencies of DRB1*1202 in populations from Java (53.4%), south Borneo (38.1%) and Taiwan (12.5%), and the low frequency in the Trobrianders (1.8%). DRB1*1202 was absent in the Roro, Tolai and the Polynesian Western Samoa samples. A high frequency of the DRB1*1408 allele (26.8%) was observed in the Papuan NAN speakers (PNG Highlanders) and in the AN-speaking Polynesian population from Western Samoa (9.1%), and a very low frequency in Trobrianders (0.8%). The DRB1*1201 allele was observed in PNG Highlanders at 14.3% and was infrequent in Trobrianders (0.6%) and Roro (3.9%) and absent in Tolai. Among the Austronesians, the DRB1*08032 allele was detected in the Trobriand sample at a high frequency of 32.8%, and at moderate frequencies in Polynesians (11.3%), Roro (9.6%) and Taiwanese (7.3%). The most common subtypes in the DR4 group were DRB1*0403 in Trobri-

anders, Roro and Polynesians (Western Samoa) and DRB1*0405 in PNG Highlanders (16.1%) and Tolai (6.5%). DRB1*0404 was only present in the Taiwanese population (12.5%). DRB1*0410 was only found in PNG Highlanders (3.5%). The distribution of the DRB1*0901 allele, most frequent in Orientals (Hashimoto *et al.*, 1994), differed amongst the Austronesians examined: a high frequency was observed in Polynesians (45.4%), and a lower frequency in the Trobrianders (17.9%), the Roro (13.5%) and Taiwanese (4.2%). In the Tolai population, the population from Borneo, and the Papuan-speaking PNG Highlanders, DRB1*0901 was absent. One remarkable feature of the Tolai population is the prevalence of the DRB1*1101 allele (54.3%). DRB1*1101 was not detectable in PNG Highlanders or in the population from south Borneo. The most common DRB1* alleles observed in the Roro were the DRB1*1502 and DRB1*1602 alleles.

Distribution of HLA-DPB1 alleles

The DPB1 allele frequencies are shown in Table 2. The Austronesian populations round the Solomon Sea analysed were characterized by significantly reduced DPB1 diversity, with a heterozygosity between 4 and 68% (in contrast, heterozygosity is 83% in the Javanese and 77%

Table 3. Most frequent haplotypes with linkage disequilibrium in studied populations of Asia-Oceania

Trobriander (<i>N</i> = 74)			Roro (<i>N</i> = 26)			Javanese (<i>N</i> = 59)			Tolai (<i>N</i> = 46)			Taiwan (<i>N</i> = 42)		
DRB1-DQB1	HF	<i>D</i>	DRB1-DQB1	HF	<i>D</i>	DRB1-DQB1	HF	<i>D</i>	DRB1-DQB1	HF	<i>D</i>	DRB1-DQB1	HF	<i>D</i>
08032-0601	34.2	14.0	1602-05	23.1	9.0	1202-0301	50.0	19.0	1101-0301	54.3	21.4	1202-0301	14.3	6.3
0901-0303	12.5	5.9	0901-03	11.6	4.7	15021-0501	11.0	5.0	1502-0601	12.0	5.7	1101-0301	11.9	5.2
0403-0302	8.6	4.4	1502-0601	11.6	4.4	0701-02	8.5	4.0	1401-05031	7.6	3.5	0403-0302	9.5	4.1
1502-0601	7.9	2.8	1502-05	11.6	1.0				0405-0402	6.5	3.2	0404-0302	8.3	3.8
1101-0301	5.9	2.9	0403-03	9.6	4.2				1401-0502	6.5	2.8	0803-06011	8.3	4.0
1401-05031	5.3	2.5	0803-0601	7.7	3.5							1401-0502	8.3	4.0
												1501-0602	7.1	3.5

HF: haplotype frequency (%) (HF < 5% is not listed); *N*: number of individuals; *D*: delta value multiplied by 100.

in the German populations). The most striking feature was the prevalence of the DPB1*0501 allele in most of the Austronesian samples, especially in Trobrianders (97.6%), followed by the Roro (72.1%), Polynesians from Western Samoa (70.4%) and Taiwanese (69.8%). Among the Tolai, the most frequent DPB1 allele was the 0201 allele (56.5%). DPB1*0201 was also relatively frequent in PNG Highlanders (26.8%). DPB1*0401 was the most frequent allele found in the Highland Papuan population (39.3%) and in Javanese (21.2%). The DPB1*1301 allele was only detected with a frequency of 19.5% in Javanese and in the population from south Borneo at a very high frequency of 42.9%.

Distribution of HLA-DQA1 alleles

The following allele frequencies were detected in the Trobriand sample: DQA1*0101/0104, $9.3 \pm 2.8\%$; DQA1*0102, $14.7 \pm 3.3\%$; DQA1*0103, $34.8 \pm 4.5\%$; DQA1*02, $0.9 \pm 0.9\%$; DQA1*03, $32.0 \pm 4.4\%$; DQA1*0501, $7.4 \pm 2.5\%$ and DQA1*0601, $0.9 \pm 0.9\%$.

DRB1-DQB1 haplotypes and linkage disequilibrium

Because family studies could only be performed in the Trobriand sample, the associations between different HLA loci in all other populations studied were estimated. The most probable DRB1-DQB1 haplotypes and delta values are listed in Table 3. The observed haplotypes were similar to those of other Oceanian populations from Melanesia and Micronesia (Serjeantson & Gao, 1995), but rarely seen in other ethnic groups. The strongly associated DRB1*08032-DQB1*0601 haplotype was found at a high frequency in Trobrianders (34.2%). The other Melanesians (Roro and Tolai) and Taiwanese examined showed a reduced frequency of this haplotype and in the Javanese this haplotype was not observed. The most common haplotype in Roro was DRB1*1602-DQB1*05. In Tolai DRB1*1101 showed a very strong association with DQB1*0301 with a high frequency of 54.3%. In PNG Highlanders this haplotype was observed less frequently (Gao *et al.*, 1992a). DRB1*1202 was strongly associated with DQB1*0301 in the Javanese examined (50%), confirming the high frequencies found in Micronesians and other Javanese.

Phylogenetic trees

Genetic distances were computed between all examined populations and relevant previously published populations using allele frequencies from the DRB1 and DPB1 loci alone (Tables 4 and 5) and from both class II loci (data not shown). The results demonstrated the shortest genetic distances for DRB1 (0.23 and 0.27) and DPB1 (0.02 and 0.006) between the population of the Trobriand islands and aboriginal populations in Australia and Taiwanese, in particular the Atayal. The Trobriander were also found to be genetically close to the Polynesian populations of Western Samoa and Raratonga (for DRB1, 0.40 and 0.59;

for DPB1, 0.40). In contrast, New Guinea Lowlanders and other Melanesian populations in the Solomon Sea, such as the Tolai and the populations from Madang and Rabaul, had a large genetic distance to the Trobriand population (0.88–1.32 for DRB1 and 0.82 for DPB1). Nevertheless, calculation of the genetic distances (around 0.4) also revealed a relatively strong genetic relationship amongst the examined populations of the Trobriand Islanders and the Roro (DRB1 0.47 and DPB1 0.02). Consistent with their linguistic differences, the Papuan population from the New Guinea Highlands differed genetically from the Austronesian populations. A phylogenetic tree constructed from Nei's genetic distance using the neighbour-joining method (Saitou & Nei, 1987) is shown in Fig. 2. The support by bootstrap tests for the tree with the neighbour-joining method was significantly higher than with the UPGMA method.

Trobriander, Roro and Polynesians from Western Samoa were separated by relatively short genetic distances — this may indicate a recent common ancestor. The examined Malayo-Polynesians from Java and south Borneo seem to be genetically remote from the other AN-speaking populations. They formed a clear-cut 'Indonesian' cluster. Trobrianders, Roro and Polynesians were separated by a short distance from the Taiwanese Aborigines included in this study.

The minimum sample size requirements according to Chakraborty (1992) are given in Table 6.

Discussion

The investigation of HLA-DRB1 and DPB1 allele distribution in eight populations of Asia-Oceania as well as the DRB1-DQB1 haplotype frequencies and linkage disequilibrium in five populations revealed restricted genetic diversity.

The number of DRB1 alleles in the examined ethnic groups varied between nine alleles (Tolai) and 17 alleles (Javanese). Analysis of the DPB1 locus revealed a significantly reduced number of DPB1 alleles, especially in the AN populations from Melanesia. We found four DPB1 alleles and a very low heterozygosity of 5% in Trobriand Islanders, five alleles in Tolai and six alleles in Roro. This low degree of HLA diversity could be evidence of the geographical and cultural isolation of these populations, especially the Trobriand Islanders. This is consistent with our analysis of genealogical data from 150 individuals, of whom only five stated that not more than one parent or grandparent originated from outside the Trobriand Islands. This low degree of diversity was confirmed by the analysis of the DRB1-DQB1 haplotypes, which revealed a low number of distinct haplotypes.

Distribution of DRB1 alleles and DRB1-DQB1 haplotypes

Only two DR2-related DRB1-DQB1 haplotypes were observed in Trobriand Islanders, in contrast to nine distinct DR2-related haplotypes found in southern Chinese (Serjeantson & Gao, 1995). The most common haplotype

Table 4. Nei's genetic distance estimated from DRB1 allele frequencies in 21 presently studied and relevant previously published populations of Asia-Oceania

	Trob (N = 81)	Roro (N = 26)	Gidr ¹ (N = 191)	Tolai (N = 48)	Mad ² (N = 65)	Rab ² (N = 60)	Fiji ² (N = 57)	NCal ² (N = 65)	PNGH (N = 28)	WSam (N = 22)	Rara ³ (N = 78)	Niue ³ (N = 70)	Nau ³ (N = 67)	Kiri ³ (N = 62)	SBor (N = 21)	Java (N = 59)	Abor ⁴ (N = 424)	Taiw (N = 48)	Atay ⁵ (N = 192)	Ami ⁵ (N = 150)	Chin ⁴ (N = 796)
Trob	0.00	0.47	0.57	1.21	1.32	0.88	0.66	0.86	1.01	0.40	0.59	0.68	0.99	0.89	1.84	1.94	0.27	0.52	0.23	1.05	0.66
Roro	0.47	0.00	0.35	1.30	0.85	0.95	0.75	1.02	0.89	0.76	0.92	0.97	0.75	1.16	1.17	1.65	0.86	0.71	0.94	0.64	0.68
Gidr ¹	0.57	0.35	0.00	1.54	1.06	1.24	0.86	0.84	0.68	1.84	1.71	1.80	0.63	1.04	0.66	1.30	0.39	0.65	0.79	0.65	0.69
Tolai	1.21	1.30	1.54	0.00	0.06	0.04	0.13	0.14	1.80	1.61	0.42	1.40	1.12	2.12	2.38	2.42	1.91	0.62	0.64	0.72	0.88
Mad ²	1.32	0.85	1.06	0.06	0.00	0.14	0.10	0.13	1.76	1.57	0.51	1.50	0.96	1.98	1.70	2.07	1.96	0.72	0.91	0.71	0.82
Rab ²	0.88	0.95	1.24	0.04	0.14	0.00	1.85	0.21	1.46	1.50	0.34	1.08	0.97	1.60	2.26	2.35	1.49	0.45	0.44	0.59	0.80
Fiji ²	0.66	0.75	0.86	0.13	0.10	1.85	0.00	0.08	1.20	0.89	0.26	0.79	0.73	1.27	1.58	1.91	1.04	0.47	0.53	0.56	0.57
NCal ²	0.86	1.02	0.84	0.14	0.13	0.21	0.08	0.00	1.31	1.13	0.38	1.08	0.80	1.40	1.45	2.09	1.18	0.50	0.51	0.75	0.57
PNGH	1.01	0.89	0.68	1.80	1.76	1.46	1.20	1.31	0.00	1.52	0.94	0.84	1.27	1.16	1.53	2.13	0.52	1.03	1.27	0.81	1.26
WSam	0.40	0.76	1.84	1.61	1.57	1.50	0.89	1.13	1.52	0.00	0.58	0.78	1.82	1.55	2.94	2.75	1.34	1.06	0.66	1.59	0.49
Rara ³	0.59	0.92	1.71	0.42	0.51	0.34	0.26	0.38	0.94	0.58	0.00	0.16	1.04	1.01	2.21	2.50	1.32	0.48	0.37	0.90	0.57
Niue ³	0.68	0.97	1.80	1.40	1.50	1.08	0.79	1.08	0.84	0.78	0.16	0.00	1.23	0.97	1.92	2.61	1.20	0.75	0.54	1.62	0.84
Nau ³	0.99	0.75	0.63	1.12	0.96	0.97	0.73	0.80	1.27	1.82	1.04	1.23	0.00	1.15	0.17	0.20	1.18	0.30	0.74	0.68	0.67
Kiri ³	0.89	1.16	1.04	2.12	1.98	1.60	1.27	1.40	1.16	1.55	1.01	0.97	1.15	0.00	0.24	0.18	1.08	0.43	0.73	1.35	0.97
SBor	1.84	1.17	0.66	2.38	1.70	2.26	1.58	1.45	1.53	2.94	2.21	1.92	0.17	0.24	0.00	0.10	1.64	0.56	1.13	1.36	0.67
Java	1.94	1.65	1.30	2.42	2.07	2.35	1.91	2.09	2.13	2.75	2.50	2.61	0.20	0.18	0.10	0.00	2.10	0.65	1.39	1.50	0.86
Abor ⁴	0.27	0.86	0.39	1.91	1.96	1.49	1.04	1.18	0.52	1.34	1.32	1.20	1.18	1.08	1.64	2.10	0.00	0.92	0.59	1.20	1.10
Taiw	0.52	0.71	0.65	0.62	0.72	0.45	0.47	0.50	1.03	1.06	0.48	0.75	0.30	0.43	0.56	0.65	0.92	0.00	0.27	0.31	0.46
Atay ⁵	0.23	0.94	0.79	0.64	0.91	0.44	0.53	0.51	1.27	0.66	0.37	0.54	0.74	0.73	1.13	1.39	0.59	0.27	0.00	1.13	0.46
Ami ⁵	1.05	0.64	0.65	0.72	0.71	0.59	0.56	0.75	0.81	1.59	0.90	1.62	0.68	1.35	1.36	1.50	1.20	0.31	1.13	0.00	0.84
Chin ⁴	0.66	0.68	0.69	0.88	0.82	0.80	0.57	0.57	1.26	0.49	0.57	0.84	0.67	0.97	0.67	0.86	1.10	0.46	0.46	0.84	0.00

Trob: Trobriand Islanders; Roro: Roro, Papua New Guinean Lowlanders; Gidr: Gidra, Papua New Guinean Lowlanders; Tolai: Tolai, population from east New Britain; Mad: Madang; Rab: Rabaul; Fiji: Fiji;

NCal: New Caledonia, all Melanesian populations; PNGH: Papua New Guinean Highlander; WSam: Western Samoa; Rara: Raratonga; Niue: Niue, both Polynesian populations; Nau: Nauru;

Kiri: Kiribati, both Micronesian populations; SBor: south Borneo; Java = Javanese; Abor: Kimberly and Cape York Australian aboriginal population; Taiw: Taiwanese; Atay: Atayal, Taiwanese aboriginal tribe;

Ami: Ami, Taiwanese aboriginal tribe; Chin: northern and southern Chinese.

N = number of typed individuals.

Low genetic distances of studied populations — Trobrianders, Roro and Tolai — are shown in bold.

¹Yoshida *et al.* (1995); ²Gao *et al.* (1992a); ³Gao *et al.* (1992d); ⁴Gao & Serjeantson (1992); ⁵Hu *et al.* (1996).

Table 5. Nei's genetic distance estimated from DPB1 allele frequencies in 13 presently studied and relevant previously published populations of Asia-Oceania

	Trob (N = 81)	Roro (N = 26)	Tolai (N = 48)	PNGH (N = 28)	PNGH ¹ (N = 142)	WSam (N = 22)	Abor ² (N = 125)	SBor (N = 21)	Java (N = 59)	Taiw (N = 48)	Atay ³ (N = 192)	Ami ³ (N = 150)	SChin ⁴ (N = 99)
Trob	0.00	0.02	0.84	1.34	1.19	0.04	0.15	1.22	0.80	0.02	0.00	0.00	0.16
Roro	0.02	0.00	0.57	0.82	0.75	0.02	0.09	1.07	0.62	0.03	0.02	0.02	0.11
Tolai	0.84	0.57	0.00	0.28	0.39	0.70	0.44	1.41	0.95	0.74	0.76	0.79	0.36
PNGH	1.34	0.82	0.28	0.00	0.02	0.74	0.62	0.99	0.45	1.18	1.23	1.25	0.63
PNGH ¹	1.19	0.75	0.39	0.02	0.00	0.63	0.60	0.88	0.38	1.11	1.14	1.15	0.64
WSam	0.04	0.01	0.70	0.74	0.63	0.00	0.11	0.98	0.53	0.06	0.05	0.05	0.14
Abor ²	0.15	0.09	0.44	0.62	0.60	0.11	0.00	1.04	0.62	0.14	0.14	0.14	0.15
SBor	1.22	1.07	1.41	0.99	0.88	0.98	1.04	0.00	0.16	1.14	1.23	1.18	0.63
Java	0.80	0.62	0.94	0.45	0.38	0.53	0.62	0.16	0.00	0.76	0.80	0.76	0.38
Taiw	0.02	0.03	0.74	1.18	1.11	0.06	0.14	1.14	0.76	0.00	0.01	0.01	0.15
Atay ³	0.01	0.02	0.76	1.23	1.14	0.05	0.14	1.23	0.80	0.01	0.00	0.00	0.14
Ami ³	0.01	0.02	0.79	1.25	1.15	0.05	0.14	1.18	0.76	0.01	0.00	0.00	0.14
SChin ⁴	0.16	0.11	0.36	0.63	0.64	0.14	0.15	0.63	0.38	0.15	0.14	0.14	0.00

Trob: Trobriand Islanders; Roro: Roro, Papua New Guinean Lowlanders; Tolai: Tolai, population from east New Britain; PNGH: Papua New Guinean Highlanders; WSam: Western Samoa; Abor: Cape York Australian aboriginal population; SBor: south Borneo; Java = Javanese; Taiwan: Taiwanese; Atay: Atayal, Taiwanese aboriginal tribe; Ami: Ami, Taiwanese aboriginal tribe; SChin: southern Chinese. N = number of typed individuals.

Low genetic distances of studied populations — Trobrianders, Roro and Tolai — are shown in bold.

¹Imanishi *et al.* (1992a); ²Gao *et al.* (1992c); ³Hu *et al.* (1996); ⁴Falco *et al.* (1993).

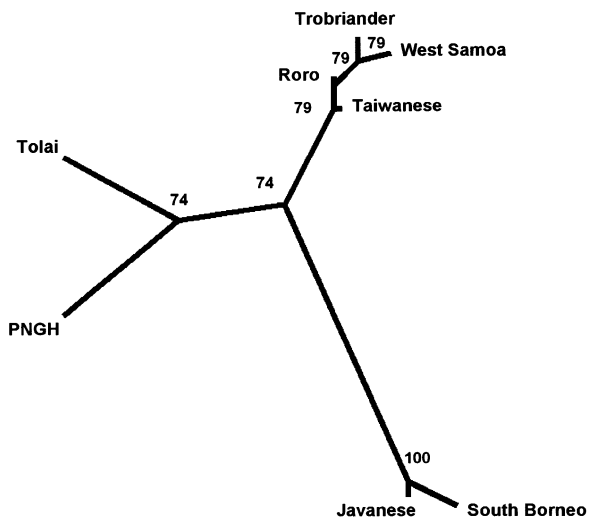


Figure 2. Unrooted neighbour-joining tree using Nei's genetic distances based on HLA-DRB1 and DPB1 allele frequencies of the populations studied. The percentage of bootstrap support for each branch is shown.

of Trobriand Islanders (DRB1*08032-DQA1*0103-DQB1*0601) has frequently been found in Australian Aborigines from East and West Cape York (Gao *et al.*, 1992b) and in Atayal, a Taiwanese aboriginal population (Hu *et al.*, 1996) and at a lower frequency in Japanese and Koreans. The DRB1*1101 and DQB1*0301 alleles observed in Tolai were prevalent in Melanesians from Madang (north coast of PNG), New Britain, New Caledonia and Fiji as well as in the Polynesian population of Raratonga (Gao & Serjeantson, 1992). The Tolai population showed, on the whole, a characteristic Mela-

nesian distribution of DRB1 alleles. The most frequent haplotype in Javanese has been observed at a similarly high frequency in Micronesians from Nauru and Kiribati (Serjeantson & Gao, 1995). The most common DRB1 alleles observed in Roro, the DRB1*1502 and 1602 alleles, were found at quite similar frequencies in the NAN Gidra population (Yoshida *et al.*, 1995), living on the south coast of New Guinea not far from the Roro. In addition, the very short genetic distance for the DRB1 locus (0.36; in comparison, in Roro/Trobriander, 0.47) between the two populations confirmed that the linguistic difference is not reflected by the genetic distance. This suggests that, although the Roro remain a distinct cultural unit, there could be genetic admixture with neighbouring Lowland groups. This is also known from oral history. There is expected to be considerable exchange of marriage partners between different linguistic neighbours so that the linguistic differences do not necessarily act as a barrier to gene flow (Nagy *et al.*, 1997).

Corresponding to their short geographic distance, the distributions of the DRB1 alleles in Javanese and in the sample from south Borneo are quite similar, but different from those in other Oceanian populations. The PNG Highlanders differed from all other examined populations, i.e. the DRB1*0410, a variant of DRB1*0405 (different by only one non-synonymous base substitution at codon position 86), was only seen in the PNGH sample. Gao & Serjeantson (1992) have found this allele in Australian Aborigines (2.8%), in Melanesians and at a very low frequency (0.3%) in Polynesians and Chinese. The most frequent DRB1 allele in PNGH, DRB1*1408, was also present in the Polynesian population from Western Samoa (9.1%) and at a very low frequent in Trobriand Islanders (0.6%). The DRB1*1408 allele differs from

Table 6. Minimum sample size requirements according to Chakraborty

	Population	Number of alleles k^* found in the sample	Analysed sample size	Minimum sample size needed to represent all alleles k for $\alpha = 0.1$ with frequency above 5%
HLA DRB1	Trobriander	14	81	48
	Roro	12	26	46
	Tolai	9	48	43
	Javanese	17	59	50
	PNGH	11	28	45
	South Borneo	12	21	46
	Taiwanese	15	48	48
	West Samoa	11	22	45
HLA DPB1	Trobriander	4	81	36
	Roro	6	26	40
	Tolai	5	46	38
	Javanese	16	59	49
	PNGH	10	28	45
	South Borneo	10	21	45
	Taiwanese	7	48	41
	West Samoa	6	22	40

*With the following assumption for an endogamous population: number of alleles for a k -allelic codominant locus = number of alleles found in the endogamous population.

DRB1*1401 found prevalently in Trobriand Islanders and Tolai by a single, second base transversion (A \rightarrow C) at codon position 57 in exon 2, as previously reported by Serjeantson & Gao (1995). The occurrence of DRB1*1408 in the present and previously investigated PNGH groups as well as in Australian Aborigines (Gao *et al.*, 1992b) does not support the hypothesis that this allele is a novel Polynesian-specific variant.

Because of the remarkably dissimilar distribution patterns of DPB1 alleles in different population groups, explicable by differential selection after the separation of the major population groups, the DPB1 locus may be used to trace back the origin and the migration of a distinct population. The distribution of DPB1 alleles in the studied populations revealed reduced diversity and marked differences between the populations (Gao *et al.*, 1992c). The Austronesian populations – Trobriand Islanders, Roro and Western Samoans – are characterized by a markedly increased DPB1*0501 allele frequency. Nearly 98% of the Trobriand Islanders carry this ‘Asian-specific’ DPB1 allele. DPB1*0501 is well represented in Singapore Chinese (48.1%; Gao *et al.*, 1992a), southern Chinese (40.9%; Falco *et al.*, 1993) and Shanghai Chinese (34.6%; Wang *et al.*, 1992), Australian Aborigines (51.2%; Gao *et al.*, 1992c) and Japanese (39%; Hashimoto *et al.*, 1994). A comparison of the DPB1*0501 frequencies found in Trobriander Islanders, Roro and Western Samoans with the frequency found in Taiwanese Aborigines in the present and a previously published study – in the Atayal tribe DPB1*0501 has a frequency of 84% (Hu *et al.*, 1996) – revealed a very close genetic relationship between Taiwanese Aborigines and the studied AN groups. This is one piece of evidence to support the theory that the origin of Austronesians is in south-east Asia, possibly Taiwan.

In field work carried out far from modern civilization, a sample of adequate size can often not be collected from endogamous single homogeneous populations, especially if only unrelated individuals have to be included. However, we should ensure that all possible alleles are represented in a sample or at least it should be taken into account that in some samples there could be a lack of information. In order not to lose information by mixing populations or by omitting very small samples, we have presented all results. We have addressed the sample size issue by asking what sample sizes are adequate for our population studies and which effects based on HLA allele frequencies can even be discussed. We have used the inequality originally described by Chakraborty (1992) for a variable number of tandem repeat loci assuming the prediction of the neutral mutation model of the infinite allele model to analyse the sample size requirements. Although we cannot assume selective neutrality for the HLA loci analysed, the application of this inequality should give a sufficient indication for our discussion of the sample size requirement. The following assumption was made for markedly isolated populations with a reduced number of alleles at each locus: the number of alleles found in the endogamous population was equated with the number of alleles for a k -allelic codominant locus. The results in Table 6 demonstrate that the alleles with frequency $\leq 5\%$ were only found by chance in the samples from the Roro population, from Papua New Guinea Highlands, south Borneo, Western Samoa and Taiwan. Admittedly, the boundary of minimum sample size to represent all alleles shown in Table 6 is too crude when the allele frequencies are not equal. Far larger sample sizes are needed to represent rare alleles in a sample. For example, to find a fourth rare allele with a frequency of 0.6% or above with 90% confidence

in the DPB1 analysis of the Trobriand population a minimum sample size of ≥ 304 individuals would be necessary. Nevertheless, because the rare alleles contribute little to genetic distance one might conclude that, if we sacrifice some rare alleles, the mistake made in calculating the genetic distances is small and should not be taken into consideration.

The significantly reduced DPB1 diversity and the diminished DRB1 allelic variation may reflect the isolation of the ethnic groups studied, a small founder population and/or bottlenecks during the migration which led to the colonization of the Pacific. The low diversity can be also the result of a loss of alleles due to illness or environmental stresses with subsequent positive directional selection in response to infectious pathogens, driving certain few alleles to high frequencies. In general, the patterns of diseases are typical for endogamous populations still adhering to traditional life-styles and customs. The most frequent diseases of the Roro and the Trobriand population are caused by infections. The influence of these diseases on HLA allele distribution in the investigated populations is poorly understood, although a specific study is currently under way.

The differences in allelic distribution between the culturally and linguistically similar AN populations Trobriand Islanders and Roro may originate from a relatively early separation during their migration from south-east Asia and an independent development of these two populations. Genetic drift is probably the main cause of differences in allele distribution, and it should be taken into consideration that drift may have its greatest effect on small populations. In our study, the population size of the Trobriand Islanders (20 000–25 000) and Roro (6000) was quite small. This low effective population size could have led to fixation of a small number of alleles, increased in frequency by drift and subsequently enhanced and redirected through both mixing and isolating and as a result of local adaptations to circumstances that were themselves highly diverse.

Different selection pressures are responsible for maintaining and generating the diversity of the HLA class II regions, focusing on the functional role of polymorphic residues in peptide binding and presentation, suggested by the structural model of the peptide binding groove (Erlich & Gyllenstein, 1991). The evolutionary history of polymorphism at class II loci reflects selection pressures for different loci and different genetic mechanisms. Because of these different evolutionary forces operating on class II loci and the probable functional differences, we first calculated the genetic distances separately for DRB1 and DPB1. The phylogenetic tree represents the summarized genetic relationships among the examined populations. The results demonstrate a short distance between the two Austronesian populations Roro and Trobrianders. Both are genetically closer to the people of Western Samoa (distance: 4000 km direct line) than to the Tolai (distance 400 km direct line) which are characterized by a 'Melanesian' distribution of HLA DRB1 alleles and a shortest genetic distance to the Melanesian

populations of Rabaul and Madang (Gao & Serjeantson, 1992; Gao *et al.*, 1992a,d; Serjeantson & Gao, 1995; Yoshida *et al.*, 1995). Thus we cannot confirm the oral history account that the first settlers came from the 'north-east', probably from New Britain. Linguistically Trobrianders and Tolai belong to the same subgroup. Such linguistic similarities do not necessarily show a common genetic ancestor, of course. And furthermore, although the physical features of the Trobriander suggest a genetic admixture of Papuan and Austronesian, this cannot be supported by HLA analysis. It is quite possible that more than one wave of Austronesian newcomers arrived on the Trobriand islands. There are four clans as on other islands in this region, representing distinct groups or immigrants who arrived either simultaneously or successively. A clan-based analysis of the HLA data could not detect any significant difference (data not shown). These results correspond with the marriage system of the Trobrianders requiring young people to find their marriage partners outside their own clan. At the DNA level this leads to mixed genes.

In conclusion, the findings of this study contribute to the reconstruction of the history of human migration in Asia-Oceania. Our data support previous studies which demonstrated a genetic, archaeological and cultural association of the AN-speaking populations in Melanesia and populations of south-east Asia. It is very likely that they share a common south-east Asian ancestor and past founder events may have led to this reduced variability, followed by genetic drift. Our results suggest that, in particular, the AN populations of the Trobriand Islands and Roro may have originated from south-east Asia; some observed divergence could be due to separation during their migration. The genetic distance between these two populations and the Tolai population from east New Britain indicates a genetic admixture of the Tolai with other populations such as Madang and Rabaul, which could be explained by a different migration pattern, and an earlier settlement of the Tolai region of New Britain. Alternatively, isolation of the Trobriand Islanders, gene flow between Roro and neighbouring Papuan groups and selection forces could have led to the divergent HLA class II allelic distribution. The genetic affinity of the Polynesians examined with Trobriand Islanders and Roro confirms the hypothesis that Polynesians derived from AN-speaking Melanesians, indicating a recent common ancestor. However, from our data an alternative hypothesis of a Polynesian back-migration into Melanesia causing the observed HLA allele distribution cannot be excluded.

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