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# D1S80 DNA profiling in five African populations

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# D1S80 DNA PROFILING IN FIVE AFRICAN POPULATIONS

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Leslie R. Adrien

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To: Dean Arthur W. Herriott
College of Arts and Sciences

This thesis, written by Leslie R. Adrien, and entitled D1S80 DNA Profiling in Five African Populations, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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## DEDICATION

I dedicate this thesis to my family and friends, whose support and love made it possible to keep my sanity while successfully completing my goals.

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My deepest indebtedness is to God whose endless grace and blessings enabled me to achieve my current feat.

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ABSTRACT OF THE THESIS

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The highly polymorphic D1S80 locus has no known genetic function. This variable number of tandem repeat (VNTR) has been valuable in forensic identification. We have obtained allelic and genotypic frequencies for five African populations (Benin, Cameroon, Egypt, Kenya and Rwanda), which could be employed as databases to identify individuals.

The polymerase chain reaction, followed by vertical polyacrylamide gel electrophoresis and silver staining was our method of analysis. Allele frequencies were used to infer genetic associations using Phylip 3.5, Principal Component and G-test statistical programs. Tests for Hardy-Weinberg equilibrium were employed. $F_{st}$ estimates and power of discrimination values were also determined for each of our populations.

Our analyses of 28 additional populations demonstrated that the D1S80 locus alone provided for the discrimination of major racial groups. Genetic homogeneity between the African groups was observed. We have generated a database useful for human differentiation and phylogenetic studies.

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## INTRODUCTION

The D1S80 locus has been utilized in paternity testing, forensic applications and phylogenetic analysis, since its description by Nakamura [1]. It is the most characterized amplified fragment length polymorphism (AmpliFLP) [2]. As a single locus, this variable number of tandem repeat (VNTR) is highly polymorphic. Observed heterozygosity has been reported as high as 78% [3]. The D1S80 locus by itself enables the discrimination of races and ethnic groups [4]. The standard procedure for D1S80 analysis involves the polymerase chain reaction (PCR) followed by polyacrylamide gel electrophoresis (PAGE) and silver staining. D1S80 is amenable to the PCR since its size ranges from 369 to over 785 base pairs [5].

We decided to focus our study on African populations and their phylogenetic relationships to non-African populations. Sub-Saharan African populations are known for their high levels of diversity [6]. Our research is concentrated on five different populations. These five groups were collected in Benin, Cameroon, Egypt, Kenya and Rwanda. All populations, except the Egyptian population, are sub-Saharan. The majority of all sub-Saharan Africans are now Bantu speaking resulting from the Bantu expansion estimated to have occurred approximately three thousand years ago [6]. The Bantu diaspora was driven by the incipient agricultural revolution in Africa. These farmers/invaders migrated from the central-western coast of Africa eastward and southward. Bantus make up one of four main extant African groups. A brief look into the history of each population provided information on the genetic composition of each. The Sahara desert has been a migration barrier in recent human evolution. Berbers and

Arabs mainly influenced the genetic make-up of groups in northern Africa, while the heritage south of the Sahara are native Africans, with limited European admixture occurring relatively recently, in the late 15th century [7].

Forty-two separate ethnic groups populate Benin [8]. Due to its location on the west coast of Africa, it was highly involved in the Atlantic slave trade. The natives of this country endured both British and French authority over them for more than a century. The Bantu dispersal is thought to have originated in an area including the country of Benin [6].

Tutsi kings reigned in Rwanda from the late 18th century up to the 1960's [8]. The Hutus, a Bantu group, penetrated the region west of Lake Victoria during the Bantu dispersal. Ethnic strife exists between these two groups and is manifested through their battles for leadership. Belgium colonized the territory following World War I for a period of approximately 40 years. During this time, the requirement for each tribe to be distinguished with identity cards kept them isolated. Rwanda is known to be one of the most densely populated countries in Africa [9]. The Hutu group makes up 90% of this country [8].

The native people of Cameroon experienced authority from the Portuguese, British, Germans and French. Cameroon has the most ethnic groups of any other country in Africa, namely 200 [8]. The country is currently divided into a northern French zone and a southern British zone. Cameroon is, geographically, just south of the focus of the Bantu expansion.

Kenya, on the east coast of Africa, has influence of several Arab groups. The cultural and genetic impact of Omani traders is clearly visible in this country. The British

arrived in the 1800's and assigned different ethnic groups to different parts of the country to live, thereby further isolating these groups. There are more than 100 different ethnic groups established in Kenya [8]. A 1989 census indicated that Arabs, Asians and Europeans made up less than 1% of the total population [10].

The Egyptian samples were collected from the area of Tanta, which is located on the Nile River delta. This north African population is largely made up of Arabs and Berbers [6]. Berbers were the earliest known inhabitants in north Africa [7]. From 711 of the common era (CE) on, Arabs have dominated the Berbers within these areas [6].

In this study, we examined the D1S80 allelic frequencies of African populations from Benin, Cameroon, Egypt, Kenya and Rwanda and compared them to 28 worldwide reference populations. This information will be the basis of databases for forensic probability estimation and population genetic studies. Allelic and genotypic frequencies were determined by utilizing the gene count method [11]. Divergence from Hardy-Weinberg equilibrium (HWE) expectations were determined, with the Fisher exact test and the Chi-Square test, as well as $F_{st}$ estimates and power of discrimination values in each of our populations. Observed and expected homozygosity and heterozygosity values were also generated. In addition, twenty-eight other populations were incorporated into our study to ascertain possible genetic similarities. Allele frequencies were used to infer genetic associations through phylogenetic, principal component and G-Test statistical analyses.

## BACKGROUND INFORMATION

The D1S80 locus is located at the telomeric position of the p arm of chromosome 1. Nakamura and collaborators first described this locus in 1988 [3]. Its designation stems from the fact that this is a DNA locus on chromosome 1, a Single copy sequence and it was the $80^{th}$ locus described on chromosome 1. D1S80 is a VNTR that is also referred to as an AmpliFLP. To date, no phenotypic trait has been associated with this locus [5]. AmpliFLPs are distinguished by differences in length between the alleles, not by sequence [3, 12], although considerable sequence differences have been reported. The overall length ranges of AmpliFLPs are from 200 base pairs (bp) to 1000 bp [3]. The D1S80 known allele size range, including the flanking regions, is from 369bp to 785bp [5]. D1S80 has a core repeat unit of 16 bps [3]. D1S80 alleles typically contain from 14 to 41 repeats of this 16 bp sequence [13]. D1S80 alleles are co-dominantly inherited [12]. Estimates of heterozygosity are generally greater than 70% [14, 15]. Although this is a single locus, it is highly polymorphic due to the large number of alleles it contains (more than 27). As many as twenty-nine different alleles have been found within one population [5, 13]. The number of possible genotypes can be calculated by the formula G = n (n+1) / 2 [3]. According to this formula, 435 genotypes are possible when 29 alleles are identified. A high number of alleles most likely result from high mutation rates and a lack of selection pressure. Alleles with greater than 41 repeats and those smaller than 14 have both been reported [16-19]. The alleles with the highest recurring frequency are those with 18 and 24 repeats [12, 20-22]. The high frequency of these alleles suggests

that these are ancestral alleles [23]. Allele 15 is not common, and therefore is not incorporated into D1S80 allelic ladders.

## MATERIALS AND METHODS

### DNA Isolation

All samples were collected as whole blood in EDTA Vacutainer tubes from unrelated individuals. These individuals were identified by biographical information traced back at least two generations. The appropriate ethical guidelines, as dictated by Florida International University, were followed during the collection of samples. Each collection was arranged through the leaders of the regions and supervised by the same. The blood samples were lysed and leukocyte nuclei were separated from the rest of the blood components. Several washes in lysis buffer were performed to rinse and isolate the leukocyte nuclei. The DNA was then purified using proteinase-K digestion and standard organic phenol-chloroform extraction. Ethanol precipitation followed to pellet the DNA. DNA was then dissolved in TE (Tris-Ethylenediamine Tetraacetate) buffer. All samples were stored in -80°C when not in use.

### Amplification of DNA

PCR was performed in a Perkin-Elmer 480 thermal cycler. Amplification parameters were 1 minute at 94°C, 1 minute at 65°C and 1 minute at 72°C for twenty-nine cycles. An additional 10-minute extension at 72°C followed [13]. PCR products were stored at 4°C until they were loaded onto a polyacrylamide gel. PCR amplifications were carried out in 25µl reactions containing 16.3µl of water, 2.5µl of 10X buffer containing

15mM $MgCl_2$, 2.5µl (0.15mM) dNTPs, 1.25µl (10µM) of each primer, 0.2µl (5U) *Taq* DNA and 10-50 nanograms of DNA sample. The sequence of the forward primer was 5' GAAACTGGCCTCCAAACACTGCCCGCCG 3' and that of the reverse primer was 5' GTCTTGTTGGAGATGCACGTGCCCCTTGC 3' [24]. The Benin samples were DOP (degenerate oligonucleotide primer) pre-amplified according to Buchanan and collaborators [25]. This method of PCR is used when the DNA is of poor quality or low yield. From the DOP-PCR product, 6µl were used to specifically amplify for D1S80. Samples were overlaid with two drops of mineral oil to protect them from evaporation throughout the amplification procedure.

**Vertical Polyacrylamide Gel Electrophoresis**

A 39:1 acrylamide stock solution was used to make an 8% polyacrylamide gel. The longer glass plate used for casting the slab gel was treated with an adherent to retain the gel as the shorter plate is detached. Gels were 35 x 45 centimeters in size with a 0.8-millimeter thickness or 38 x 46 centimeters in size with a 1-millimeter thickness. An Applied Biosystems AmpliFLP D1S80 Allelic Ladder was electrophoresed every four samples. The gels were run at 1000-1200 volts for five to six hours, depending upon the migration of the xylene-cyanol tracking dye. Electrophoresis was stopped 45 minutes after the xylene-cyanol dye ran out of the bottom of the gel. All gels were silver stained to visualize the alleles.

Silver staining consisted of a 5-minute slow agitation in 1% nitric acid. Following a water rinse, the gel was placed in fresh 0.2% silver nitrate and shaken for 20

minutes. Another water rinse was done prior to the development step in fresh 0.28 M sodium carbonate, 0.05% formaldehyde solution. Development consisted of several rinses in the sodium carbonate solution until it no longer became "milky". Once the desired band intensities were achieved, the gel was placed in 10% acetic acid stop solution for 5 minutes. Gels were then placed in water for another 5 minutes. After drying, gels were photographed under visible light. All gels were scored to ascertain genotypes independently by at least two people. Genotyping was performed directly from the gels by comparison to the allelic ladder. Any discrepancies were repeated.

**Population Sampling**

A total of five African populations were analyzed in our study. They were Benin, Cameroon, Egypt, Kenya and Rwanda. All the sub-Saharan populations belong to the Niger-Congo language family. A total of one hundred samples were analyzed from Benin. These individuals belong to the Fon ethnic group. They were collected in the southern part of the country from the Zagnanado population. The Kenya group consisted of 106 Bantu individuals from small villages 100 kilometers northeast of Nairobi. The Rwanda population was made up of 100 individuals of the Hutu tribe. Hutus are a Bantu group. The Egyptian group was collected in the region of Tanta located at the center of the Nile delta. Tanta is approximately 94 kilometers north of Egypt's capital city, Cairo. This population is primarily Arab/Berber in origin. Arabia and north Africa both share the Afro-Asiatic language. This north African population shares their ancestry to a lesser extent with Greeks, Turks and other Mediterranean Basin people. Tantas are therefore

Caucasians. There were 100 samples from Tanta processed. The Cameroon group consisted of 16 Bantu individuals from villages 50 kilometers southwest of the city of Yaounde, in the southern region of the country. In our study, these 16 samples were analyzed together with the 34 individuals reported by Araujo da Silva in 1999 [26]. Another twenty-eight populations were analyzed along with these in order to ascertain genetic relationships. These are all extracted from published information as indicated in Table 1.

**Table 1. Populations included**

| Population | 3 Letter Code* | N | Location | Reference |
|---|---|---|---|---|
| Andalucia | Anc | 120 | Southern province of Spain | [27] |
| Andalusian | Ans | 147 | Southern province of Spain | [16] |
| ArabMoslem | Ara | 94 | Gaza Strip, Judaea, Samaria, Israel | [28] |
| Australia | Aus | 250 | Victoria | [29] |
| Bahama | Bah | 88 | Throughout Bahamas | [4] |
| Bari | Bar | 24 | NE Colombia, S. America | [4] |
| Basque | Bas | 257 | North Central Spain | [28] |
| Benin | Ben | 100 | S. Benin, West Africa | Present Study |
| BWHAlaska | BWH | 109 | Bethel-Wade Hampton, Alaska | [30] |
| Cameroon | Cam | 34 | Yaounde City | [26] |
| Cameroon2 | Ca2 | 16 | SW of Yaounde City, W. Africa | Present Study |
| CanaryIsl | Can | 123 | General population from Canary Islands | [16] |
| Chimila | Chm | 46 | NE Colombia, S. America | [4] |
| China (Han) | Chn | 216 | Xian & Shijiazhuang districts of China | [28] |
| Congo | Con | 34 | Lubumbashi City | [26] |
| Denmark | Den | 210 | General population from Denmark | [28] |
| DubaiArab | Dub | 93 | United Arab Emirates | [31] |
| Egypt | Tan | 100 | Tanta, N. Africa | Present Study |
| Galicia | Gal | 149 | NW province of Spain | [28] |
| Greece | Gre | 107 | Cyprus | [32] |
| Haiti | Hai | 83 | Caribbean | [28] |
| Kenya | Ken | 106 | NE of Nairobi, E. Africa | Present Study |
| Korea | Kor | 116 | Seoul | [28] |
| MapucheArg | Map | 61 | Argentina, S. America | [33] |
| Navajo | Nav | 28 | New Mexico, USA | [4] |
| Nigeria | Nig | 67 | W. Africa | [28] |
| N.SlopeAlas | NSA | 92 | North Slope Borough, Alaska | [30] |
| Philippines | Phi | 103 | Metro Manila | [34] |
| Rwanda | Rwa | 100 | E. Africa | Present Study |
| SaudiArabia | Sau | 220 | Riyadh | [35] |
| Taiwan (Han) | Tai | 105 | General population of Taiwan | [28] |
| Turkey | Tur | 112 | General population of Turkey | [36] |
| Zimbabwe | Zim | 101 | Mashonaland Province | [28] |
| Totals 33 | | 3611 | | |

*These codes are used in Table 6.

**Statistical Analysis**

The gene counting method [11] was used to generate genotypic and allelic frequencies. Allelic frequencies were analyzed with PHYLIP 3.5 [37], Numerical Taxonomy and Multivariate Analysis System (NTSys) principal component [38], and Carmody's G-test [39]. The depiction of homogeneic clustering within PHYLIP and NTSys suggests adequate population sampling. The SEQBOOT, CONSENSE, GENDIST, CONTML, UPGMA, NJ and ML programs are all within the PHYLIP software. Phylogenetic trees were used to visualize the general phylogenetic relationships between the populations and as a proof of the integrity of the data. Three distance-based analyses were used to generate dendrograms, the Unweighted Pair Group Method with Averages (UPGMA), Neighbor Joining (NJ) and Maximum Likelihood (ML). UPGMA assumes that the rates of mutation are equal across time for all lineages [40]. Nei genetic distances were calculated through the use of the GENDIST program. This tree was then created with the UPGMA program, 1000 bootstrap replications were generated with SEQBOOT and CONSENSE configured a consensus tree. The NJ method, on the other hand, produces an un-rooted network. When rooted, a constant rate of evolution is assumed. First, GENDIST was used to calculate Nei's genetic distances. The NJ program was then run, SEQBOOT generated 1000 bootstrap replications and a consensus tree was built with CONSENSE. The NJ method offers rapid analysis with large data sets. The final tree was done with the ML method, which does not assume constant evolutionary rates, thereby enhancing the validity of the relationships in the other trees. SEQBOOT produced a large number (1000) of bootstrapped data sets that

were then inputted into the CONTML program. The CONTML and CONSENSE programs configured the best-fit tree directly from the allele frequencies. The principal component (PC) test was performed to generate a two dimensional plot of PC 1 and 2. In PC analysis, allelic frequencies are inputted into the NTSys program to segregate and cluster groups within by plotting according to their variability. PCs 1 and 2 usually contain the most amount of variability, with subsequent PCs decreasing in value. The G-test calculates likelihood ratios according to the obtained frequencies. It contrasts the observed and expected frequencies [15]. One thousand simulations were done for each 2 x 2 contingency table. The G-test analyzes each 2 x 2 table and indicates which populations are homogeneous with each other. Probability values less than 0.050 indicate significantly different populations.

In addition to these phylogenetic and statistical analyses, several other parameters were computed to describe the five African populations. Expected homozygosity and heterozygosity values were compared to those reported in the literature. Conformance to Hardy-Weinberg equilibrium was tested by use of the Fisher exact and Chi-Square tests within the Genetic Data Analysis (GDA) software [41]. This software was also used to calculate theta-P values which are equivalent to $F_{st}$ values [41]. This analysis estimates the probability that two alleles are identical by descent [42]. It is an attempt to offset the substructure effects on heterozygote genotypic frequencies [43]. Assumptions are no mutation rate and the sampling of an effective population size. If these assumptions are met, these values range between 0 and 1. This measure indirectly indicates gene flow [44].

## RESULTS

Table 2 shows the allelic frequencies for the five African populations. The 24 allele was most frequent in the Benin, Kenya, Rwanda and Tanta populations with frequencies of 0.2150, 0.2028, 0.2200 and 0.4150, respectively. In the Cameroon population the 34 allele was most common with a frequency of 0.3125. African groups have been shown to have the highest frequencies for the 24, 28 and 34 alleles [45]. All five populations shared alleles 17, 20, 21, 23, 24, 25, 28, 31 and 34. Only within the Rwandan population was allele 16 detected and allele 36 was found in a single Cameroon sample.

There were 16 different alleles and 50 different genotypes found in the Benin population. Within the Kenyan and Rwandan groups, 17 various alleles and 49 various genotypes were distinguished. The Tanta samples exhibited 18 alleles and 40 genotypes. There were 11 alleles and 14 genotypes discerned in the Cameroon samples. All genotypic frequencies are contained in Table 3. Within the Benin population, the 22/24 genotype was most frequent representing 6% of the samples. In the Kenya population, 24/28 was the most recurring genotype representing 7.55% of the total. Both 24/34 and 17/24 were most abundant within the Rwandan samples, each with a frequency of 6%. The homozygous genotype, 24/24, was highly repetitive within the Tanta population, displaying a frequency of 18%. Cameroon also had two most recurrent genotypes. These were 21/34 and 25/34, both with a frequency of 12.5%. The number of alleles and genotypes observed were not exceptionally high compared to the number of genotypes that are possible. Utilizing the formula $G=n(n+1)/2$, the most genotypes attainable for

**Table 2. Allelic Frequencies**

| Alleles | Benin (N=100) | Kenya (N=106) | Rwanda (N=100) | Tanta (N=100) | Cameroon2 (N=16) |
|---|---|---|---|---|---|
| 14 | ... | ... | ... | ... | ... |
| 15 | ... | ... | ... | ... | ... |
| 16 | ... | ... | 0.0100 | ... | ... |
| 17 | 0.0550 | 0.0283 | 0.1050 | 0.0150 | 0.0625 |
| 18 | 0.0550 | 0.0613 | 0.0900 | 0.1400 | ... |
| 19 | ... | ... | 0.0050 | 0.0050 | ... |
| 20 | 0.0550 | 0.0236 | 0.0100 | 0.0150 | 0.0313 |
| 21 | 0.1100 | 0.0991 | 0.1000 | 0.0300 | 0.1563 |
| 22 | 0.1000 | 0.0896 | 0.1200 | 0.0450 | ... |
| 23 | 0.0300 | 0.0283 | 0.0050 | 0.0350 | 0.0313 |
| 24 | 0.2150 | 0.2028 | 0.2200 | 0.4150 | 0.2188 |
| 25 | 0.0650 | 0.0377 | 0.0300 | 0.0600 | 0.0625 |
| 26 | 0.0050 | 0.0142 | ... | 0.0300 | ... |
| 27 | 0.0100 | 0.0236 | 0.0550 | 0.0200 | ... |
| 28 | 0.1100 | 0.1981 | 0.0700 | 0.0650 | 0.0313 |
| 29 | 0.0200 | 0.0330 | 0.0100 | 0.0500 | ... |
| 30 | 0.0100 | ... | ... | 0.0050 | ... |
| 31 | 0.0800 | 0.0330 | 0.0400 | 0.0400 | 0.0313 |
| 32 | 0.0050 | 0.0283 | 0.0200 | 0.0050 | ... |
| 33 | ... | 0.0047 | ... | 0.0050 | 0.0313 |
| 34 | 0.0750 | 0.0802 | 0.1000 | 0.0200 | 0.3125 |
| 35 | ... | ... | ... | ... | ... |
| 36 | ... | ... | ... | ... | 0.0313 |
| 37 | ... | ... | ... | ... | ... |
| 38 | ... | ... | ... | ... | ... |
| 39 | ... | ... | ... | ... | ... |
| 40 | ... | 0.0142 | 0.0100 | ... | ... |
| 41 | ... | ... | ... | ... | ... |

...=allele not detected; N=total number of individuals

Theta–P values: Benin=0.032, Kenya=0.027, Rwanda=0.029, Tanta=0.009, Cameroon2=0.021

## Table 3. Genotypic Frequencies

| Genotype | Benin | | Kenya | | Rwanda | | Tanta | | Cameroon2 | | Genotype | Benin | | Kenya | | Rwanda | | Tanta | | Cameroon2 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Freq | N | Freq | N | Freq | N | Freq | N | Freq | | N | Freq | N | Freq | N | Freq | N | Freq | N | Freq |
| **16/16** | | ... | | ... | 1 | 0.010 | | ... | | ... | **20/24** | 2 | 0.0200 | 2 | 0.018 | 1 | 0.0100 | 2 | 0.0200 | | ... |
| **17/17** | 3 | 0.0300 | | ... | 3 | 0.030 | | ... | | ... | **20/25** | 2 | 0.0200 | | | | ... | | ... | | ... |
| **17/18** | | ... | | ... | 1 | 0.010 | | ... | | ... | **20/29** | 1 | 0.0100 | | | | ... | 1 | 0.0100 | | ... |
| **17/21** | | ... | | ... | 3 | 0.030 | | ... | | ... | **20/31** | 1 | 0.0100 | 1 | 0.009 | | ... | | ... | | ... |
| **17/22** | 2 | 0.0200 | 1 | 0.0094 | 2 | 0.020 | | ... | | ... | **20/32** | 1 | 0.0100 | | | | ... | | ... | | ... |
| **17/24** | 1 | 0.0100 | 2 | 0.0189 | 6 | 0.060 | 2 | 0.0200 | 1 | 0.0625 | **20/33** | | ... | | | | ... | | ... | 1 | 0.0625 |
| **17/25** | | ... | | ... | 1 | 0.010 | | ... | | ... | **20/34** | | ... | 2 | 0.018 | | ... | | ... | | ... |
| **17/28** | | ... | 2 | 0.0189 | | | | ... | | ... | **21/21** | 1 | 0.0100 | | | | ... | 1 | 0.0100 | 1 | 0.0625 |
| **17/29** | | ... | 1 | 0.0094 | | | 1 | 0.0100 | | ... | **21/22** | 3 | 0.0300 | 3 | 0.028 | 5 | 0.0500 | | ... | | ... |
| **17/31** | 1 | 0.0100 | | ... | | | | ... | | ... | **21/24** | 5 | 0.0500 | 5 | 0.047 | 5 | 0.0500 | | ... | 1 | 0.0625 |
| **17/34** | 1 | 0.0100 | | ... | 2 | 0.020 | | ... | 1 | 0.0625 | **21/25** | | ... | 2 | 0.018 | | ... | | ... | | ... |
| **18/18** | 1 | 0.0100 | | ... | 2 | 0.020 | 5 | 0.0500 | | ... | **21/27** | | ... | 1 | 0.009 | | ... | | ... | | ... |
| **18/21** | | ... | 2 | 0.0189 | 2 | 0.020 | | ... | | ... | **21/28** | 5 | 0.0500 | 6 | 0.056 | 2 | 0.0200 | | ... | | ... |
| **18/22** | | ... | 1 | 0.0094 | 3 | 0.030 | | ... | | ... | **21/29** | | ... | | | | ... | 2 | 0.0200 | | ... |
| **18/23** | 1 | 0.0100 | | ... | | | | ... | | ... | **21/31** | 1 | 0.0100 | | | | ... | 1 | 0.0100 | | ... |
| **18/24** | 4 | 0.0400 | 3 | 0.0283 | 4 | 0.040 | 11 | 0.1100 | | ... | **21/32** | | ... | | | 1 | 0.0100 | | ... | | ... |
| **18/25** | 1 | 0.0100 | 1 | 0.0094 | | | 2 | 0.0200 | | ... | **21/34** | 5 | 0.0500 | 2 | 0.018 | 2 | 0.0200 | 1 | 0.0100 | 2 | 0.1250 |
| **18/27** | | ... | | ... | 1 | 0.010 | 1 | 0.0100 | | ... | **22/22** | 3 | 0.0300 | | | 1 | 0.0100 | | ... | | ... |
| **18/28** | 2 | 0.0200 | 4 | 0.0377 | | | 3 | 0.0300 | | ... | **22/23** | | ... | 1 | 0.009 | | ... | | ... | | ... |
| **18/29** | | ... | | ... | | | 1 | 0.0100 | | ... | **22/24** | 6 | 0.0600 | 2 | 0.018 | 4 | 0.0400 | 4 | 0.0400 | | ... |
| **18/31** | 1 | 0.0100 | | ... | 1 | 0.010 | | ... | | ... | **22/25** | 1 | 0.0100 | | | | ... | 2 | 0.0200 | | ... |
| **18/32** | | ... | 1 | 0.0094 | | | | ... | | ... | **22/27** | | ... | | | 3 | 0.0300 | 1 | 0.0100 | | ... |
| **18/34** | | ... | 1 | 0.0094 | 2 | 0.020 | | ... | | ... | **22/28** | 1 | 0.0100 | 5 | 0.047 | 1 | 0.0100 | | ... | | ... |
| **19/22** | | ... | | ... | 1 | 0.010 | | ... | | ... | **22/29** | | ... | 2 | 0.018 | | ... | | ... | | ... |
| **19/24** | | ... | | ... | | | 1 | 0.0100 | | ... | **22/31** | | ... | 2 | 0.018 | | ... | 2 | 0.0200 | | ... |
| **20/20** | 1 | 0.0100 | | ... | | | | ... | | ... | **22/32** | | ... | | | 1 | 0.0100 | | ... | | ... |
| **20/21** | 1 | 0.0100 | | ... | | | | ... | | ... | **22/34** | | ... | 2 | 0.018 | 1 | 0.0100 | | ... | | ... |
| **20/22** | 1 | 0.0100 | | ... | 1 | 0.010 | | ... | | ... | **23/23** | 1 | 0.0100 | | | | ... | | ... | | ... |

## Table 3. Genotypic Frequencies (Con't)

| Genotype | Benin | | Kenya | | Rwanda | | Tanta | | Cameroon2 | | Genotype | Benin | | Kenya | | Rwanda | | Tanta | | Cameroon2 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Freq | N | Freq | N | Freq | N | Freq | N | Freq | | N | Freq | N | Freq | N | Freq | N | Freq | N | Freq |
| **23/31** | | ... | 1 | 0.0094 | 1 | 0.0100 | | ... | | ... | **26/31** | | ... | | | | ... | 1 | 0.0100 | | ... |
| **23/32** | | ... | 1 | 0.0094 | | ... | | ... | | ... | **27/27** | | ... | | | 1 | 0.0100 | 1 | 0.0100 | | ... |
| **23/34** | 1 | 0.0100 | 1 | 0.0094 | | ... | | ... | | ... | **27/33** | | ... | 1 | 0.009 | | ... | | ... | | ... |
| **24/24** | 2 | 0.0200 | 3 | 0.0283 | 3 | 0.0300 | 18 | 0.1800 | 1 | 0.0625 | **27/34** | 1 | 0.0100 | 1 | 0.009 | 2 | 0.0200 | | ... | | ... |
| **24/25** | 2 | 0.0200 | 3 | 0.0283 | 2 | 0.0200 | 4 | 0.0400 | | ... | **28/28** | 1 | 0.0100 | 4 | 0.037 | 2 | 0.0200 | 1 | 0.0100 | | ... |
| **24/26** | 1 | 0.0100 | 2 | 0.0189 | | ... | 2 | 0.0200 | | ... | **28/29** | | ... | 3 | 0.028 | | ... | 1 | 0.0100 | | ... |
| **24/27** | 1 | 0.0100 | 2 | 0.0189 | 3 | 0.0300 | | ... | | ... | **28/30** | 2 | 0.0200 | | | | ... | 1 | 0.0100 | | ... |
| **24/28** | 5 | 0.0500 | 8 | 0.0755 | 1 | 0.0100 | 5 | 0.0500 | | ... | **28/31** | 1 | 0.0100 | | | 1 | 0.0100 | | ... | | ... |
| **24/29** | 2 | 0.0200 | 1 | 0.0094 | 2 | 0.0200 | 3 | 0.0300 | | ... | **28/32** | | ... | | | 1 | 0.0100 | | ... | | ... |
| **24/31** | 5 | 0.0500 | 1 | 0.0094 | 3 | 0.0300 | 2 | 0.0200 | | ... | **28/34** | | ... | 2 | 0.018 | 3 | 0.0300 | | ... | 1 | 0.0625 |
| **24/32** | | ... | | ... | | ... | 1 | 0.0100 | | ... | **28/40** | | ... | 1 | 0.009 | | ... | | ... | | ... |
| **24/33** | | ... | | ... | | ... | 1 | 0.0100 | | ... | **29/34** | 1 | 0.0100 | | | | ... | | ... | | ... |
| **24/34** | 3 | 0.0300 | 4 | 0.0377 | 6 | 0.0600 | 2 | 0.0200 | 1 | 0.0625 | **31/31** | 1 | 0.0100 | | | | ... | | ... | | ... |
| **24/36** | | ... | | ... | | ... | | ... | 1 | 0.0625 | **31/32** | | ... | 2 | 0.018 | 1 | 0.0100 | | ... | | ... |
| **24/40** | | ... | 2 | 0.0189 | 1 | 0.0100 | | ... | | ... | **31/34** | 1 | 0.0100 | | | | ... | 1 | 0.0100 | 1 | 0.0625 |
| **25/25** | | ... | | ... | 1 | 0.0100 | 1 | 0.0100 | | ... | **31/40** | | ... | | | 1 | 0.0100 | | ... | | ... |
| **25/26** | | ... | 1 | 0.0094 | | ... | | ... | | ... | **32/34** | | ... | 2 | 0.018 | | ... | | ... | | ... |
| **25/28** | 4 | 0.0400 | 1 | 0.0094 | 1 | 0.0100 | 1 | 0.0100 | | ... | **34/34** | 1 | 0.0100 | | | 1 | 0.0100 | | ... | 1 | 0.0625 |
| **25/31** | 3 | 0.0300 | | ... | | ... | 1 | 0.0100 | | ... | | | | | | | | | | | |
| **25/34** | | ... | | ... | | ... | | ... | 2 | 0.1250 | | | | | | | | | | | |
| **26/26** | | ... | | ... | | ... | 1 | 0.0100 | | ... | | | | | | | | | | | |
| **26/29** | | ... | | ... | | ... | 1 | 0.0100 | | ... | | | | | | | | | | | |

...=allele not detected; N=Number of genotypes

each population is calculated to be 136 for Benin, 153 for the Kenyan and Rwandan populations, 171 for Tanta and 66 for Cameroon.

The observed homozygosity for Benin, Cameroon, Kenya, Rwanda and Tanta were 0.1500, 0.1880, 0.0660, 0.1500 and 0.2800, respectively. The corresponding heterozygosity values were 0.8500, 0.8130, 0.9340, 0.8500 and 0.7200 (Table 4). These values did not vary greatly from the expected ones. These high levels of heterozygosity are consistent with genetic diversity within African populations at other loci [46].

Figure 1 shows an NJ tree using Nei distances [47]. Four main clusters of populations can be discerned. Native Americans are seen in a cluster at one end of the tree. Caucasians are grouped in the adjacent clade. The Mapuche Argentin population segregated with the Caucasians. Hutz and collaborators also placed this population with Caucasians [33]. The following clade exhibits all the African groups as well as the Saudi Arabia and Moslem Arab populations. The last clade clusters the Oriental populations.

In the UPGMA depiction (Figure 2), there are two main branches after the bifurcation between the Andalusian population sample and all the other groups. These two branches have Denmark and Australia versus the rest of the populations in our study. Five clusters can be found here. Dubai Arabs head off the first cluster that incorporates other southwest Asian populations along with two southeast European populations, Tanta and Mapuche Argentina. Once again, Tanta appears to be most closely related to those countries across the adjoining Mediterranean Sea. This would suggest that southern European and southwest Asian countries neighboring the Mediterranean Sea contributed greatly to the genetic constitution of this group. The next cluster holds

**Figure 1. Neighbor Joining Tree**

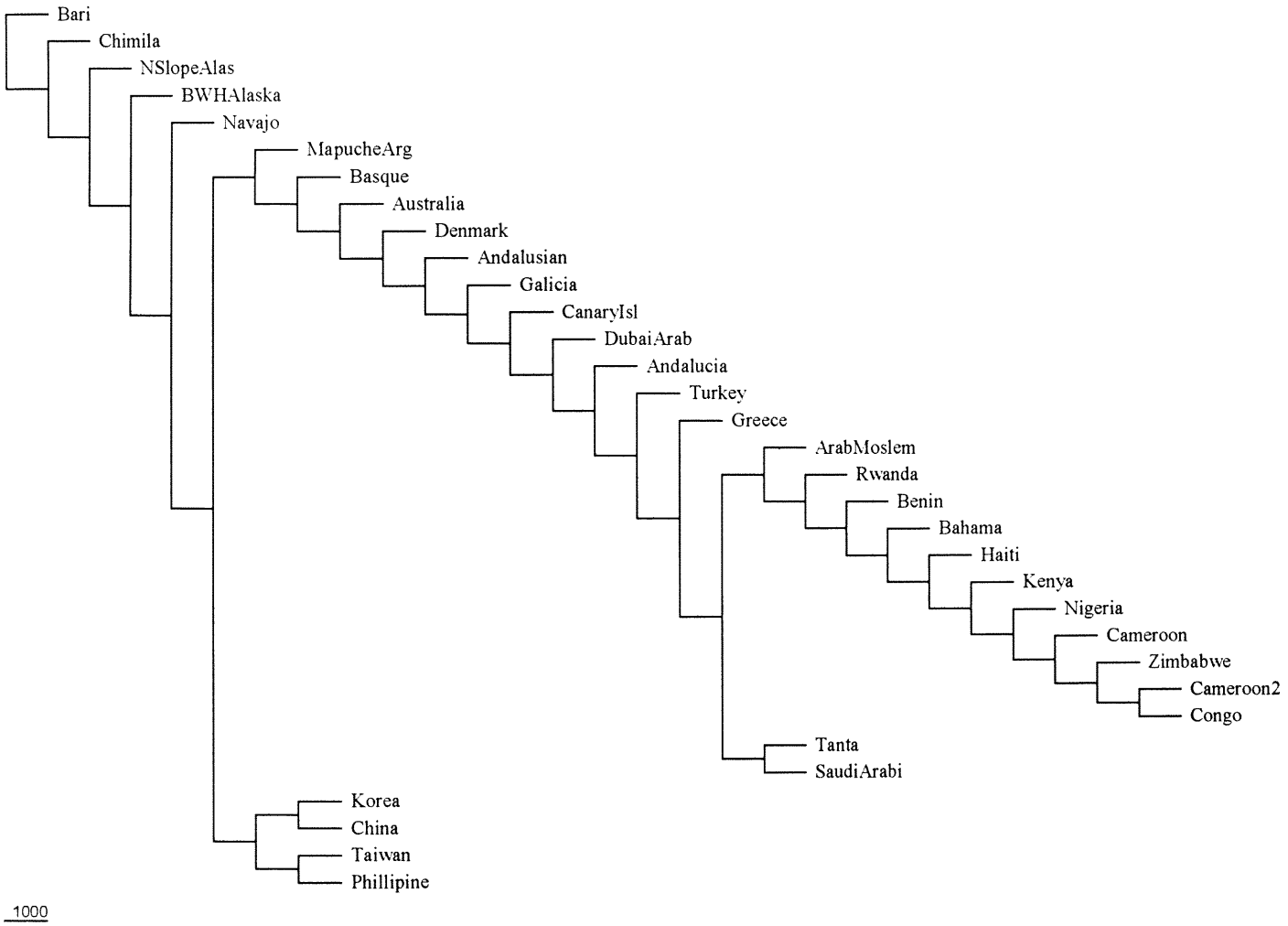
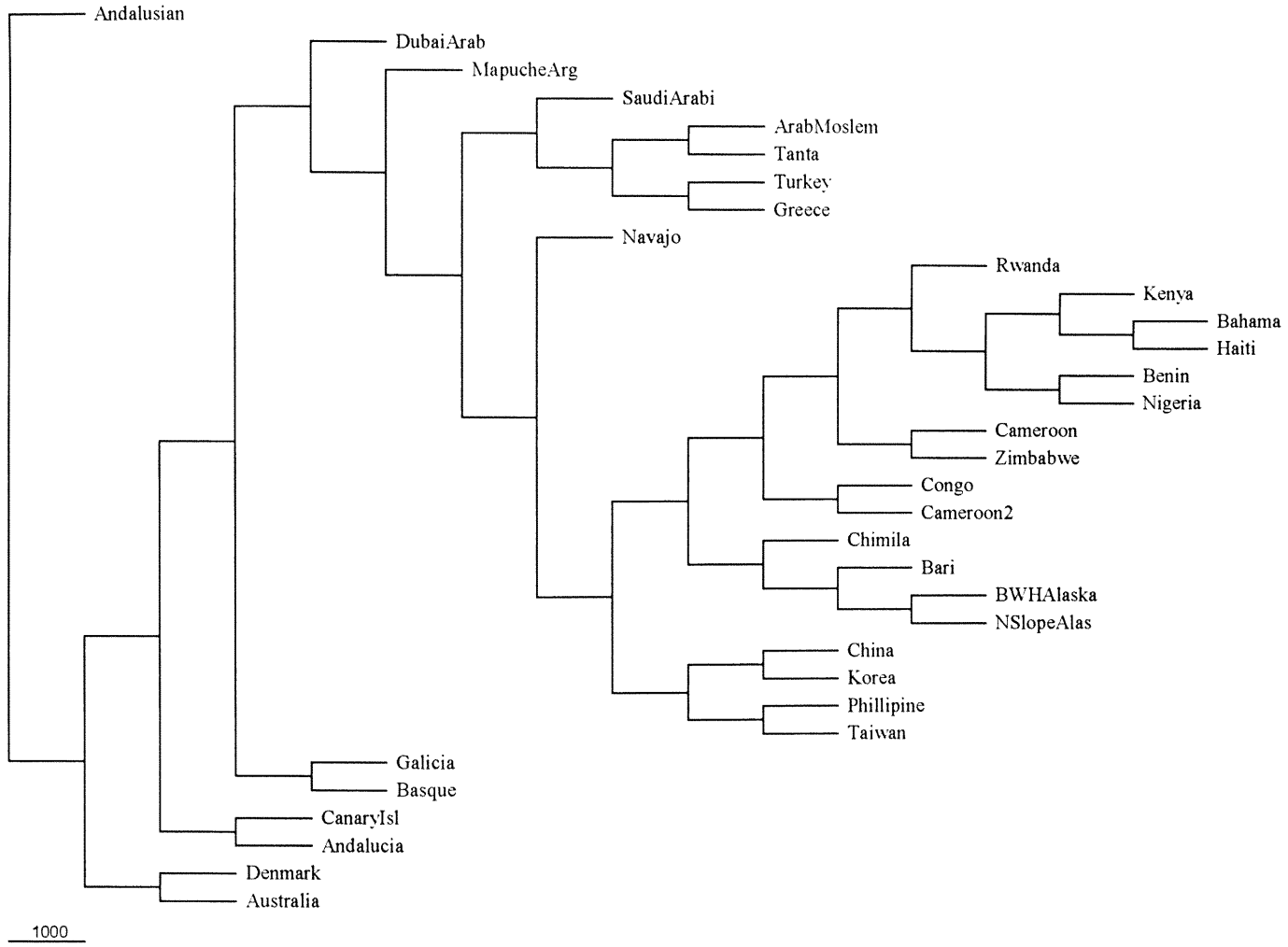


**Figure 2. UPGMA Tree**



the Navajo population branched with the Africans and other Mongoloids. Lastly, the rest of the Caucasians are depicted.

Figure 3 shows a radial representation of the Maximum Likelihood Tree. This tree's primary bifurcation is between the southwest Asian populations and all other populations. All of the African groups are clustered together along with the Greek population. The Caucasians also segregate together. The Mongoloids make up the next assemblage. The bootstrap values in this figure indicate the likelihood that the depicted relationships are statistically significant. Bootstrap values greater than 50 percent (indicated as 500 or greater in the figure) are considered credible indicators of phylogenetic relationships.

Figure 4 displays the variations in all 27 alleles amongst the 33 populations included in the study. Each allele frequency is standardized upon input into the NTSys program. Two by two contingency tables were generated and eigenvalues and eigenvectors were calculated and then plotted. PC 1 indicated 21% of the variability while 14% was held in PC 2. PC 1 shows delineation between African populations and all other populations. PC 2 separates west African populations from the rest of the African groups and the southeast Asians from the American Indian groups. There is also a separation between the southwest Asians and European groups.

The data represented in Table 5 indicates the results of tests for adherence to Hardy-Weinberg equilibrium expectations. A Fisher exact test, along with a Chi-Square test was performed. The Benin population did not conform to Hardy-Weinberg equilibrium expectations according to the Fisher exact test. Its probability (p) value was

0.019. The Rwanda population was also shown to be marginally out of Hardy-Weinberg equilibrium by the Chi-Square test, with a p-value of 0.036.

The G-test analysis of the 33 different populations (Table 6) provided data consistent with the other statistical tests previously mentioned. In the 2 x 2 comparisons, statistically insignificant values and low G-scores indicate homogeneity among populations, while statistically significant values with high G-scores are indicative of distinctiveness. G-score p-values are significant below 0.05 and indicate non-homogeneity among populations. Generally, groups within the major races were not found to be significantly different. Notable exceptions were the Tanta population when compared to the sub-Saharan African groups and the Mapuche and Navajo populations when compared to other Native Americans. No significant differences were seen between the Tanta population and the Caucasoid groups. Similarly, the Mapuche and Navajo populations were not significantly different when compared to the Caucasian groups.

**Figure 3. Maximum Likelihood Tree with Bootstrap Values**

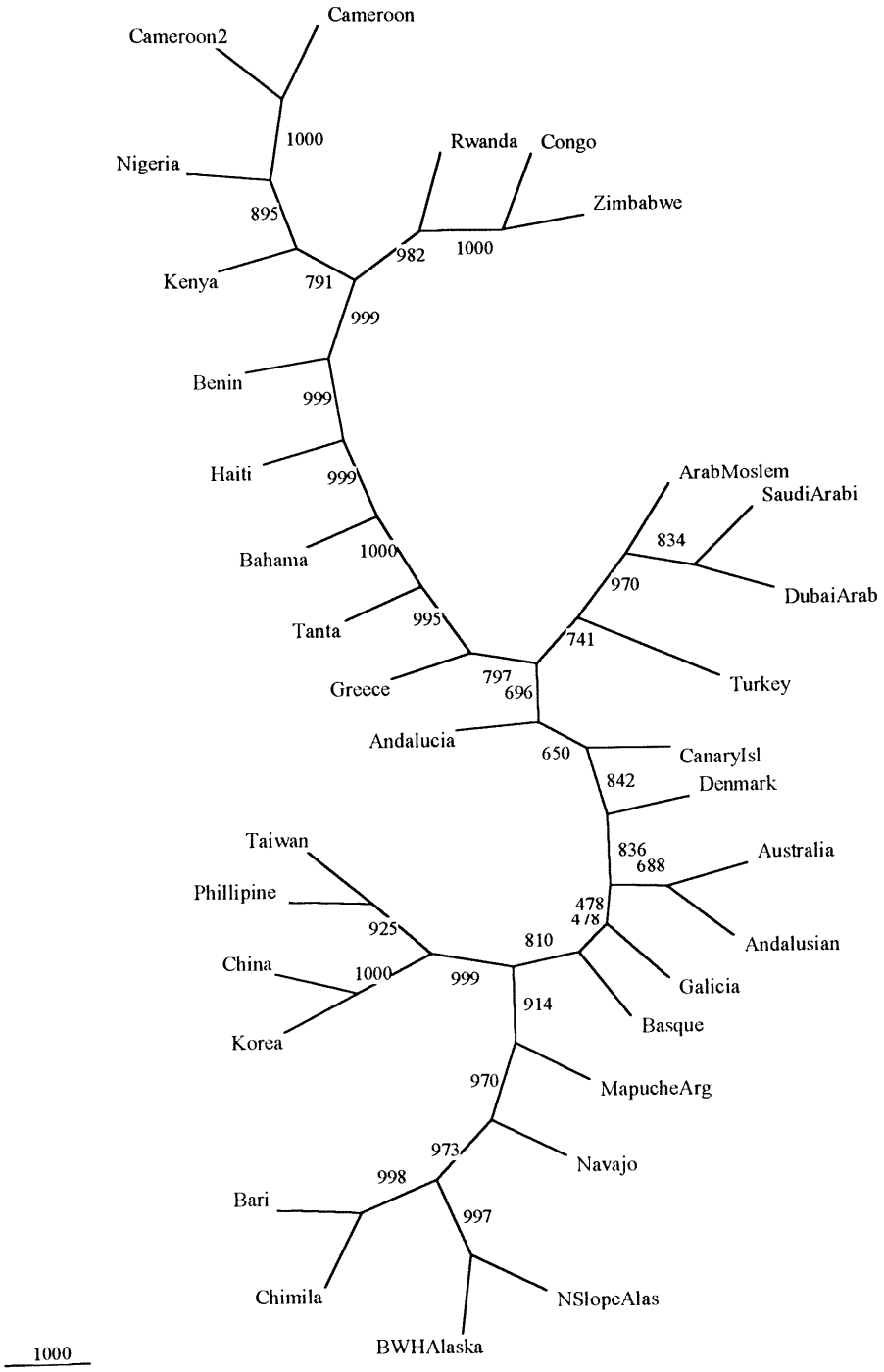


**Figure 4. Principal Component Plot**

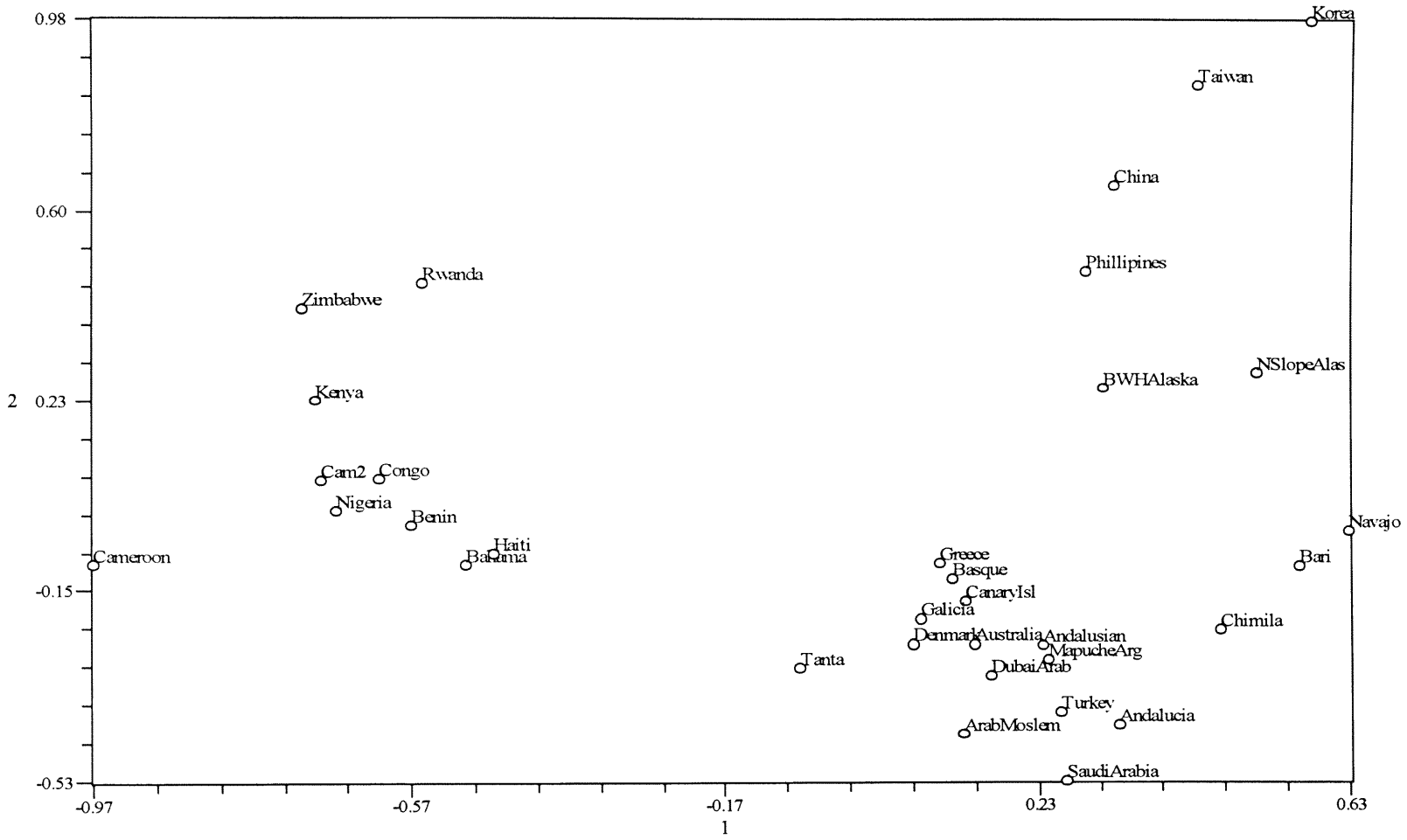


**Table 4. Observed/Expected homozygosity and heterozygosity values**

| Population | N | Observed Homozygosity % | Expected Homozygosity Unbiased (%) | Observed Heterozygosity % | Expected Heterozygosity Unbiased (%) |
|---|---|---|---|---|---|
| Benin | 100 | 15 | 10.28 | 85 | 89.72 |
| Kenya | 106 | 6.6 | 11.18 | 93.4 | 88.82 |
| Rwanda | 100 | 15 | 10.87 | 85 | 89.13 |
| Tanta | 100 | 28 | 20.62 | 72 | 79.38 |
| Cameroon | 16 | 18.8 | 15.73 | 81.3 | 84.27 |

**Table 5. Test for Hardy-Weinberg Equilibrium and Power of Discrimination**

| Population | N | Fisher Exact (3200 shufflings) (p) | Chi Square (3200 shufflings) (p) | Power of Discrimination % |
|---|---|---|---|---|
| Benin | 100 | 0.019 | 0.109 | 96.98 |
| Kenya | 106 | 0.704 | 0.212 | 97.03 |
| Rwanda | 100 | 0.247 | 0.036 | 97.06 |
| Tanta | 100 | 0.049 | 0.377 | 93.48 |
| Cameroon | 16 | 0.879 | 0.492 | 92.19 |

**Table 6. G Scores with probability values between all 33 populations**

| | Anc | Ans | Ara | Aus | Bah | Bar | Bas | Ben | BWH | Cam | Ca2 | Can | Chm | Chn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Andalucia | | 39.607 | 37.366 | 38.200 | 113.841 | 110.568 | 44.118 | 143.180 | 152.635 | 130.007 | 100.841 | 26.710 | 242.916 | 123.671 |
| Andalusian | 0.904 | | 47.932 | 25.949 | 93.360 | 117.457 | 49.165 | 113.126 | 144.274 | 111.033 | 77.863 | 28.462 | 212.149 | 131.290 |
| ArabMoslem | 0.895 | 0.732 | | 58.012 | 75.265 | 129.990 | 78.713 | 102.207 | 179.643 | 100.351 | 74.700 | 33.556 | 230.491 | 146.251 |
| Australia | 0.937 | 1.000 | 0.322 | | 130.877 | 118.601 | 38.410 | 172.272 | 162.712 | 150.267 | 108.843 | 30.595 | 275.305 | 156.095 |
| Bahama | 0.000 | 0.000 | 0.008 | 0.000 | | 151.487 | 140.302 | 17.390 | 162.303 | 31.186 | 43.138 | 98.566 | 231.274 | 163.705 |
| Bari | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | 103.104 | 167.631 | 65.014 | 141.848 | 106.582 | 113.677 | 65.327 | 117.950 |
| Basque | 0.803 | 0.744 | 0.012 | 0.971 | 0.000 | 0.001 | | 178.190 | 141.540 | 152.011 | 110.707 | 42.610 | 274.587 | 152.081 |
| Benin | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | | 176.910 | 31.453 | 37.415 | 121.300 | 251.940 | 218.647 |
| Bethel-Wade | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.009 | 0.001 | 0.000 | | 162.327 | 111.761 | 160.778 | 171.059 | 144.319 |
| Cameroon | 0.000 | 0.000 | 0.000 | 0.000 | 0.967 | 0.000 | 0.000 | 0.958 | 0.000 | | 21.447 | 119.943 | 180.598 | 163.019 |
| Cameroon2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.677 | 0.000 | 0.000 | 0.812 | 0.000 | 1.000 | | 94.847 | 121.522 | 120.043 |
| CanaryIsland | 0.996 | 0.998 | 0.963 | 0.995 | 0.000 | 0.000 | 0.888 | 0.000 | 0.000 | 0.000 | 0.000 | | 233.634 | 109.141 |
| Chimila | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | 279.437 |
| China | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| Congo | 0.000 | 0.000 | 0.000 | 0.000 | 0.176 | 0.000 | 0.000 | 0.425 | 0.000 | 0.934 | 0.993 | 0.000 | 0.000 | 0.000 |
| Denmark | 0.994 | 0.995 | 0.294 | 1.000 | 0.000 | 0.000 | 0.993 | 0.000 | 0.000 | 0.000 | 0.000 | 0.997 | 0.000 | 0.001 |
| DubaiArab | 0.674 | 0.723 | 0.975 | 0.575 | 0.000 | 0.000 | 0.157 | 0.000 | 0.000 | 0.000 | 0.000 | 0.935 | 0.000 | 0.000 |
| Galicia | 0.902 | 0.990 | 0.146 | 0.976 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.998 | 0.000 | 0.001 |
| Greece | 0.814 | 0.629 | 0.830 | 0.112 | 0.000 | 0.000 | 0.424 | 0.000 | 0.000 | 0.000 | 0.000 | 0.999 | 0.000 | 0.000 |
| Haiti | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.992 | 0.773 | 0.000 | 0.000 | 0.000 |
| Kenya | 0.000 | 0.000 | 0.000 | 0.000 | 0.995 | 0.000 | 0.000 | 0.957 | 0.000 | 0.975 | 0.645 | 0.000 | 0.000 | 0.000 |
| Korea | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.998 |
| Mapuche | 0.714 | 0.773 | 0.661 | 0.827 | 0.000 | 0.000 | 0.689 | 0.000 | 0.001 | 0.000 | 0.000 | 0.565 | 0.000 | 0.034 |
| Navajo | 0.366 | 0.697 | 0.112 | 0.753 | 0.001 | 0.004 | 0.588 | 0.000 | 0.166 | 0.000 | 0.004 | 0.289 | 0.000 | 0.079 |
| Nigeria | 0.000 | 0.000 | 0.009 | 0.000 | 1.000 | 0.000 | 0.000 | 1.000 | 0.000 | 1.000 | 0.960 | 0.000 | 0.000 | 0.000 |
| NorthSlope | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.024 | 0.000 | 0.000 | 0.319 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Philippines | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 0.243 |
| Rwanda | 0.000 | 0.000 | 0.000 | 0.000 | 0.061 | 0.000 | 0.000 | 0.566 | 0.000 | 0.349 | 0.678 | 0.000 | 0.000 | 0.000 |
| SaudiArabia | 0.054 | 0.001 | 0.329 | 0.001 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.005 | 0.000 | 0.001 |
| Taiwan | 0.000 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.002 | 0.000 | 1.000 |
| Tanta | 0.944 | 0.978 | 0.995 | 0.843 | 0.134 | 0.000 | 0.799 | 0.005 | 0.000 | 0.002 | 0.032 | 0.966 | 0.000 | 0.000 |
| Turkey | 0.999 | 0.694 | 0.999 | 0.751 | 0.000 | 0.000 | 0.247 | 0.000 | 0.000 | 0.000 | 0.000 | 0.983 | 0.000 | 0.000 |
| Zimbabwe | 0.000 | 0.000 | 0.000 | 0.000 | 0.227 | 0.000 | 0.000 | 0.546 | 0.000 | 0.922 | 0.891 | 0.000 | 0.000 | 0.000 |

* G Scores are read with the vertical population columns at the side named first then the horizontal population columns at the top.

Probability values are read with the horizontal population columns at the top first, followed by the vertical population columns at the side.

**Table 6. G Scores with probability values between all 33 populations (Con't)**

| | Con | Den | Dub | Gal | Gre | Hai | Ken | Kor | Map | Nav | Nig | NSA | Phi | Rwa |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Andalucia | 146.468 | 30.276 | 45.073 | 40.870 | 40.924 | 125.557 | 153.487 | 126.254 | 41.104 | 49.716 | 131.656 | 169.899 | 108.519 | 168.469 |
| Andalusian | 111.572 | 31.112 | 45.934 | 30.928 | 47.290 | 106.217 | 135.584 | 138.957 | 43.099 | 43.987 | 109.624 | 157.981 | 120.831 | 142.548 |
| ArabMoslem | 97.702 | 56.502 | 31.172 | 62.444 | 39.725 | 89.025 | 99.273 | 139.397 | 41.504 | 56.448 | 78.158 | 192.991 | 120.772 | 117.153 |
| Australia | 171.063 | 20.131 | 50.561 | 34.893 | 65.816 | 155.570 | 191.049 | 168.103 | 41.717 | 42.383 | 156.745 | 172.030 | 147.608 | 223.569 |
| Bahama | 56.588 | 121.455 | 97.947 | 115.624 | 91.023 | 9.918 | 26.481 | 130.172 | 84.808 | 80.533 | 20.551 | 227.178 | 159.925 | 65.632 |
| Bari | 142.303 | 123.785 | 112.306 | 109.924 | 120.636 | 170.148 | 181.735 | 120.155 | 76.470 | 61.784 | 175.901 | 59.775 | 115.429 | 166.623 |
| Basque | 179.871 | 32.600 | 62.875 | 25.339 | 53.222 | 167.673 | 209.024 | 158.468 | 45.585 | 46.337 | 168.817 | 159.639 | 128.989 | 223.925 |
| Benin | 46.469 | 148.110 | 128.501 | 140.188 | 110.725 | 18.432 | 32.126 | 183.707 | 107.236 | 92.984 | 18.197 | 229.203 | 174.025 | 46.187 |
| Bethel-Wade | 187.132 | 156.341 | 150.532 | 165.211 | 151.856 | 173.671 | 221.464 | 147.171 | 75.158 | 51.168 | 193.508 | 46.936 | 160.884 | 207.418 |
| Cameroon | 33.435 | 136.752 | 113.046 | 128.385 | 105.311 | 27.216 | 30.811 | 137.238 | 90.682 | 112.572 | 22.213 | 206.225 | 157.942 | 50.547 |
| Cameroon2 | 29.011 | 101.393 | 81.840 | 84.684 | 93.787 | 37.972 | 42.955 | 100.573 | 77.951 | 71.980 | 32.567 | 118.487 | 106.827 | 41.962 |
| CanaryIsland | 119.099 | 26.296 | 35.715 | 29.656 | 26.248 | 112.040 | 126.744 | 119.247 | 44.023 | 51.156 | 107.602 | 160.159 | 81.753 | 132.269 |
| Chimila | 179.004 | 280.336 | 228.222 | 256.023 | 232.589 | 249.148 | 276.121 | 254.587 | 147.515 | 93.234 | 239.257 | 186.523 | 223.738 | 264.791 |
| China | 186.581 | 152.396 | 131.487 | 148.493 | 117.489 | 180.447 | 221.648 | 32.800 | 72.374 | 63.737 | 194.085 | 159.601 | 60.732 | 230.538 |
| Congo | | 161.252 | 117.265 | 130.518 | 108.194 | 55.783 | 52.436 | 152.371 | 125.675 | 102.051 | 35.139 | 191.858 | 142.699 | 38.328 |
| Denmark | 0.000 | | 48.415 | 29.092 | 57.369 | 141.032 | 179.522 | 166.526 | 50.298 | 47.231 | 140.014 | 157.979 | 130.925 | 196.173 |
| DubaiArab | 0.000 | 0.604 | | 40.832 | 45.101 | 110.938 | 126.084 | 130.129 | 45.437 | 55.877 | 102.245 | 145.521 | 128.256 | 122.977 |
| Galicia | 0.000 | 1.000 | 0.851 | | 52.790 | 135.106 | 161.797 | 137.335 | 62.296 | 47.986 | 134.122 | 156.447 | 121.664 | 157.906 |
| Greece | 0.000 | 0.260 | 0.595 | 0.384 | | 101.656 | 113.479 | 123.019 | 48.039 | 64.533 | 95.708 | 177.959 | 96.639 | 107.389 |
| Haiti | 0.127 | 0.000 | 0.000 | 0.000 | 0.000 | | 21.703 | 142.693 | 95.959 | 93.759 | 20.347 | 238.417 | 171.147 | 67.002 |
| Kenya | 0.294 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | | 177.234 | 119.720 | 113.156 | 13.760 | 282.176 | 202.894 | 49.228 |
| Korea | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | 96.236 | 71.044 | 162.908 | 171.779 | 98.165 | 185.059 |
| Mapuche | 0.000 | 0.468 | 0.611 | 0.121 | 0.365 | 0.000 | 0.000 | 0.000 | | 33.734 | 109.913 | 103.612 | 81.819 | 136.517 |
| Navajo | 0.000 | 0.547 | 0.132 | 0.544 | 0.020 | 0.000 | 0.000 | 0.040 | 0.770 | | 101.281 | 53.518 | 56.840 | 105.015 |
| Nigeria | 0.926 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | | 234.626 | 166.791 | 30.837 |
| NorthSlope | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.135 | 0.000 | | 138.991 | 228.179 |
| Philippines | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.103 | 0.000 | 0.000 | | 173.564 |
| Rwanda | 0.850 | 0.000 | 0.000 | 0.000 | 0.000 | 0.047 | 0.425 | 0.000 | 0.000 | 0.000 | 0.983 | 0.000 | 0.000 | |
| SaudiArabia | 0.000 | 0.001 | 0.361 | 0.001 | 0.027 | 0.000 | 0.000 | 0.000 | 0.001 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 |
| Taiwan | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.693 | 0.072 | 0.304 | 0.000 | 0.000 | 0.995 | 0.000 |
| Tanta | 0.000 | 0.971 | 0.894 | 0.918 | 0.989 | 0.010 | 0.001 | 0.000 | 0.617 | 0.211 | 0.017 | 0.000 | 0.000 | 0.000 |
| Turkey | 0.000 | 0.687 | 0.766 | 0.433 | 0.973 | 0.000 | 0.000 | 0.000 | 0.788 | 0.145 | 0.000 | 0.000 | 0.000 | 0.000 |
| Zimbabwe | 0.969 | 0.000 | 0.000 | 0.000 | 0.000 | 0.415 | 0.646 | 0.000 | 0.000 | 0.000 | 0.942 | 0.000 | 0.000 | 0.183 |

* G Scores are read with the vertical population columns at the side named first then the horizontal population columns at the top.

Probability values are read with the horizontal population columns at the top first, followed by the vertical population columns at the side.

**Table 6. G Scores with probability values between all 33 populations (Con't)**

| | Sau | Tai | Tan | Tur | Zim |
|---|---|---|---|---|---|
| Andalucia | 65.720 | 103.030 | 36.641 | 26.075 | 222.281 |
| Andalusian | 104.940 | 103.976 | 33.689 | 48.917 | 193.150 |
| ArabMoslem | 51.768 | 107.591 | 29.973 | 27.009 | 159.421 |
| Australia | 128.394 | 120.909 | 42.397 | 46.275 | 285.404 |
| Bahama | 175.476 | 121.110 | 59.671 | 147.989 | 54.610 |
| Bari | 176.081 | 122.218 | 133.725 | 119.068 | 191.230 |
| Basque | 137.352 | 114.901 | 44.065 | 59.029 | 298.696 |
| Benin | 218.823 | 145.400 | 75.585 | 152.219 | 44.397 |
| Bethel-Wade | 296.155 | 126.507 | 148.347 | 117.485 | 245.616 |
| Cameroon | 159.837 | 133.847 | 80.703 | 139.143 | 35.580 |
| Cameroon2 | 110.536 | 97.612 | 65.794 | 106.624 | 36.584 |
| CanaryIsland | 76.757 | 82.041 | 33.659 | 32.125 | 189.462 |
| Chimila | 344.003 | 230.828 | 232.619 | 241.360 | 273.669 |
| China | 308.712 | 23.733 | 129.085 | 137.124 | 272.791 |
| Congo | 173.634 | 136.409 | 98.742 | 137.854 | 31.104 |
| Denmark | 99.333 | 119.295 | 33.970 | 46.820 | 265.064 |
| DubaiArab | 47.847 | 120.199 | 37.462 | 45.368 | 207.168 |
| Galicia | 97.748 | 117.993 | 38.109 | 54.235 | 233.191 |
| Greece | 67.310 | 86.571 | 27.537 | 32.431 | 175.021 |
| Haiti | 183.290 | 133.163 | 71.018 | 144.069 | 48.656 |
| Kenya | 228.369 | 166.715 | 81.392 | 156.318 | 43.631 |
| Korea | 277.317 | 48.672 | 133.840 | 149.635 | 213.142 |
| Mapuche | 81.088 | 61.267 | 45.510 | 39.556 | 162.977 |
| Navajo | 74.869 | 50.194 | 56.984 | 56.483 | 119.820 |
| Nigeria | 164.973 | 143.123 | 70.595 | 131.079 | 34.258 |
| NorthSlope | 303.174 | 140.537 | 179.921 | 186.407 | 296.454 |
| Philippines | 209.880 | 27.685 | 111.666 | 115.354 | 208.422 |
| Rwanda | 236.932 | 159.017 | 100.531 | 163.188 | 55.842 |
| SaudiArabia | | 213.788 | 46.167 | 49.613 | 342.655 |
| Taiwan | 0.000 | | 96.118 | 107.290 | 179.009 |
| Tanta | 0.568 | 0.000 | | 36.962 | 147.280 |
| Turkey | 0.497 | 0.000 | 0.937 | | 225.073 |
| Zimbabwe | 0.000 | 0.000 | 0.000 | 0.000 | |

* G Scores are read with the vertical population columns at the side named first then the horizontal population columns at the top.
Probability values are read with the horizontal population columns at the top first, followed by the vertical population columns at the side.

## DISCUSSION

To aid in our comparison of allelic frequencies, we generated allelic frequency graphs from Table 2 for each of our five African populations (Figures 5-9). Graphs were also created for African American and Caucasian samples from the United States (Figures 14 & 15) as well as the four previously data-banked African populations (Figures 10-13).

The African populations examined, including the African Americans, exhibited alleles 18, 24, 28, 31 and 34, indicating genetic similarity. Exceptions to this include both Cameroon samples (Cameroon at allele 31 and Cameroon2 at allele 18), which may be due to sampling error. The two alleles most prevalent in US Caucasians were the same two that were found most frequently in the Tanta population.

The observed heterozygosity for the five African populations in our study was greater than 70%. These heterozygosity values are indicative of the genetic diversity of this locus. Of the five African populations, the Kenya population appears to be the most diverse with a heterozygosity value of 93.4%, compared to reported heterozygosity of 87.6% in the African American population [48]. The discriminatory power of the D1S80 locus was evident, with values above 90% for all five populations. Previous reports have indicated that the discrimination power for this locus is between 94 and 98% [49].

Of the five populations in our study, only the Benin population was significantly out of conformance to Hardy–Weinberg equilibrium expectations, with a Fisher exact test p-value of 0.019. Preferential amplification of specific alleles does not seem to be an explanation since a low number of samples tested were homozygous (15 of the 100

samples). Our Theta values ranged from 0.009 in the Tanta population sample to 0.032 in the Benin samples. High values of theta indicates greater genetic substructure within a population. The overall theta-P value, indicating the variance present in the combination of all the alleles detected in the 5 African populations, for the D1S80 locus was 0.023. This was comparable to published values of 0.019 [50]. The National Research Council II recommends the use of such a factor in all population studies. Recommendation 4.1 of this council's 1996 report suggests an inbreeding coefficient of 0.01 be used for United States populations, while an increased value of 0.03 would better suit more isolated populations [51].

The topology of the NJ tree illustrates the similarities between different populations by clustering them into clades. The Oriental and American Indian groups were separated from Caucasians and sub-Saharan Africans. Caucasians and sub-Saharan Africans are also separated from each other. This tree further discriminated between east Asians, with the two northern samples (Korea and China) grouped independently of the two southern ones (Taiwan and Philippine), and American Indians. The American Indian groups segregate together even though they may not be geographically close to each other, their origins ranging from North America to South America. The sample from Mapuche Argentina is genetically grouped with the Caucasians. The Tanta and Saudi Arabia populations segregated together indicating the historic influence that Saudi Arabian populations had on Tanta.

The UPGMA tree also clustered the populations based on their genetic similarities. Within this tree, the southeastern Europeans (Turkey and Greece) and the southwestern Asians (Saudi Arabia, Dubai Arab and Arab Moslem) were split from the

**Figure 5. Allelic Distribution Graph of the Benin Population**

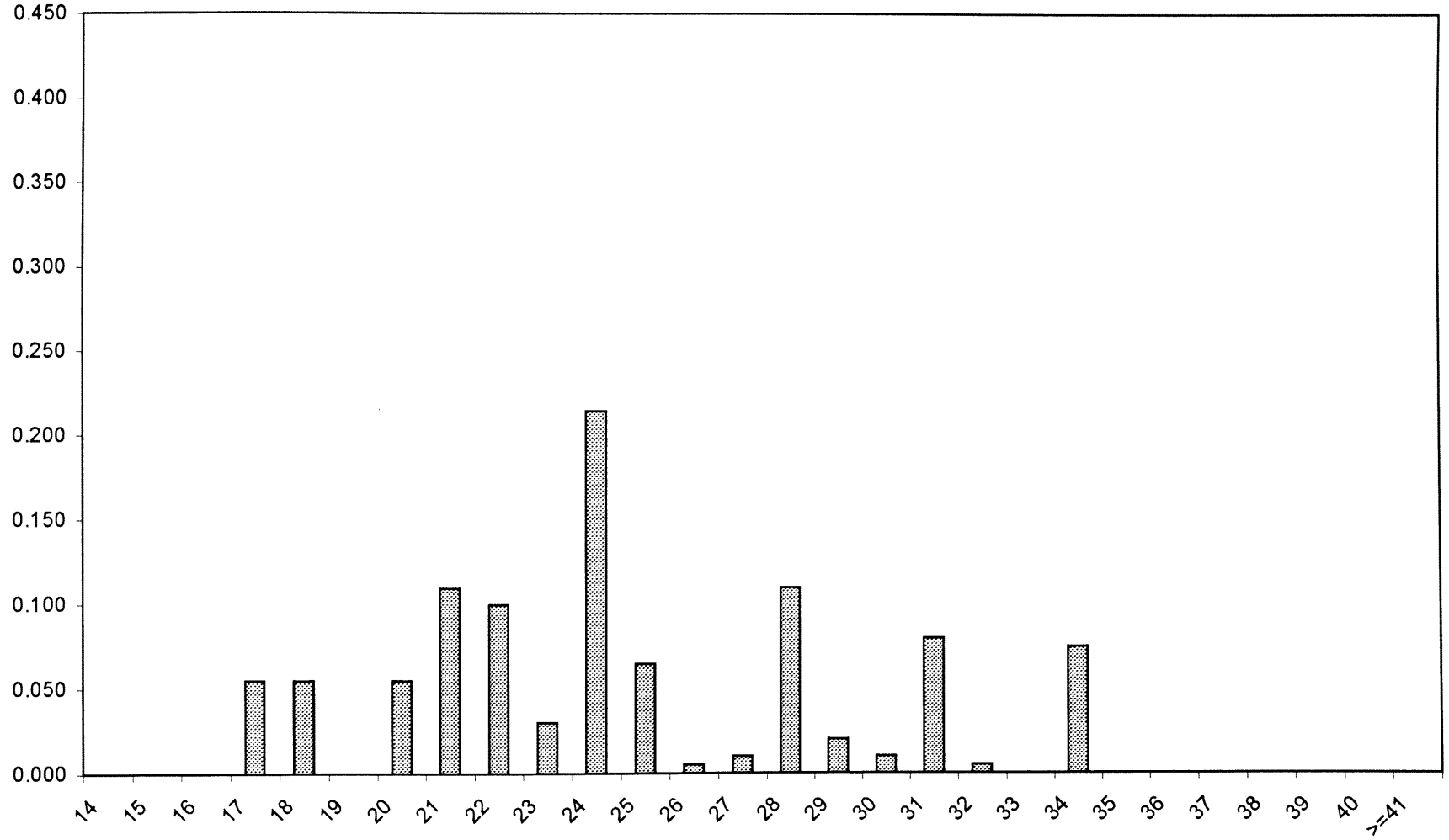


**Figure 6. Allelic Distribution Graph of the Kenya Population**

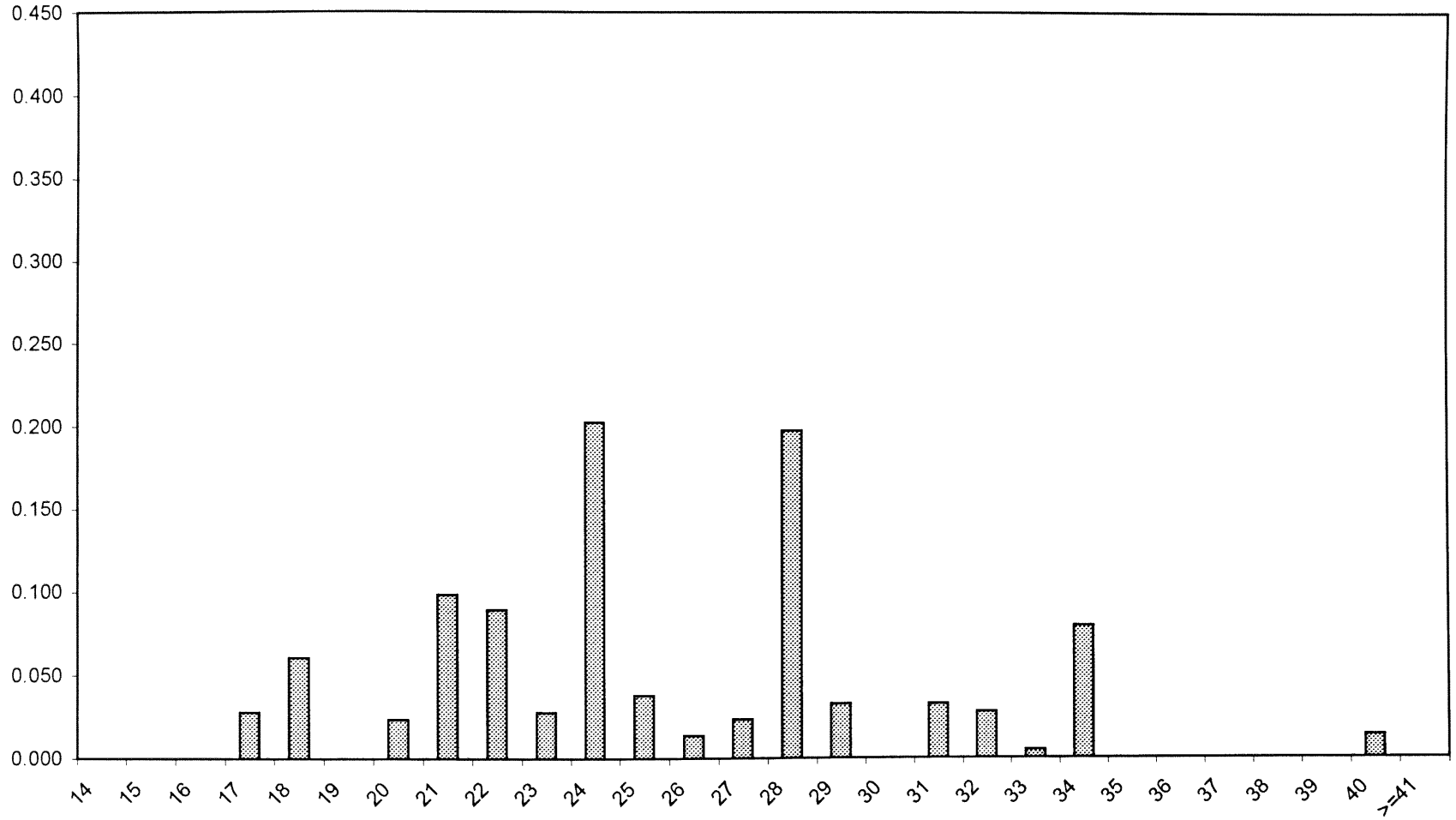


**Figure 7. Allelic Distribution Graph of the Rwanda Population**

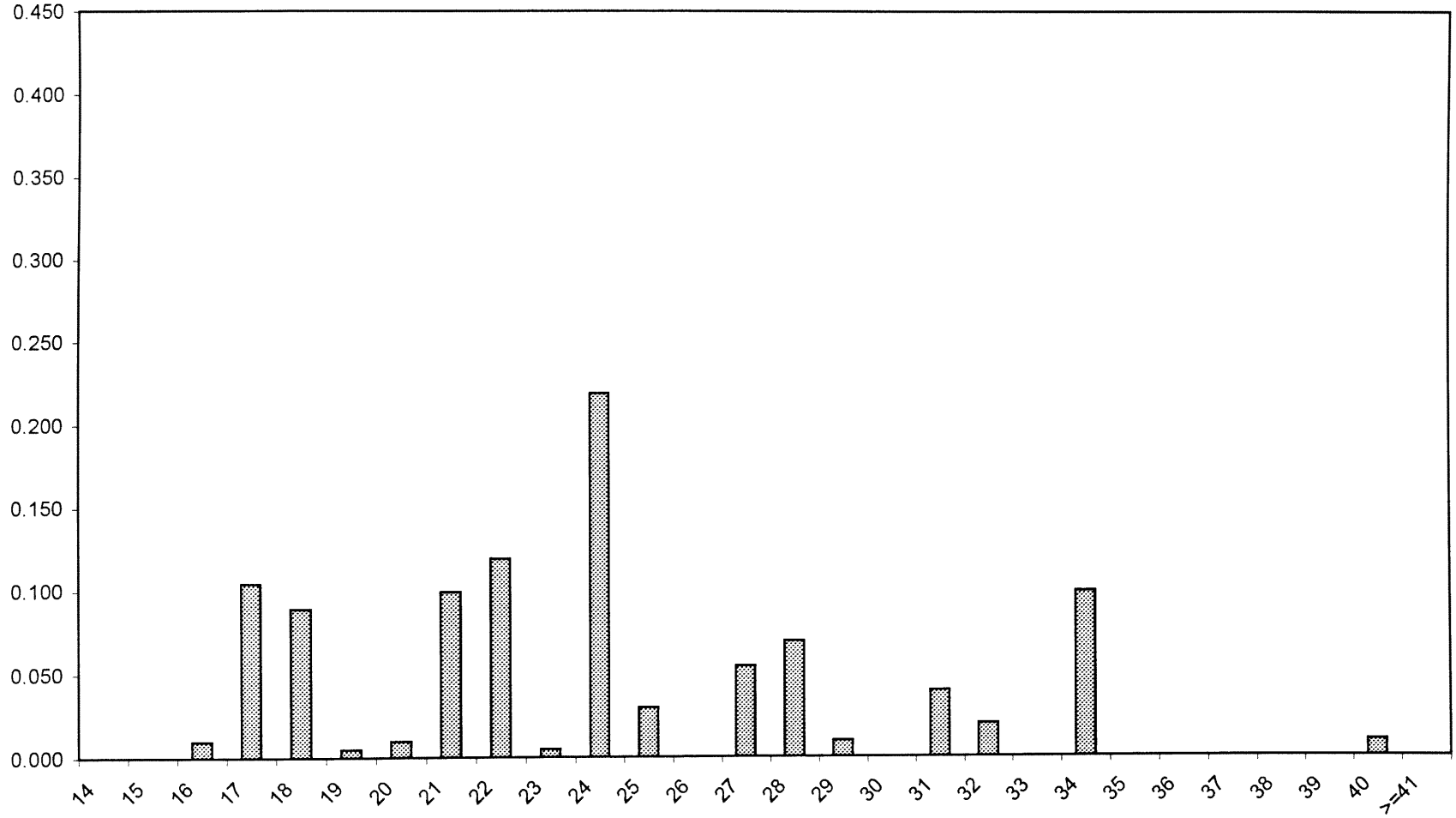


**Figure 8. Allelic Distribution Graph of the Tanta Population**

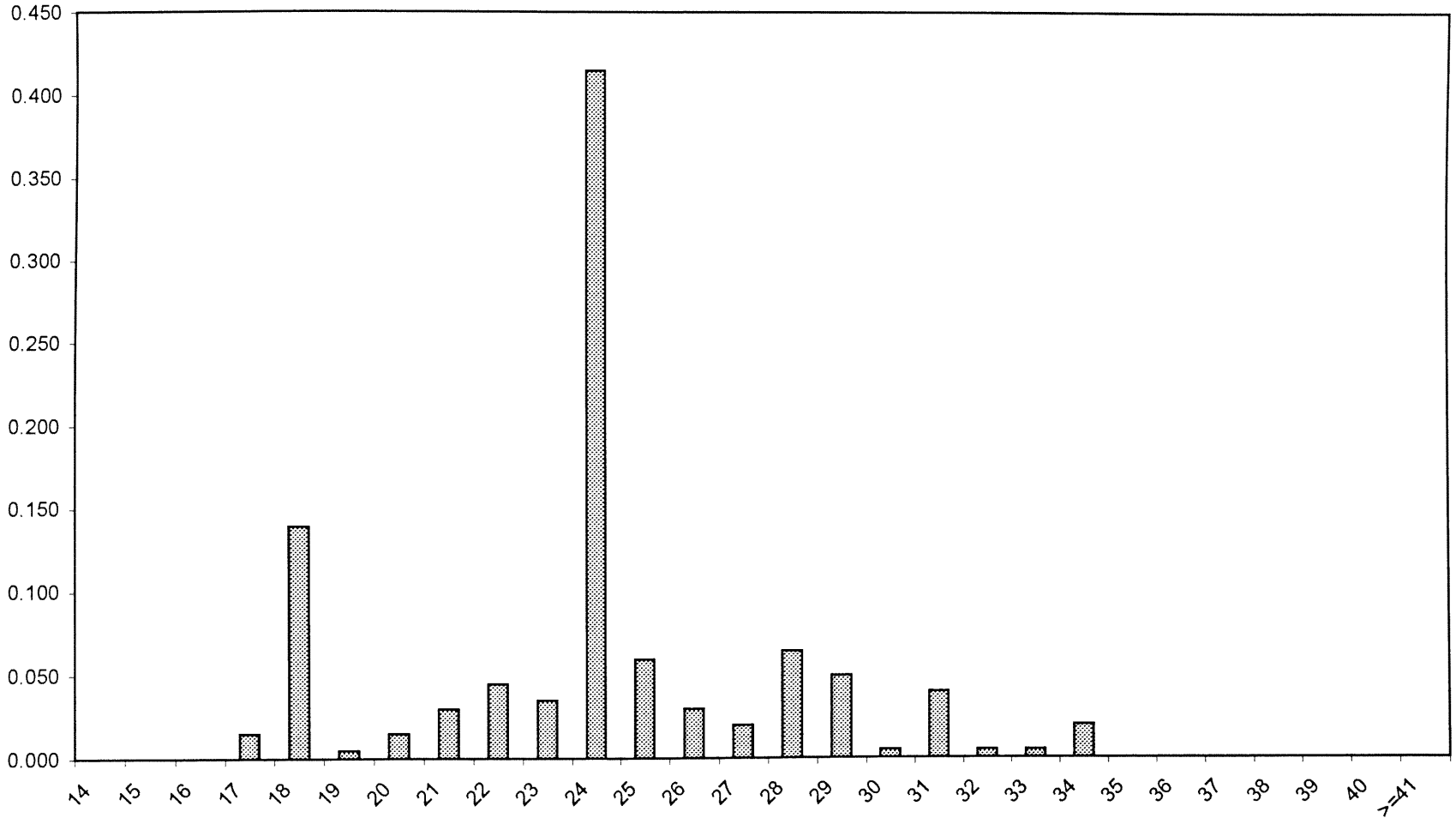


**Figure 9. Allelic Distribution Graph of the Cameroon2 Population**

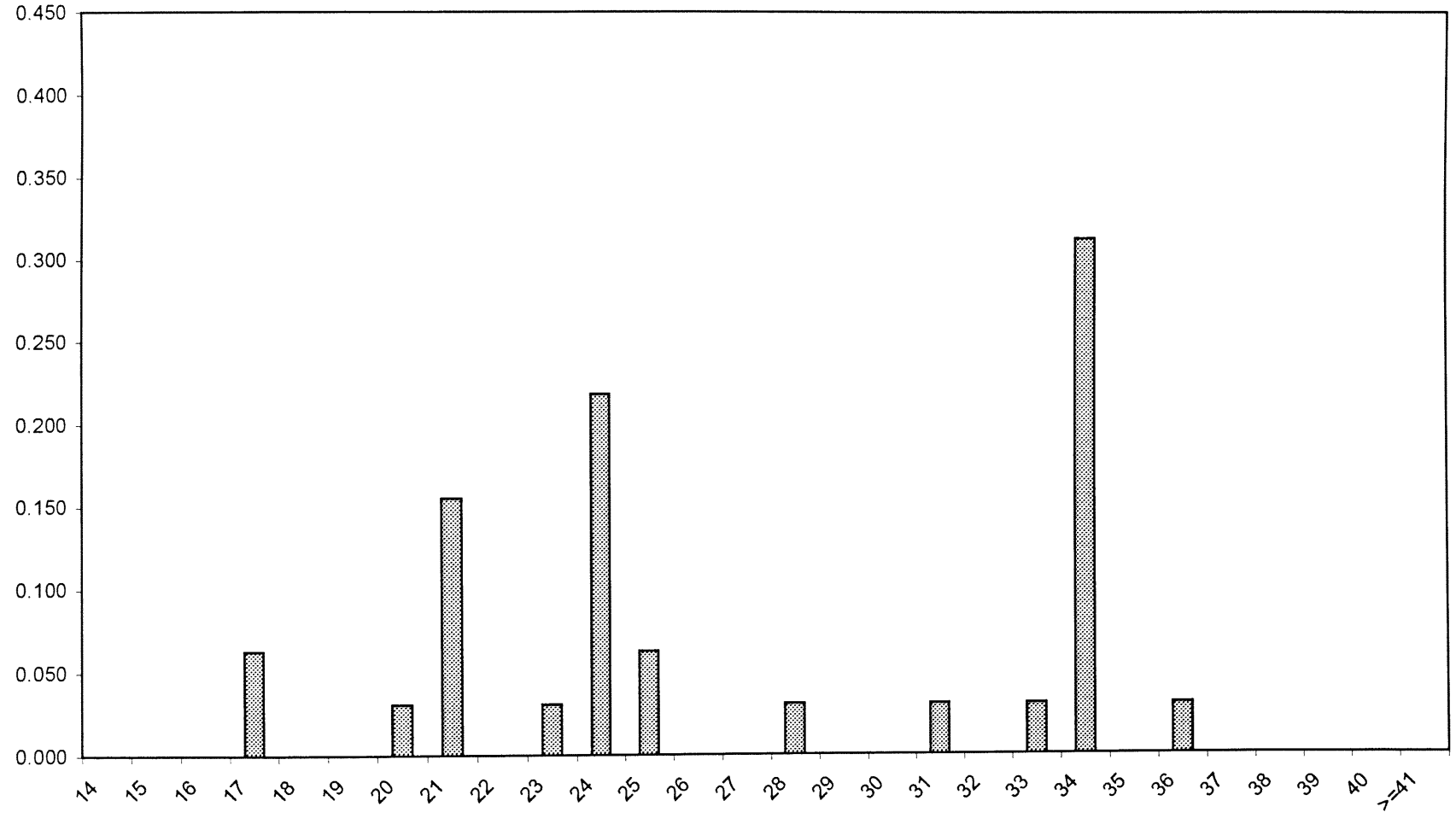


**Figure 10. Allelic Distribution Graph of the Cameroon Population [26]**

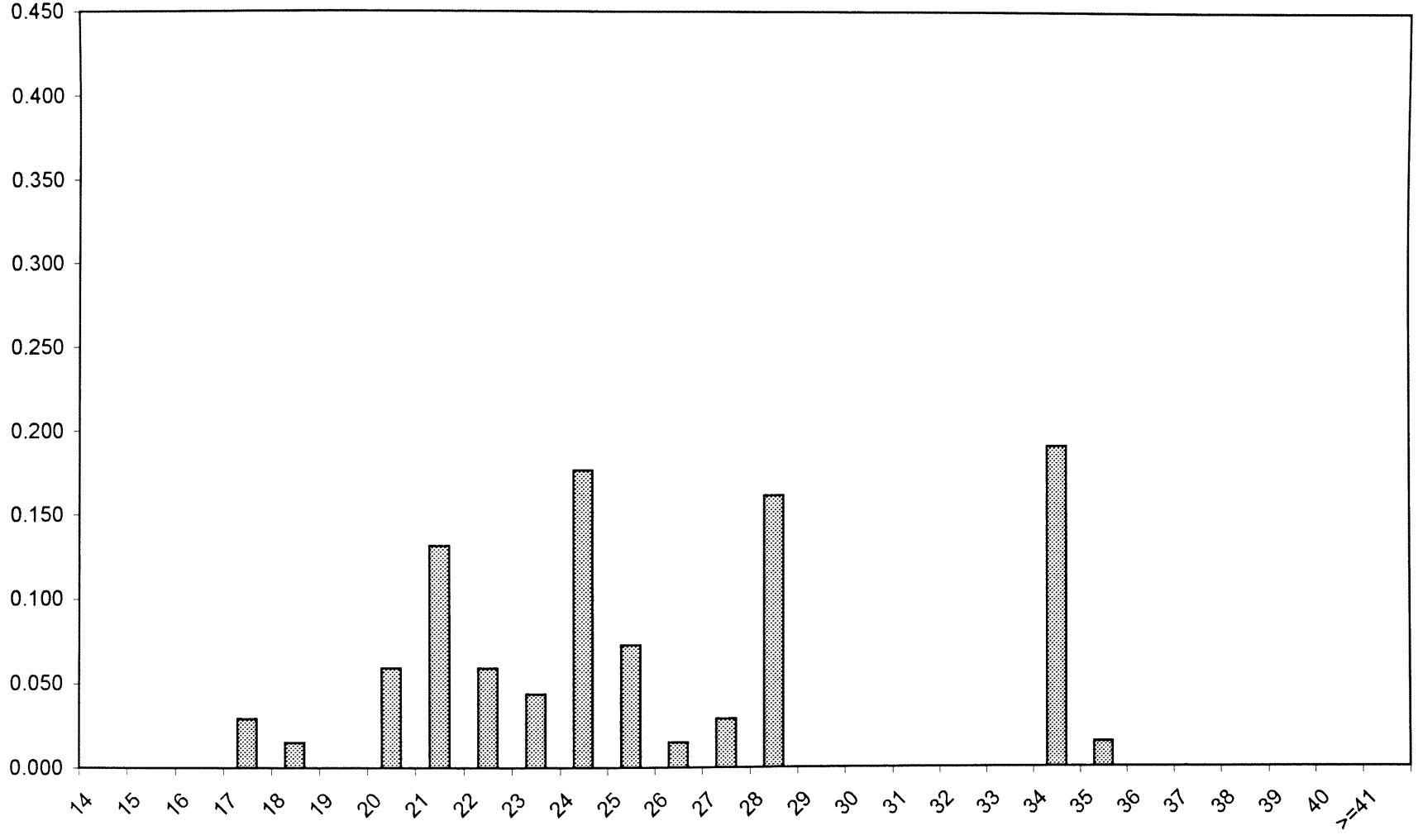


**Figure 11. Allelic Distribution Graph of the Congo Population [26]**

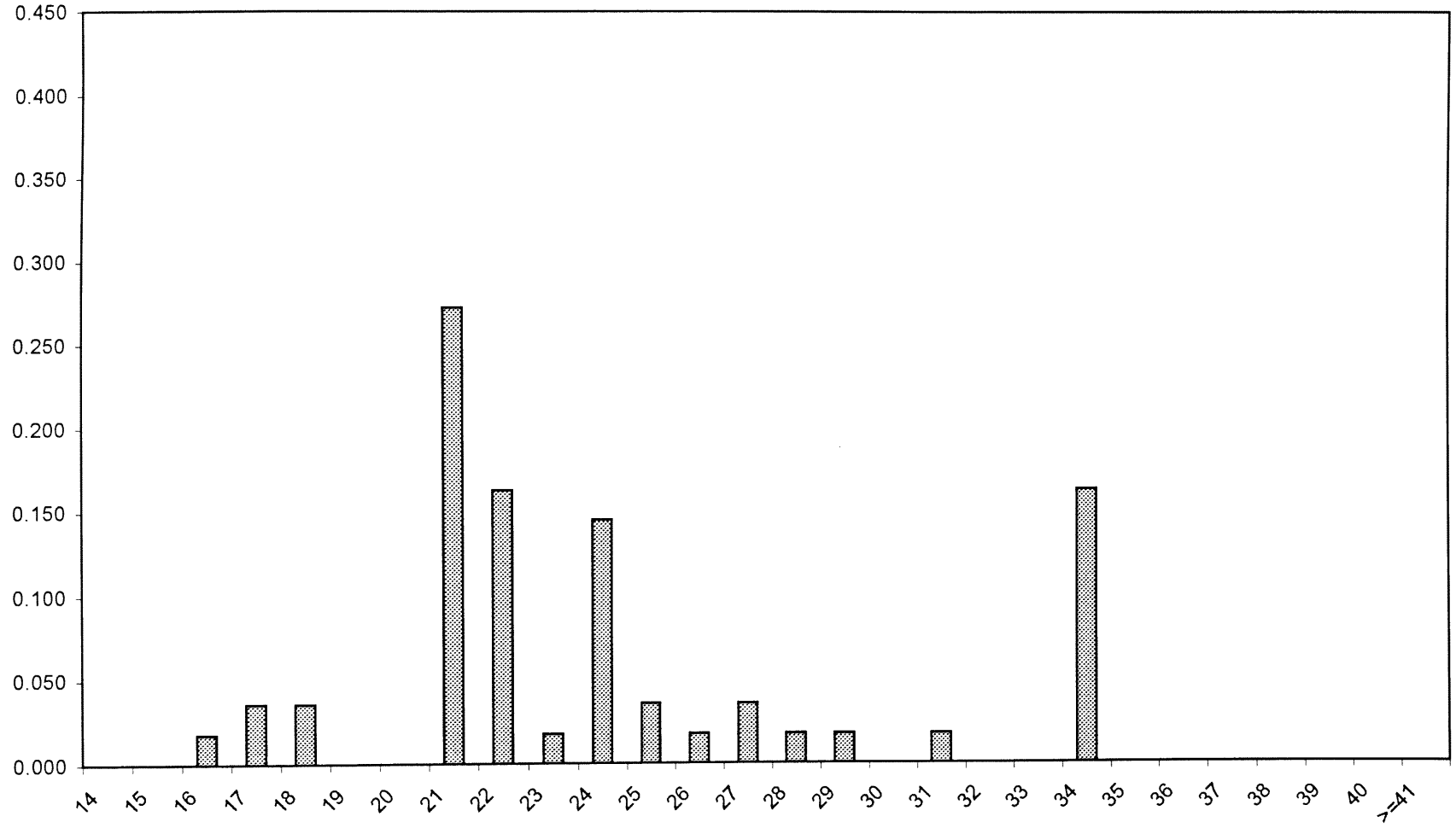


**Figure 12. Allelic Distribution Graph of the Nigeria Population [28]**

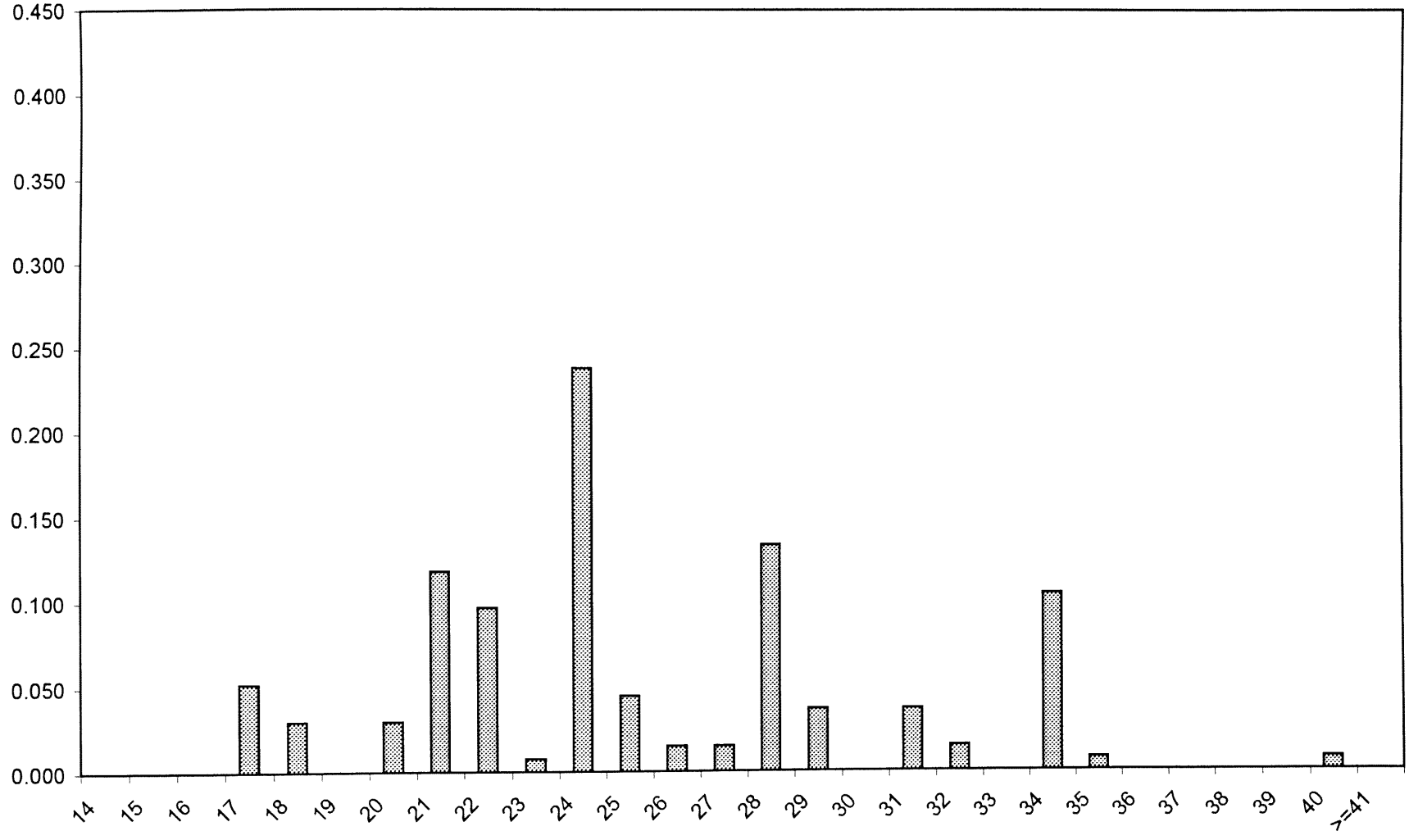


**Figure 13. Allelic Distribution Graph of the Zimbabwe Population [28]**

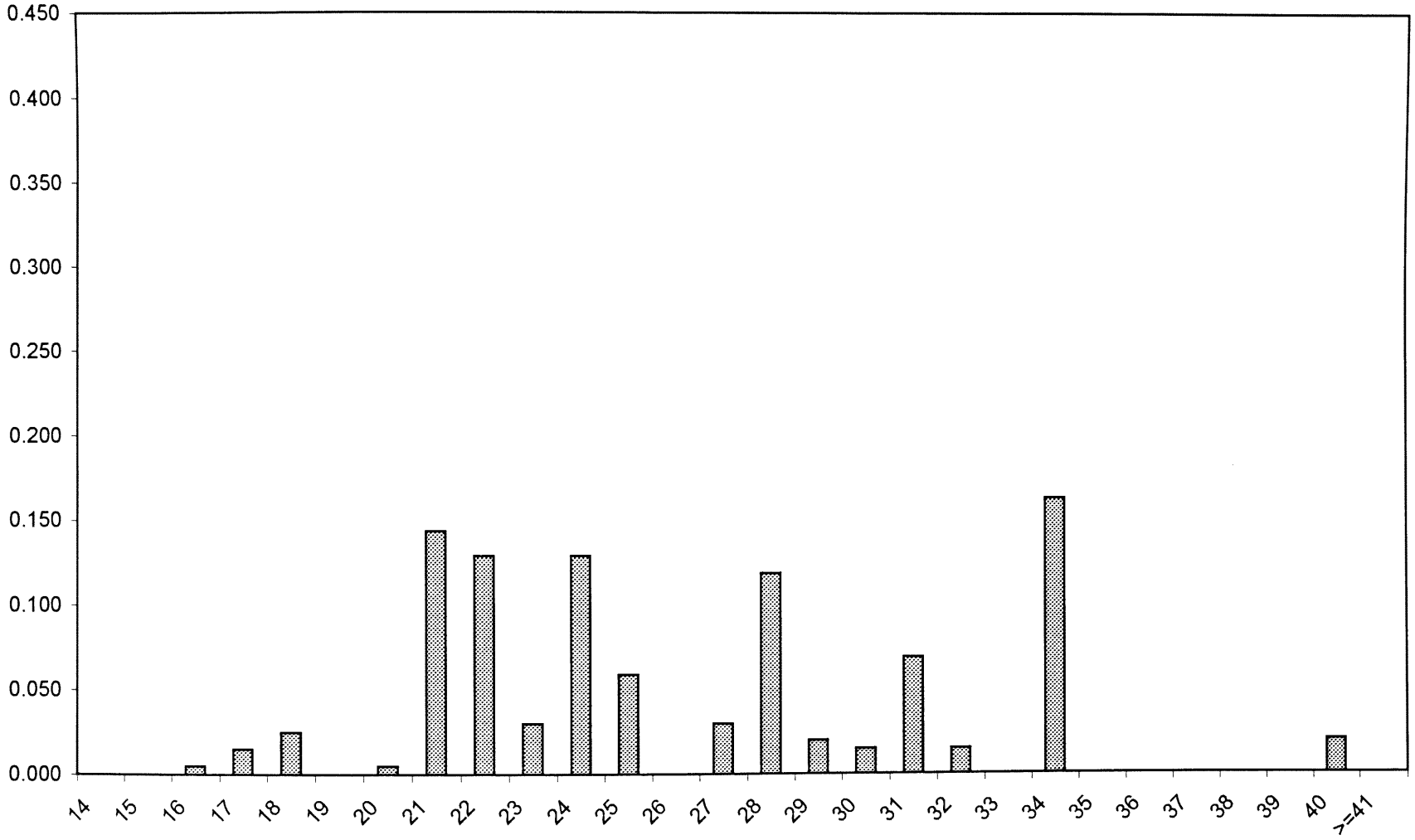


**Figure 14. Allelic Distribution Graph of the African American Population [48]**

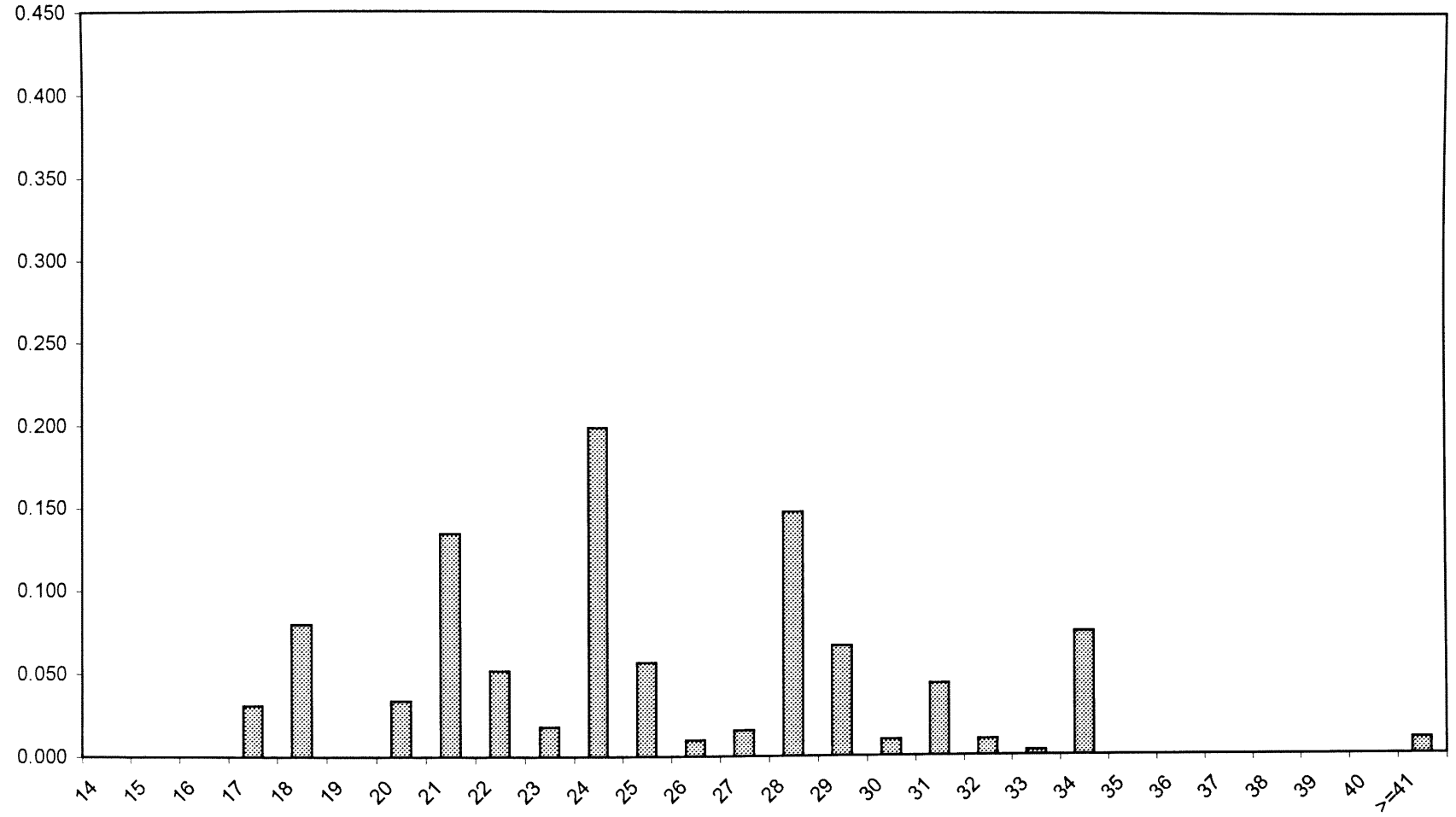
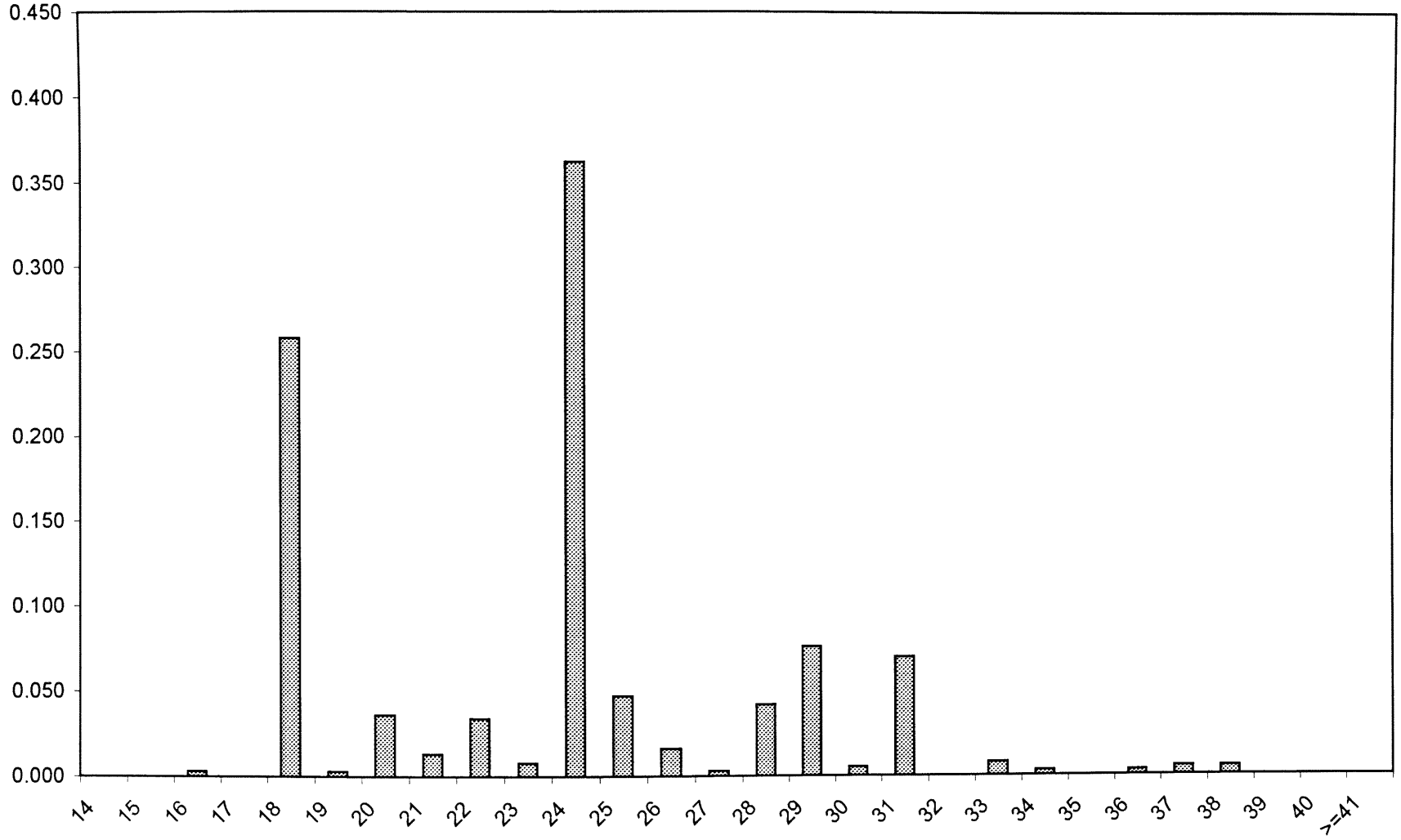


**Figure 15. Allelic Distribution Graph of the Caucasian Population [48]**



rest of the Caucasians. This is indicative of the variation detected between these groups. The Mapuche sample was more closely grouped to the southwest Asians than to the rest of the Caucasians. The two Alaskan groups show great homogeneity by segregating together. The east Asians are once again separately grouped into northern and southern regions.

Unlike the other two tree building methods, the ML dendrogram represented calculated distances based on the differences between the populations. Within this depiction, the southwest Asian groups (Saudi Arabia, Dubai Arab and Arab Moslem) are again separated from the other Caucasian populations. The east Asians are also shown to be distinct from the Native American groups even though they appear in the same cluster. The east Asians are segregated into northern and southern groups. The two Columbian Indian populations and the two Alaskan populations are shown to be genetically similar.

Throughout the previous analyses, consistent segregations were observed. The five African populations examined in our study were invariably grouped with the sub-Saharan African cluster, with the exception of the one Egyptian sample. The 16 Cameroon samples of our study segregated closely with the previously published Cameroon population [26].

The PC plot yielded a visual representation of allelic variability between the groups. This two-dimensional plot represented 35% of the total variation among the 27 alleles inputted. The x-axis of the PC clearly delineates the sub-Saharan African populations from the other populations. The north African population, Tanta, falls between this group and the Caucasians, nearer to the latter. Along the y-axis, the west Africans and west African derived populations are further segregated within the sub-

Saharan cluster. The y-axis also separates east Asians from the American Indians and middle eastern populations from the rest of the Caucasians. Negroids showed the greatest variability between the alleles with a value of 0.5046. This is indicated in the figure by the sparse arrangement of these populations. The Mongoloids represent the second most diverse group and are also sparsely gathered on the right side of the PC plot. The east Asians segregate together, as do the American Indians. Homogeneity is shown between the two Alaskan populations and the two Columbian populations that segregate near to each other. Similar to the NJ and UPGMA phylogenies, the Mapuche population segregates with the Caucasians, nearer to the southwest Asians. The Caucasian populations are clustered together closely denoting less diversity between the groups. However, segregation is still visible between the southwest Asians and the rest of the Caucasians. The Caucasians showed the least variability between their alleles with a value of 0.2662.

The G-Test displayed homogeneity within the major races. It is in line with the other analyses that we performed in regard to the racial groupings of the populations. This analysis also grouped the Tanta and Mapuche populations with the Caucasians.

In this study we report on D1S80 databases from five African populations. The D1S80 locus provided us with a quick view of the phylogenetic relationships of the 33 populations examined. Although broad categorization of the populations was possible, clearly more polymorphic loci are required to provide for more significant comparisons.

## CONCLUSION

Our study has shown that this single locus is highly polymorphic. It was capable of separating 33 different populations into their constituent ethnic groups of Negroid, Mongoloid or Caucasoid. Each of the phylogenetic analyses performed indicated homogeneity between our African samples amongst themselves and in comparison to the few samples that previously existed from Africa.

The allelic distributions in our African populations were similar to that of other African and African derived populations in the literature. The Tanta population, which is believed to have been descendents of Caucasians, showed a similar distribution of alleles as the United States Caucasians [48].

Although D1S80 is an extensively studied locus, the majority of the data is focused on European populations (sparse database information currently exists for the following African nations: Cameroon, Congo, Nigeria and Zimbabwe). This study is the first to include greater than three African populations. A database useful for human differentiation has been created for four additional African populations.

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