Universidade de Lisboa
Faculdade de Ciências
Departamento de Química e Bioquímica

CHARACTERIZATION OF THE GENETIC STRUCTURE OF THE AZOREAN POPULATION

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Doutoramento em Genética Molecular

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Hospital do Divino Espírito Santo de
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Tese orientada pela Investigadora Doutora Luisa Mota Vieira
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Branco CC, Mota-Vieira L. Surnames in Azores: Analysis of the isonymy structure. *Hum Biol*. 2005; 77: 37-44.

Cabral R, Branco CC, Costa S, Caravello GU, Tasso M, Peixoto BR, Mota-Vieira L. Geography of surnames in Azores: specificity and spatial distribution analysis. *Am J Hum Biol*. 2005; 17: 634-645.

Pacheco PR, Branco CC, Cabral R, Costa S, Araújo AL, Peixoto BR, Mendonça P and Mota-Vieira L. The Y-chromosomal heritage of the Azores Islands population. *Ann Hum Genet*. 2005; 69: 145-156.

Branco CC, Mota-Vieira L. Population structure of São Miguel Island (Azores, Portugal): A surname study. *Hum Biol*. 2003; 75: 929-939.

No cumprimento do disposto na referida deliberação, esclarecemos serem da nossa responsabilidade a execução das experiências que estiveram na base dos resultados apresentados (excepto quando referido em contrário), assim como a sua interpretação e discussão.

PREFACE

Genomic medicine, a biomedical research area which uses the individual information to provide better health care, has been considerably developed since the Human Genome Project. One of its current challenges is the identification of the risk or susceptibility for multifactorial diseases and the study of their frequency in populations. The knowledge produced in this research area, will, most certainly, be responsible for new treatment strategies, such as pharmacogenomics, resulting in more effective and less toxic drugs. This PhD thesis had as major objective contribute to the characterisation of the genetic background and population structure of the Azorean population. The information retrieved from this work is essential in the comprehension of the Azorean diversity and ancestry, which, on the other hand, will be important for the development of genomic medicine, in particular, for the design of future mapping studies in this population.

A detailed overview of the literature concerning human diversity markers, population structure and the advantages of isolated *versus* outbred populations are given in chapters I, II and III, respectively. Chapter I focuses briefly on the contribution of molecular and non-molecular markers, where an introduction of the importance of surnames and of human genome polymorphisms is shown. The use of linkage disequilibrium and its importance in the human genome architecture is demonstrated. Chapter II describes the evolutionary forces, such as genetic drift, selection, mutation and migration, which play a relevant role in the population's structure. Moreover, genetic distance measures and inbreeding are also presented. Chapter III compares isolated and outbred populations in terms of advantages for genetic studies. Examples of five human isolated populations are exhibited.

Chapter IV is devoted to the characterization of the study population, the Azores. Its geographic location, demography, discovery and settlement are introduced. A brief description of other genetic studies in this population and the objectives of this scientific research are given.

Chapters V, VI and VII assemble the scientific work performed in this PhD thesis, which are object of publication in international journals. Chapter V concerns the structure of Azorean population through the analysis of surnames. Chapter VI approaches the Azorean ancestry, with studies of Y-chromosome lineages and *Alu*

insertion polymorphisms. Finally, chapter VII reports the Azorean diversity and structure based on genetic markers located both in autosomes and X-chromosome.

The last chapter of this thesis, chapter VIII, provides a general integrative discussion of the results placing them in perspective with state-of-the-art data in population genetics field. Perspectives for future work are also highlighted.

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"Sometimes our light goes out but is blown into flame by another human being. Each of us owes deepest thanks to those who have rekindled this light."

Albert Schweitzer

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ABREVIATIONS

AD	Alzheimer's disease
AMH	Anatomically modern human
ARSACS	Autosomal recessive spastic ataxia of Charlevoix-Saguenay
ASD	Autism spectrum disorder
BMI	Body mass index
bp	Base pairs
BRCA	Breast cancer gene
CEPH	Centre d' Etude du Polymorphisme Humain
CEU	CEPH project in Utah
CHB	Han Chinese population of Beijing
CHD	Congenital heart disease
cM	CentiMorgan
CNPs	Copy number polymorphisms
CNVs	Copy number variations
D	Depression
D-leut	Dariusleut
DM1	Myotonic dystrophy
DNA	Deoxyribonucleic acid
FMR	Fragile X mental retardation
HEXA	Hexosaminidase A gene
HIV	Human imunodeficiency virus
HG	Haplogroups
HLA	Human leucocyte antigen
HOGA	Gyrate atrophy of choroids and retina
HVR	Hypervariable regions
HWE	Hardy-Weinberg equilibrium
I	Intrusion
IAM	Infinite allele model
IBD	Identical by descent
IBD+D	Isolation by distance and depression
IBD+DDP	Isolation by distance and double depression
IBDM	Isolation by distance model
IDE	Insulin degrading enzyme
ISVs	Intermediate-sized variants
JC	Jukes-Cantor model
JPT	Japanese ancestry from the Tokyo area
kb	Kilobases
LCT	Lactase gene
LCVs	Large-scale copy number variants
LD	Linkage disequilibrium
LDD	Long-distance differentiation
L-leut	Leherleut

LINES	Long interspersed nuclear elements
MAF	Minor allele frequency
Mb	Megabases
MDS	Multi dimensional scaling
MHC	Major histocompatibility complex
MJD	Machado-Joseph disease
mtDNA	Mitochondrial DNA
Ne	Population size
NF1	Neurofibromin 1 gene
NIDDM	Non-insulin-dependent diabetes mellitus
NJ	Neighbor-Joining
NPL	Non-parametric linkage
NRY	Nonrecombining portion of the Y-chromosome
Numts	Nuclear mitochondrial pseudogenes
PAH	Hepatic phenylalanine hydroxylase
PDHc	Pyruvate dehydrogenase complex
PKU	Phenylketonuria
OMIM	Online mendelian inheritance in man
OOA	Old Order Amish
PCR	Polymerase chain reaction
RC-L1s	Retrotransposition-competent L1s
REV	General reversible model
RNA	Ribonucleic acid
SA	Spatial autocorrelation
S-leut	Schmiedeleut
SGCG	Gamma-sarcoglycan gene
SINES	Short interspersed nuclear elements
SLSJ	Saguenay-Lac-Saint-Jean
SMM	Stepwise mutation model
SNPs	Single nucleotide polymorphisms
SPSS	Statistical package for social Sciences
STRs	Short tandem repeats
Ta	Transcribed active
TPMT	Thiopurine S-methyltransferase
tSNPs	tag single nucleotide polymorphisms
UPGMA	Unweighted pair group method with arithmetic mean
US	United States
UTM	Universal transverse mercator
VNTRs	Variable number of tandem repeats
YBP	Years before present
YHRD	Y-Chromosome haplotype reference database
YRI	Yoruba people of Ibidan Peninsula in Nigeria

LIST OF USEFUL WEBSITES

ALFRED - Allele Frequency Database	http://alfred.med.yale.edu/alfred/index.asp
American Society of Human Genetics	http://www.ashg.org/genetics/ashg/ashgmenu.htm
Arlequin (software)	http://lgb.unige.ch/arlequin/
Copy Number Variation Project	http://www.sanger.ac.uk/humgen/cnv
Database of Nuclear DNA	http://www.ertzaintza.net/cgi-bin/db2www.exe/adn.d2w
European Directory DNA Diagnostic Laboratories	http://www.eddnal.com/
Ensembl Database	http://www.ensembl.org/index.html
European Society of Human Genetics	http://www.eshg.org
Genetic Data Analysis (software)	http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php
GENEPOP (software, web version)	http://genepop.curtin.edu.au
Gold (software)	http://www.sph.umich.edu/csg/abecasis/GOLD/
Human Gene Mutation Database	http://www.hgmd.cf.ac.uk/ac/index.php
Human Genome Database	http://www.gdb.org
Human Genome Project	http://www.ornl.gov/sci/techresources/Human_Genome/home.shtml
Human Genome Variation Database	http://hgvbase.cgb.ki.se
IMGT/HLA Database	http://www.ebi.ac.uk/imgt/hla
National Centre for Biotechnology Information	http://www.ncbi.nlm.nih.gov
Online Mendelian Inheritance in Man	http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM
Orphanet	http://www.orphanet.pt/
Portuguese Society of Human Genetics	http://www.spgh.net
Rare diseases database	http://www.rarediseases.org/
Single Nucleotide Polymorphism Database	http://www.ncbi.nlm.nih.gov/projects/SNP
SPSS (software)	http: //www.spss.com
STRBase	http://www.cstl.nist.gov/biotec/strbase
Structure (software)	http://pritch.bsd.uchicago.edu/software.html
The International HapMap Project	http://www.hapmap.org
UCSC Genome Bioinformatics	http://genome.ucsc.edu
Wikipédia	http://pt.wikipedia.org/wiki/P%C3%A1gina_principal
Y-Chromosome Consortium	http://ycc.biosci.arizona.edu
Y-STR haplotype Database	http://www.ystr.org

RESUMO

O estudo da diversidade genética humana possibilita um melhor conhecimento dos padrões de distribuição das doenças genéticas numa população, bem como contribui para a caracterização da evolução humana. O arquipélago dos Açores (Portugal), situado no norte do oceano Atlântico, é composto por nove ilhas vulcânicas distribuídas desigualmente por três grupos geográficos: o oriental com duas ilhas – São Miguel e Santa Maria –, o central que inclui cinco ilhas – Terceira, Pico, Faial, São Jorge e Graciosa –, e o ocidental com Flores e Corvo. A fim de compreender e determinar o fundo genético da população açoriana, a presente tese teve por base duas abordagens principais: os nomes de família (sobrenomes) e os marcadores genéticos localizados em diferentes cromossomas.

A avaliação da origem genética da população dos Açores foi realizada através da análise de linhagens paternas (cromossoma Y) e marcadores *Alu*. O cromossoma Y apresenta algumas vantagens que possibilitam traçar linhagens, nomeadamente não sofre recombinação e é transmitido de pais para filhos. Contudo, quando um pai apenas tem filhas essa linhagem pode-se perder. Assim, o estudo das origens de uma população deve ser complementado com marcadores localizados nos cromossomas autossómicos, por exemplo, os polimorfismos de inserção *Alu*. Estes polimorfismos possibilitam a inferência directa do estado ancestral (ausência de inserção), e a sua aplicação aos estudos da evolução populacional é vantajosa. Além disso, as inserções *Alu* representam ambas as contribuições – paterna e materna –, uma vez que estão sujeitas a eventos de recombinação e outras forças evolutivas. Os resultados das linhagens paternas na população Açoriana revelaram nove haplogrupos (HG) diferentes, na sua maioria frequentes na Europa. Assim, os dados apontam para uma grande contribuição de indivíduos do continente português, bem como, embora em frequências mais baixas, de indivíduos do Médio-Oriente (HG J*) e do norte de África (HG E*(xE3)). Igualmente, os resultados baseados nos marcadores *Alu* indicam uma proximidade elevada entre populações portuguesas, marroquinas e espanholas, nomeadamente, Catalãos e Andaluzos. Esta proximidade reflecte-se na árvore filogenética, onde os Açores e Portugal continental ramificam com Catalunha, Andaluzia, Marrocos e Argélia, bem como corrobora com os resultados obtidos nas análises do cromossoma Y e dos marcadores autossómicos.

A determinação da diversidade genética com marcadores neutros permite conhecer se as forças evolutivas, designadamente, a deriva genética e a selecção, imprimem a sua influência na assinatura genética de uma população. Na presente tese, a diversidade da população Açoriana foi calculada com base em diferentes marcadores, a saber: sobrenomes, *Short Tandem Repeats* (STRs autossómicos, Y e X) e polimorfismos de inserção *Alu*. Os valores médios de diversidade obtidos nos diferentes estudos mostram que, no general, a população açoriana é muito diversa, apresentando valores mais elevados do que os encontrados no continente português. O estudo de abundância dos sobrenomes e de variabilidade dos microssatélites em cada ilha açoriana revelou que as ilhas mais diversas são Terceira e São Miguel. Ambos os estudos apontam para que as ilhas mais pequenas – Corvo, Graciosa e Santa Maria –, apresentem, como esperado, valores mais baixos de variabilidade. A análise de parentesco entre ilhas foi avaliada usando os sobrenomes e 15 STRs. Duas imagens diferentes emergem: os sobrenomes mostram uma proximidade maior entre os grupos central e ocidental, e os STRs posicionam o grupo central mais próximo do oriental. Esta dualidade pode ser explicada pelo facto dos sobrenomes exibirem uma imagem mais recente, que considera as características sócio-económicas das ilhas, enquanto os dados dos microssatélites revelam a evolução baseada nas características do povoamento do arquipélago, onde se evidenciam São Miguel e Terceira como agentes principais no povoamento das restantes ilhas. Ambas as abordagens são complementares. Em termos de desequilíbrio de ligação (LD), o grupo ocidental apresentou um valor de LD multialélico (D') mais elevado (0,328), no entanto, este valor indica a ausência de LD neste grupo de ilhas. Os grupos central e oriental mostram valores semelhantes, ambos com ausência de LD. Em suma, os Açores, bem como Portugal continental, evidenciam LD apenas para distâncias físicas curtas. Estes dados sugerem que será necessário um número elevado de marcadores para realizar estudos de mapeamento fino de genes de susceptibilidade para doenças complexas. No entanto, outras características (por exemplo, o mesmo ambiente e a possibilidade de construir grandes pedigrees através de registos civis e da igreja) fazem desta população um recurso possível para futuros estudos genéticos.

O coeficiente de consanguinidade populacional tem um papel determinante na identificação da subdivisão de populações humanas. As estimativas baseadas em STRs e sobrenomes evidenciam valores diferentes. O coeficiente de consanguinidade calculado a partir dos nomes de família para a ilha de São Miguel é cerca de sete vezes

menor do que o obtido com base nos 21 STRs. Ambas as determinações têm inconsistências e nenhum valor preciso é conseguido; no entanto, todas as análises demonstram que a população açoriana é uma população aberta. De acordo com Wright (1984), valores inferiores a 0.05, como os verificados nas populações de Portugal continental e Açores, indicam pouca diferenciação genética. A presença de estrutura genética numa população pode conduzir a dados falsos e, possivelmente, a erros de interpretação. Assim, apesar de estarem dispersos por três grupos geográficos e constituírem uma população *admixed*, os Açores não apresentam subdivisão genética, e podem, portanto, ser considerados como um todo homogéneo, uma vez que as diferenças genéticas entre ilhas não são estatisticamente significativas.

Os padrões de dispersão dos indivíduos têm impacto significativo na *admixture* e na estrutura genética de uma população. As taxas de migração foram calculadas a partir de sobrenomes e microssatélites. O valor de migração para a ilha do Corvo baseado em STRs sugere que esta população está sedentária. Um valor controverso foi obtido a partir dos sobrenomes, onde esta ilha apresenta o valor mais elevado de migração indicando a saída de indivíduos desta para as outras ilhas. Ambos os estudos, sobrenomes e STRs, evidenciam o movimento dos indivíduos para as ilhas maiores, a saber, São Miguel e Terceira. Os resultados de dispersão espacial dos sobrenomes revelam que o movimento dos indivíduos ocorre essencialmente entre ilhas mais próximas (isolamento pela distância).

Em conclusão, os dados apresentados ao longo desta tese melhoram o conhecimento do fundo genético da população açoriana: os açorianos são uma população aberta com diversidade genética elevada, fluxo genético relativo e sem extenso desequilíbrio de ligação. Além disso, os padrões da diversidade são uma consequência directa da história do povoamento do arquipélago. Os resultados aqui explanados complementam o passado, estabelecendo a ponte entre a genética e a história; melhoram o conhecimento do presente; e contribuem para compreender o futuro, uma vez que o fundo genético, bem como o ambiente, influenciam certamente o tipo e a distribuição das doenças na população açoriana.

Palavras-chave: Fundo genético, diversidade genética, estrutura populacional, desequilíbrio de ligação, Açores.

SUMMARY

The study of human genetic variation allows a better understanding of disease patterns of a population, as well as, contributes to the comprehension and description of human evolution. In the present thesis, we present a broader view of the genetic structure of the Azorean population. The Azores is composed of nine volcanic islands unevenly distributed by three geographic groups: Eastern, Central and Western. We address the diversity and genetic background of this population considering surnames, SNPs, *Alu* insertion polymorphisms and different STR markers, located in different chromosomes (autosomal, Y and X).

The assessment of the genetic ancestry of the Azoreans, based on *Alu* insertion polymorphisms and Y-chromosome lineages, shows that the main contributors were the mainland Portuguese with an important participation of Middle eastern and north African populations. Additionally, the results of migration using surnames and STRs evidence relative gene flow among islanders. Considering molecular markers, the Azoreans generally present a higher genetic diversity when compared to mainland Portugal and other European populations. The surnames and molecular markers reveal no genetic structure, although the Azores are dispersed through three geographical groups and constitute an admixed population. In terms of linkage disequilibrium (LD), which was estimated in the HLA, Xq13.3 and NRY regions, the archipelago, similarly to mainland Portugal, shows LD only for short physical distances. All analyses suggest that the Azoreans are an outbred population, where the identification of IBD regions will require high density of genetic markers. Thus, the results demonstrate that both surnames and molecular markers are complementary and aid in the genetic characterization of a population.

In general, this thesis improved the knowledge of the genetic signature of Azoreans, complement the past by connecting genetics and history and will contribute to predict the future in terms of disease distribution in this population.

Keywords: Genetic signature, genetic diversity, population structure, linkage disequilibrium, Azores Islands.

"Why not let people differ about their answers to the great mysteries of the Universe? Let each seek one's own way to the highest, to one's own sense of supreme loyalty in life, one's ideal of life. Let each philosophy, each world-view bring forth its truth and beauty to a larger perspective, that people may grow in vision, stature and dedication."

Algernon Black

CHAPTER I

UNDERSTANDING HUMAN DIVERSITY: CONTRIBUTION OF MOLECULAR AND NON-MOLECULAR MARKERS

I. Understanding human diversity: contribution of molecular and non-molecular markers

In the animal kingdom, some species, such as, Asian lion, puma and cheetah, show very little genetic diversity (Driscoll *et al.* 2002); however, most organisms, inc1uding humans, have a considerable amount of genetic variation (Li and Sadler 1991). The proportion of genetic diversity that exists between human populations is relatively low. An early study, based on protein polymorphisms, estimated a 15% diversity between groups (Lewontin 1972). More recently, autosomal variation studies have shown that ~83-88% is found within populations and ~9-13% between continental populations (Jorde *et al.* 2000; Romualdi *et al.* 2002).

Around the world, genetic variation is geographically structured. Several scenarios for this strucutre are possible; for example, there are species in which it is observed sharp regional/ continental discontinuities, making variation different between groups, and those who are geographically undifferentiated, where variation is due to differences between individuals (Barbujani and Goldstein 2004).

An understanding of how genetic diversity is structured in the human species is not only of anthropological and political importance, but also of medical relevance with important implications for human evolution, forensics and distribution of genetic diseases in populations (Cavalli-Sforza and Feldman 2003; The International HapMap Consortium 2005; Tishkoff and Kidd 2004; Foster and Sharp 2004; Jorde *et al.* 2000). For instance, if major differences in allele frequencies exist between populations, individuals from different origins may often be expected to respond differently to medical treatments (Wilson *et al.* 2001).

Studies of genetic diversity from restricted geographical areas, where large numbers of individuals are sampled and a reasonable geographic coverage of the variation is achieved, generally reveal spatial gradients of allele frequencies (Barbujani *et al.* 1995; Rosser *et al.* 2000; Karafet *et al.* 2001) that are only occasionally disrupted by local discontinuities corresponding to linguistic or geographical barriers (Barbujani and Sokal 1990). This suggests that isolation by distance (*i.e.* decreasing gene flow with increasing geographical distances) may be the most appropriate description of human genetic

diversity (Cavalli-Sforza *et al.* 1994). In contrast, worldwide studies of human diversity based on "populations" generally find that individuals cluster discretely depending on their continents of origin (Cavalli-Sforza *et al.* 1988; Bamshad *et al.* 2003; Rosenber *et al.* 2002; Lao *et al.* 2006), and this is sometimes taken to mean that human genetic diversity is structured according to etnia (Risch *et al.* 2002; Burchard *et al.* 2003). The discrepancy in results between regional and global surveys of human genetic diversity could suggest that gradients in allele frequencies are restricted to smaller geographic regions, whereas the continents are distinguished by discontinuities in genetic diversity. Alternatively, the discrepancies may result from differences in study design as suggested, for example, by Kittles and Weiss (2003). Serre and Paabo (2004) demonstrated that when individuals are sampled homogeneously from around the globe, the pattern seen is one of gradients of allele frequencies that extend over the entire world, rather than discrete clusters. Therefore, there is no reason to assume that major genetic discontinuities exist between different continents or "races"[1].

To understand the population genetic structure it is necessary the description of the differences in polymorphism content and diversity patterns between different groups, subpopulations or metapopulations. The most obvious way to attain this characterization is through the study of molecular markers. However, approaches using cultural, demographic and socioeconomic information may also play an important role in the understanding of diversity, inbreeding and migration.

I.1. What can we learn from surnames

Cultural traits are transmitted from ancestors to their descendants, in a process analogous to inheritance, and are subject to changes, similar to mutations, by interaction between individuals, such as, teaching and imitation. In fact, they enhance the relationships within human groups, defining social entities comparable to certain biological species and populations (Manrubia and Zanette 2002).

Surnames are cultural traits (Cavalli-Sforza and Feldman 1981) whose transmission bears strong similarity with that of some biological features. In systems where surname

[1] This is a strong support against those who still believe in the existence of "races" or even "superior races". However, to group humans according to their common features, the most accepted term is etnia or ancestry.

attribution is through the paternal line, surnames simulate neutral alleles of a gene transmitted by the Y-chromosome. Thus, the expectations of the neutral theory of evolution, which is entirely described by random genetic drift, mutation, selection and migration, are satisfied (Zei *et al.* 1983). This property of surnames, together with their availability in large numbers, from present, as well as, from historical populations, makes them useful for the study of population structure (Pettener *et al.* 1998).

In recent decades, surnames have been used as genetic markers to estimate inbreeding changes in a population (Crow and Mange 1965; Pinto Cisternas *et al.* 1985; Gueresi *et al.* 2001; Boattini *et al.* 2006; Colantonio *et al.* 2006), to measure the degree of population subdivision (Lasker and Kaplan 1985; Madrigal *et al.* 2001; Colantonio *et al.* 2002; Esparza *et al.* 2006), and to analyze changes in genetic relationships between populations (Lasker 1977; Weiss 1980; Chen and Cavalli-Sforza 1983; Relethford 1988; Pettener *et al.* 1998; Calderon *et al.* 2006).

Surnames began to be used for studying the genetic structure of populations after Crow and Mange (1965) published an article on the measurement of inbreeding from frequency of isonymous marriages. Twelve years later, Lasker (1977) described a method for estimating the genetic relationship between populations through isonymy (*Ri*). This method has been widely used (Lasker and Mascie-Taylor 1983; Pinto-Cisternas *et al.* 1990; Rodríguez-Larralde 1993) and new aspects of population genetics were approached (Rodriguez-Larralde *et al.* 2000). Others, for example, Chen and Cavalli-Sforza 1983; Relethford 1988; Morton and Yasuda 1980 and Zei *et al.* 1983, have studied similarities between populations adapting Malécot's genetic kinship between populations to surnames (Malécot 1950). Furthermore, Pinto-Cisternas *et al.* (1990) and Barrai *et al.* (1990) have derived variances for parameters estimated from surnames (Rodriguez-Larralde 1993).

The use of surnames models, similarly to other genetic models, is dependent of some assumptions. The method of Crow and Mange (1965) assumes, among other things, that surnames are monophyletic, that non-random mating is symmetrical with respect to sex, and that changes of spelling, illegitimacy, or adoption do not occur. However, in large heterogeneous societies these assumptions do not hold, therefore, "... less confidence can be placed in precise estimates of kinship…" Relethford (1988). Nevertheless, the relative value of these estimates is still informative, especially when large sample sizes

and the same source of information and methodology are used in an entire country. In reality, isolation by distance has been determined with the use of surnames as well as the existence of population clusters within countries, where surname distribution and, presumably, genetic composition are more homogeneous (Barrai *et al.* 1997; Rodriguez-Larralde *et al.* 2000).

Nowadays, in many countries, millions of surnames of telephone users, often available on CD-Roms or online, can be efficiently analyzed in a short time. As examples, the surname structure of Switzerland (Barrai *et al.* 1996), Germany (Barrai *et al.* 1997), Italy (Barrai *et al.* 1999), Austria (Barrai *et al.* 2000), France (Mourrieras *et al.* 1995), and the Netherlands (Barrai *et al.* 2002) were studied, taking into account, in total, more than 20 million surnames. Investigated at different geographic scales, surname-inferred genetic structures were sometimes regarded with a certain suspicion because they are simulated markers for a single *locus*. A good example of the doubts about surname studies was expressed by Rogers (1991) "*The method ... requires an assumption that has not been appreciated: it is necessary to assume that all males in some ancestral generation, the founding stock, had unique surnames. Because this assumption is seldom justified in real populations, the applicability of the isonymy method is extremely limited. Even worse, the estimates it provides refer to an unspecified founding stock, and this implies that these estimates are devoid of information*". Nonetheless, the isonymy method was applied to genealogical databases (Gagnon and Heyer 2001; Gagnon and Toupance 2002), and consanguinity was estimated both from surnames and genealogies. Results indicate that random isonymy, estimated from family names, is not devoid of information; on the contrary, it fits well with consanguinity estimates obtained from the genealogical records (Manni *et al.* 2005).

Manrubia and Zanette (2002) have shown that results for the stationary distribution of surnames frequency are in good agreement with field data for modern human populations in different countries. Through an analysis of the transient time required for this distribution to reach its asymptotic shape, they demonstrated that some deviations observed in real data might actually reflect the composition of the founder population. This result has implications in the study of polyphyletism. Indeed, if the same surname can have multiple origins and, consequently, the individuals carrying it are not always phylogenetically related, the shape of the surname distribution will be affected. The

strong resemblance between the cultural inheritance of the surname and the biological process in which nonrecombining neutral alleles are passed to offspring has justified applying results from field data (Barrai *et al.* 1996). In the few cases, where data on genetic diversity was available, it was possible to retrieve information on past populations by comparing both sets of data (Sykes and Irven 2000). A specific example comes from the small island of Tristan da Cunha, where 300 inhabitants represent only seven surnames and five mitochondrial lineages reflects without doubt the small size of the founder population (Soodyall *et al.* 1997; Manrubia and Zanette 2002).

I.1.1. Isonymy, inbreeding and relationship coefficients

Isonymy is the possession of the same surname. The proportion of isonymy is the frequency in which this happens; interpopulation isonymy occurs between two samples and marital isonymy takes place between spouses considering both given surnames. Figure I.1 shows how intrapopulation and interpopulation isonymy are calculated.

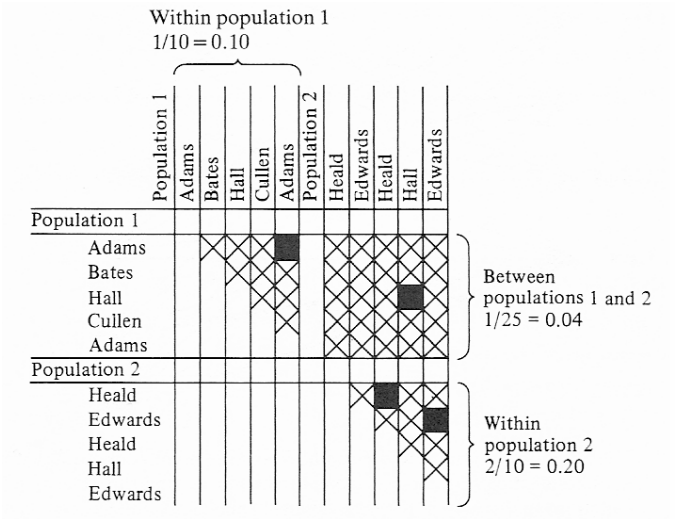


Figure I.1. Isonymy within and between population. Black squares represent isonymous pairs; crosses represent all other possible pairs (adapted from Lasker 1985).

The term isonymy is sometimes limited to marital isonymy or used as an estimate of inbreeding from the proportion of isonymy, but such limitations and extensions may be confusing and the term should not be used in these ways without all explanation.

There are several methods to calculate isonymy. According to Relethford (1988), the random isonymy between populations *i* and *j* is

$$I_{ij}=\Sigma p_{ki}p_{kj}$$

where p_{ki} and p_{kj} are the relative frequencies of surname *k* in the populations *i* and *j*, respectively. On the other hand, according to Rodriguez-Larralde *et al.* (1993) unbiased random isonymy within the population is calculated by the formula:

$$I_{ii}=\Sigma_k(p_{ik})^2-1/N_i$$

where p_{ik} is the relative frequency of surname *k* in the *i*th population, and N_i is the sample size of the same population.

Population structure constitutes deviations from panmixia, such as, those due to limited number of ancestors, to gender, to preference of certain types of consanguineous marriage, and to limited migration in social or geographic space (Rodriguez-Larralde *et al.* 2003). Several studies have shown that cultural, demographic and socioeconomic factors (religious beliefs, pattern of between-generation transfer of familial property, and increased number of relatives following a demographic expansion) influence the consanguinity level of populations (Manni *et al.* 2005; Rodriguez-Larralde *et al.* 2003). Inbreeding has been extensively analyzed by the use of surnames in populations with different degrees of isolation in Europe, Asia and north America (Colantonio *et al.* 2003 and references therein). In 1965, Crow and Mange used the marital isonymy to estimate the frequency of consanguineous marriages as a measure of inbreeding. Based on Wright's hierarchical model, they defined the total inbreeding by isonymy and its random and non-random components, in order to describe the effects of subdivision of a population in causing deviations from random mating. Currently, it is widely accepted that the calculation of the random component of inbreeding (F_{ST}) within the subpopulation is obtained from the formula, where *I* is the isonymy within subpopulation *i*:

$$F_{ST}=I_{ii}/4$$

The calculation of F_{ST} for the whole population is based on the formula suggested by Relethford (1988):

$$F_{ST}=\Sigma w_i\varphi_{ii}$$

where φ_{ii} is the random component of inbreeding ($I_{ii}/4$) of the *i*th subpopulation, and w_i is the weight due to sample size, N_i/N_t, being N_t the sample size of the whole

population.

The random component of the inbreeding coefficient when calculated from surnames is merely a statement concerning the average commonality of surnames between males and females in the population multiplied by a constant. The constant used is one-quarter, because this is the likelihood of a gene being shared by the homologous autosomal chromosomes of an offspring of first-degree relatives. The same fraction applies to other degrees of relationship following the logic adopted by Crow and Mange (1965). The likelihood of a gene being shared by first-degree relatives themselves is one in two. Therefore, their coefficient of relationship by isonymy, *Ri*, is the proportion of isonymy multiplied by one half. As applied to the males and females of a population this is,

$$Ri=\Sigma p_i q_i/2$$

if one extends the logic and the assumption of the monophyly of surnames to two populations this can be expressed as

$$Ri=\Sigma(S_{i1}S_{i2})/2p_i q_i/2\Sigma S_{i1}\,\Sigma S_{i2}$$

in which S_{i1} is the number of occurrences of the *i*th surname in a sample from population 1 and S_{i2} is the number of occurrences of the same surname in a sample from population 2. Unlike the inbreeding coefficient by isonymy, the coefficient of relationship by isonymy is not divided into random and non-random components, it is a measure of the random component.

I.1.2. Surname diversity and migration

Human migration has been studied from many points of view. When using a surname model to study its effects, it is only considered as the mechanism that redistributes genes geographically. Human migration draws pedigree lines on maps. The pattern of those lines depicts an aspect of human population structure with significance to population genetics – inbreeding. Moreover, such mapping of pedigree lines can be used to explain distributions of human genetic polymorphisms. Human genes cannot move except by the movements of people who carry them (at least before artificial insemination). Therefore, historically, human migration accounted for all the movement of genes (Lasker 1985).

Gene movement may be seen in the distances from birthplaces of parents to the birthplaces of their children. Tracing individual pedigrees has been done by geneticists and others, but such studies inevitably have a geographic aspect. Pedigrees, however, are not representative of the population as a whole. Male ancestors are usually easier to identify and trace than female, so the male line is usually more complete than female and mixed lines. As consequence the picture based on a collection of pedigrees is likely to be biased or to cover only the very few recent generations that can be completely ascertained (Lasker 1985).

The identification of the various evolution agents of the genetic structure of human populations and the assessment of their relative weight are one of the main aims of population genetics. The high level of genetic polymorphism observed in human populations has led to a search for adaptative explanations of genetic variation. However, microevolutionary events often seem better explained by migration effects, particularly immigration. Immigration implies addition of genes, which may profoundly affect gene frequencies of the receiving population, thus, becoming a driving evolutionary force. The amount of immigration has relatively little significance compared to the structure of the phenomenon, since, for instance, genetic difference between immigrants and receiving populations is believed to increase with geographical distance. One of the immigration determining elements is the choice of mates. In order to predict the nature of genetic changes, selective mating can be studied by analysing the shape and the central tendency of the distribution of distances between the places of birth of spouses (Biondi *et al.* 1993).

In 1983, Zei and collaborators proposed a method to estimate migration based on the observation that surnames generate, at equilibrium, a distribution that fits the model introduced by Karlin and McGregor (1967). This model presents the distribution of alleles expected according to the neutral theory of evolution. In a population of constant finite size, the equilibrium is reached when the number of surnames entering the population by mutation and migration equals that lost by drift. Surname mutation is relatively rare, so it can be assumed that new surnames enter into a population mainly by immigration. Moreover, in a very large population, the statistical properties of the surname distribution can be strongly correlated with genetic diversity (Barrai *et al.* 1996; Manrubia and Zanette 2002). Zei *et al.* (1983) observed that Fisher's logarithmic

distribution (Fisher 1943) derived to represent the variation in the abundance of surnames, that is, diversity. The use of that distribution to predict the number of surnames in a sample represents an excellent approximation of the Karlin-McGregor distribution. Fisher's distribution is theoretically more satisfactory for surnames than Pareto's, since it is easier to fit. Finally, Zei *et al.* (1983) were able to integrate the parameters introduced by Fisher (α) with the parameters of the Karlin-McGregor distribution (ν) combining ease of computation with meaningful theoretical interpretation through the following formulas:

Fisher's α and

$$\alpha = 1/I_{ii}$$

Karlin-McGregor's ν

$$\nu = \alpha/(N_i+\alpha)$$

establishing the relationship between Fisher's α, Karlin-McGregor's ν and population size.

Additionally, the study of the spatial distribution of genetic variation has been considered important in population studies (Rosenberg *et al.* 1999; Lefevre-Witier 2006). Spatial autocorrelation (SA) is the dependence of the values of a variable at specified geographic locations on the values of the same variable at neighbouring locations. Spatially autocorrelated data violate the assumption of independence required for most standard statistical tests, calling for special tests designed to remove the dependence of the variable on geography. Although the analysis of SA is often associated with removing the internal dependence of variables on the underlying spatial structure during hypothesis testing, the SA analysis can lead to important discoveries about the scale where spatial patterns occur, which in turn may suggest underlying factors with similar patterns. Spatial autocorrelation analysis has been used to study a variety of phenomena, such as, the genetic structure of plant, animal and human populations (Sokal *et al.* 1986; Epperson 1992; Barbujani and Sokal 1991), mortality (Setzer 1985) and their morphological patterns (Epperson and Clegg 1986; Sokal and Uyherschaut 1987).

Spatial autocorrelation summarizes the genetic similarity between populations in relation to their geographical proximity. In particular, spatial autocorrelation helps to focus on the similarity of values of a variable, *i.e.* the frequency of a surname, between

pairs of populations within arbitrary classes of distance (Caravello and Tasso 1999). This method allows estimation of the spatial distribution of surnames in the considered territory, in order to emphasize the specific processes of diffusion of individuals. It was developed by Moran (1950), perfected by Ripley (1981), as well as by Cliff and Ord (1973), whereas Sokal and Oden (1978a,b) were the first to apply it to biological problems. The following formula allows an estimate of this autocorrelation coefficient:

$$I=\frac{n\sum_{i=1}^{n}\sum_{j=1}^{n} w_{ij}(p_i-p)(p_j-p)}{W\sum_{i=1}^{n}(p_i-p)^2}$$

where p_i and p_j are the relative frequency of surnames at the ith and jth localities, p is the mean across the n municipalities, w_{ij} is equal to 1 for all the pairs of municipalities falling in the studied distance class and equal to 0 for all the other pairs, and W is the sum of all w_{ij} values in that distance class. In large samples Moran's I coefficient varies between -1 to +1, where positive significant values ($I>0$) indicate similar frequencies and negative significant values ($I<0$) indicate dissimilarity (Barbujani *et al.* 1992).

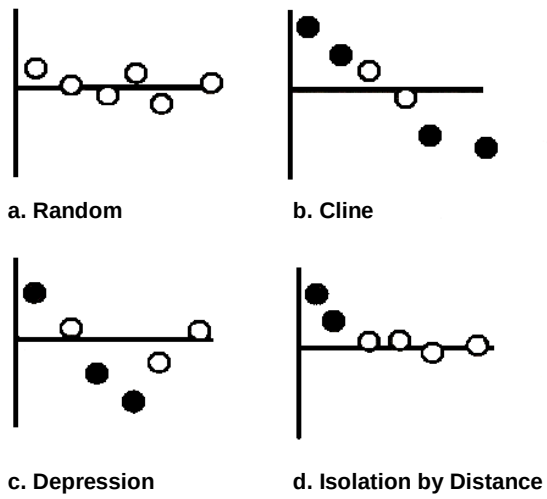


Figure I.2. Scheme of typical correlograms and of their likely interpretation. X-axis represents geographic distance and the Y-axis autocorrelation values. Shaded circles are significant autocorrelation coefficients; open circles are insignificant coefficients (adapted from Barbujani 2000).

Autocorrelation coefficients can be assembled in a plot named correlogram, which allows a better summary of the variation. The main classes of correlograms can be related with the likely evolutionary processes generating them. Clines affecting the

entire study area (Figure I.2b) or only a part of it (Figure I.2c) can be discriminated from the patterns expected under random genetic variation (Figure I.2a). In statistical terms, the null hypothesis is clearly random distribution of allele frequencies in space. In population genetics terms, however, geographic randomness would be surprising. As a rule, geographically close populations exchange more migrants than distant populations and the degree of relative isolation between localities is roughly proportional to their geographic distance (Barbujani and Sokal 1991; Barbujani 2000).

I.2. The human genome polymorphisms

The success of the Human Genome Project[2] has given us an exceptional understanding of the structure and organization of our genome (Figure I.3). Variability is observed in the human genome through single nucleotide polymorphisms (SNPs), variable number of tandem repeats (VNTRs; e.g. mini and microsatellites), presence/ absence of transposable elements (e.g. *Alu* elements) and structural alterations (e.g. deletions, duplications and inversions; Freeman *et al.* 2006).

I.2.1. Single nucleotide polymorphisms

Variations in DNA (deoxyribonucleic acid) sequence can have a major impact on how humans respond to disease, to environment and to drugs or other therapies. This makes single nucleotide polymorphisms of great value for biomedical research, for medical diagnostics and for developing pharmaceutical products (Jobling *et al.* 2004).

A SNP is a DNA sequence variation occurring when a single nucleotide – A, T, C or G – in the genome, or other shared sequence, differs between members of a species or between paired chromosomes in an individual (Figure I.4).

[2] http://www.ornl.gov/sci/techresources/Human_Genome/home.shtml. Begun formally in 1990, the Human Genome Project was a 13-year effort coordinated by the U.S. Department of Energy and the National Institutes of Health. The project originally was planned to last 15 years, but rapid technological advances accelerated the completion date to 2003. During the early years of the project, the Wellcome Trust (United Kingdom) became a major partner, but additional contributions came from Japan, France, Germany, China, and others.

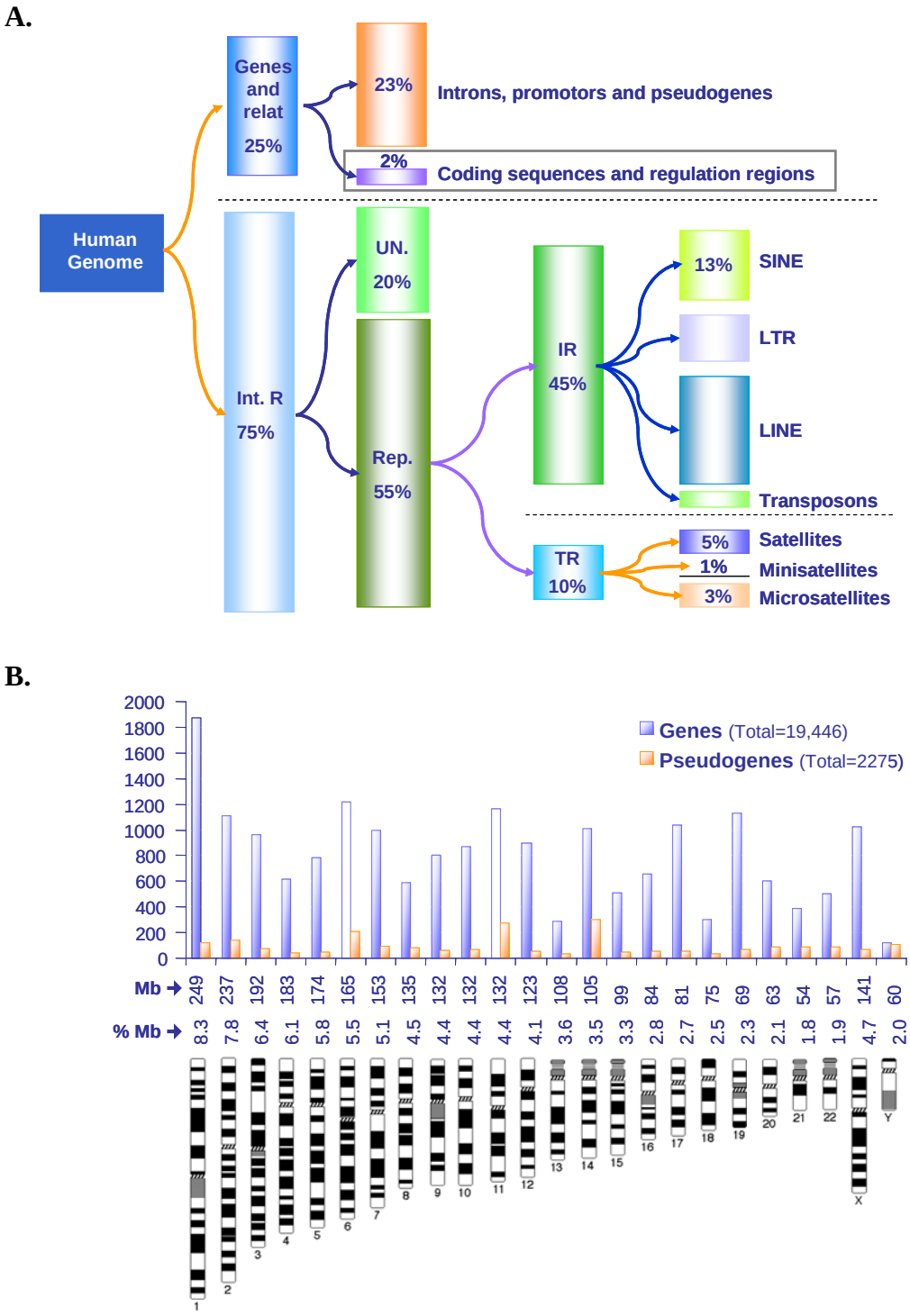


Figure I.3. Characterization of the human genome. **A.** General composition, Genes and relat: genes and associated sequences; Int.R: intergenic regions; UN: unique intergenic sequences; Rep: repetitive intergenic sequences; IR: repetitive dispersed intergenic sequences; TR: tandem repeats (Adapted from Ameziane *et al.* 2006). **B.** Genes and pseudogenes content (Adapted from Human Genome Database, last update 27 August 2007, GDB, http://www.gdb.org/gdbreports/CountGeneByChromosome.html.)

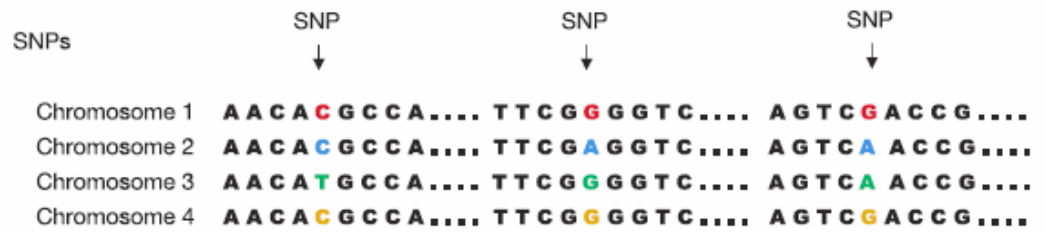


Figure I.4. Schematic representation of SNPs (adapted from International HapMap Consortium 2003[3]).

SNPs are evolutionarily stable, this is, they change very little from generation to generation. This makes them easier to follow in population studies. Several studies have used SNPs to identify genes associated with complex diseases (e.g. Pearson *et al.* 2007; Abel *et al.* 2006). These associations are difficult to establish with conventional gene-hunting methods, because a single altered gene may make only a small contribution to the disease. SNPs in the coding regions of genes or in regulatory regions are more likely to cause functional differences than SNPs elsewhere. Although most SNPs do not affect gene function, a large number of them will be valuable as markers throughout the genome for finding SNPs that affect gene function or are in linkage disequilibrium (LD) with the gene causing disease (Patil *et al.* 2001). It has been estimated that, in the world's human population, about 10 million sites (that is, one variant *per* 300 bases on average) constitute 90% of the variation in the population and differ in a way that both alleles are observed at a frequency of 1% (Crawford *et al.* 2005). The remaining 10% of variation is due to a vast array of variants that are rare in the population.

Overall, the average nucleotide diversity (π), representing the likelihood that a given nucleotide position differs across two randomly sampled sequences, is about $8x10^{-4}$ in both genome-wide and *locus*-specific studies (Przeworski *et al.* 2000; International SNP Map Working Group 2001; Venter *et al.* 2001). This means that, on average, it is expect

[3] http://www.hapmap.org. The International HapMap Project is a partnership of scientists and funding agencies from Canada, China, Japan, Nigeria, the United Kingdom and the United States to develop a public resource that will help researchers find genes associated with human disease and response to pharmaceuticals.

to find one SNP about every 1250 bp. The value of π varies significantly between chromosomes, from $5.19x10^{-4}$ for chromosome 22 to $8.79x10^{-1}$ for chromosome 15. Additionally, there is some suggestion that SNP density varies along chromosomes (Venter *et al.* 2001), and explanations have been put forward based on variation in GC-content or in the efficiency of DNA mismatch repair.

It has been estimated that >5 million common SNPs, each with a frequency varying from 10% to 50%, account for the bulk of human DNA sequence difference. Alleles making up blocks of such SNPs in close physical proximity are often correlated and define a limited number of SNP haplotypes, each of which reflect descendence from a single, ancient ancestral chromosome. New haplotypes are formed by additional mutations, or by recombination when the maternal and paternal chromosomes exchange corresponding segments of DNA, resulting in a chromosome that is a mosaic of the two parental haplotypes. The coinheritance of SNP alleles on these haplotypes leads to associations between these alleles in the population, known as linkage disequilibrium, LD (Patil *et al.* 2001).

The strong associations between SNPs in a region have a practical value, this is, genotyping only few, carefully chosen in the region, will provide enough information to understand the remainder of the common SNPs in that region. As a result, only a few of these 'tag' SNPs are required to identify each of the common haplotypes in a region (International HapMap Consortium 2003, 2005). On the basis of empirical studies, it has been estimated that most of the information about genetic variation represented by the 10 million common SNPs in the population could be provided by genotyping 200,000 to 1,000,000 tag SNPs across the genome (International HapMap Consortium 2003, 2005). For common SNPs, which tend to be older than rare SNPs, the patterns of LD largely reflect historical recombination and demographic events. Some recombination events occur repeatedly at "hotspots". The result of these processes is that current chromosomes are mosaics of ancestral chromosome regions. This explains the observations that haplotypes and patterns of LD are shared by apparently unrelated chromosomes within a population and generally among populations (International HapMap Consortium 2003, 2005; Gray *et al.* 2000).

I.2.2. Variable Number of Tandem Repeats

Variable Number of Tandem Repeats (VNTRs) constitute a class highly heterogeneous of genetic markers, more dynamic and common in eukaryotic genomes. The variation of these markers involves changes in the numbers of repeated DNA sequences arranged in tandem arrays. While the high variability of these multiallelic markers is a useful property in many aspects, the underlying high mutation rates mean that, in contrast to SNPs, alleles with the same size and sequence may not reflect identity by descent, but identity by state, and, therefore, the ancestral state cannot be determined (Naslund *et al.* 2005).

VNTRs are classified according to the size of their repeat units, the typical number of units in arrays, and sometimes their level of variability. Because their nomenclature is not systematic, three major divisions emerge: (*i*) satellite, where a single repeat sequence family can constitute several percent of the total genome, and can occur in individual repeat arrays as large as 5 Mb (megabases); (*ii*) minisatellites, which may be present at hundreds to thousands of different *loci per* genome; and (*iii*) microsatellites, that are extremely abundant in short repeat sequences (Armour *et al.* 1999). Many VNTRs are considered as neutral markers. However, there are well known examples in every class of VNTRs that play functional roles, and in which variation in repeat copy number can have phenotypic effects. Various mini and microsatellites that lie within the coding regions of genes, or in regulatory regions, affect gene expression or the function of gene products. Some satellites located in centromeres and telomere repeat arrays are important functional components of chromosomes (Naslund *et al.* 2005).

I.2.2.1. Satellites

Satellites, sometimes named macrosatellites, are large tandem arrays spanning hundreds of kilobases to megabases, and composed of repeat units of a wide range of sizes that can display a higher-order structure. A good example is alpha satellite with a repeat monomer of 171 bp, which forms a component of centromeres. This higher-order structure can be repeated hundreds or thousands of times to form an array several Mb in size. Innitially, satellites were used to genotype individuals but, because of their large size and repetitive nature, its use declined (Jobling *et al.* 2004; Warburton *et al.* 1993).

The mutation processes at these *loci* cannot be studied directly, probably it involves unequal crossing over between homologous chromosomes misaligned. Historically, some satellite polymorphisms have been used in human evolutionary studies (e.g. Oakey and Tyler-Smith 1990), but nowadays they have been superseded by *loci* which are easier to type, analyze and understand.

I.2.2.2. Minisatellites

Minisatellites consist of repeat units from about 8 to 100 bp in length, with copy numbers from as low as 5 to well over 1000. Minisatellites are qualitatively different in their variability, mutation rates, mutation processes and chromosomal locations. They are among the most dynamic *loci* in the genome, some displaying hypervariability, with very large numbers of alleles of different lengths and structures, mutation rates as high as 14% *per* generation, and complex mutation processes involving both inter- and intra-allelic events (Denoeud *et al.* 2003). They provided the first highly polymorphic, multiallelic markers for linkage studies (Bell *et al.* 1982; Nakamura *et al.* 1987) and were used in the early stages of human genome mapping (NIH/ CEPH Collaborative Mapping Group 1992). Although the abundance of polymorphic minisatellites suggests that they are fast-evolving sequences, most of them are, in fact, quite stable. Chromosomal distribution of minisatellites in the human genome is highly skewed toward telomeres and ancestrally telomeric regions (Amarger *et al.* 1998). When allele length variation is considered, minisatellites show high levels of diversity, with typical heterozygosity values of well over 90%. Sequence analysis reveals an additional level of diversity – all minisatellites examined contain not homogeneous repeat units, but variant repeats differing by base substitutions and small indels (Denoeud *et al.* 2003).

GC-rich minisatellites tend to be clustered towards the ends of chromosomes (Royle *et al.* 1988), suggesting that they might be associated with recombination hotspots either as cause or consequence (Jarman and Wells 1989).

I.2.2.3. Microsatellite or short tandem repeats

Microsatellites are sequences of a single motif (1-6 bp) which is repeated many times in tandem. They are also called simple sequences and short tandem repeats (STRs; Edwards *et al.* 1991). Historically, the term microsatellite has been used to describe only repeats of the dinucleotide motif CA/GT (Litt and Luty 1989). If these repeats are long enough and uninterrupted, STRs are excellent genetic markers due to their high level of polymorphism. Microsatellites are generally assumed to be evenly distributed over genomes but rare within coding regions. There are, however, some human diseases caused by expansions of polymorphic trinucleotide repeats in genes, such as, fragile X[4] and myotonic dystrophy[5] (e.g. Fu *et al.* 1991, Aslanidis *et al.* 1992, Rubinsztein 1999).

STR markers were first used for genetic mapping (e.g. Weissenbach *et al.* 1992) and as diagnostic tools to detect human diseases (e.g. Mills *et al.* 1992). Nowadays, microsatellites are regularly used in population and ecological studies. Additionally, microsatellites are excellent markers for studying gene flow, effective population size (Ne), paternity and relatedness. They can also be used to study the level and effects of inbreeding. However, there are also some drawbacks. The reduction or complete loss of amplification of some alleles, due to base substitutions or indels within the priming site, constitutes a main problem. These so called missing alleles will not necessarily be recognized when there is a product from the other homologue allele. This can lead to an underestimation of heterozygosity, compared with that expected on the basis of Hardy-Weinberg equilibrium (HWE).

Studies of evolutionary processes of microsatellites have shown that (*i*) the mutation of repeat units depends on the allele size and purity; (*ii*) the mutation process is upwardly biased; and (*iii*) there are some constraints on allele length (Ellegren 2000). To estimate population differentiation measures and genetic distances using STRs, theoretical

[4] The fragile X syndrome is a dominant genetic disorder with reduced penetrance caused by mutation of the *FMR1* gene (Xq27.3). Mutation at that site is found in 1 in about 2000 males and 1 in about 259 females (for revision, please, see Abbeduto *et al.* 2007).

[5] Myotonic dystrophy (DM) is a autosomal dominant, chronic, slowly progressing, highly variable inherited multisystemic disease that can manifest at any age from birth to old age. There are currently two known types of adult onset DM: Myotonic dystrophy type 1 (DM1, 19q13-2), also known as Steinert's disease, and Myotonic dystrophy type 2 (DM2, 3q13.3-q24), commonly referred to as PROMM or proximal myotonic myopathy (for revision, please, see Heatwole and Moxley 2007).

mutation models for the evolutionary processes of microsatellites are needed. Two theoretical models have been considered (Deka *et al.* 1991): the infinite allele model (IAM, Kimura and Crow 1964) and the stepwise mutation model (SMM, Kimura and Ohta 1978). Both models will be described in section II.1.2.3. (Mutation and Recombination) of the present thesis.

I.2.3. Transposable elements

While it is widely recognized that the majority of the human genome is not directly involved in the production of proteins, our understanding of the noncoding regions spanning between genes remains far from complete. The role of mobile elements in the shaping of eukaryotic genomes is becoming more and more recognized. Mobile elements make up over 45% of the human genome. These elements continue to amplify and, as a result of negative effects of their transposition, they contribute to some human diseases, for example, neurofibromatosis[6] (Wallace *et al.* 1991), haemophilia[7] (Ganguly *et al.* 2003) and breast cancer[8] (Teugels *et al.* 2005). All eukaryotic genomes contain mobile elements, although the proportion and activity of the classes of elements varies widely between genomes. Mobile elements are important in insertional mutagenesis and unequal homologous recombination events. They use extensive cellular resources in their replication, expression and amplification. There is considerable debate as to whether they are primarily an intracellular plague that attacks the host genome and exploits cellular resources, or whether they are tolerated because of their occasional positive influences in genome evolution. These repeat elements present copy numbers ranging from a few hundred to several hundred thousand including the 868,000 LINES (Long Interspersed Nuclear Elements) and 1,558,000 SINES (Short Interspersed

[6] Neurofibromatosis is an autosomal dominant genetic disorder. It encompasses a set of distinct genetic disorders that cause tumors to grow along nervous tissues and, in addition, can affect the development of non-nervous tissues, such as, bones and skin. Neurofibromatosis type 1 gene (17q11.2) produces neurofibromin (a GTPase activating enzyme). Neurofibromatosis type 2 gene (22q12) produces merlin, a cytoskeletal protein (for revision, please, see Field *et al.* 2007).

[7] Haemophilia is a disorder of the blood-clotting system. There are different types of haemophilia. Hemophilia A and B are X-linked recessive disorders. Haemophilia A is a deficiency of clotting factor VIII (Xq28) and is also known as classical haemophilia and is the cause of about 80% of cases. Haemophilia B is a deficiency of clotting factor IX (Xq27.1-q27.2) and is the cause of about 20% of cases (for revision, please, see Dargaud and Negrier 2007).

[8] Breast cancer is a malignant tumor that forms from the uncontrolled growth of abnormal breast cells. The cause of most breast cancers is unknown; however, 5-10% of breast cancers tend to cluster in families. These cancers can be caused by mutations in particular genes, such as, *BRCA1* (17q21) or *BRCA2* (13q12.3). These genes belong to a class of genes known as tumor suppressor genes (for revision, please, see Goldberg and Borgen 2006).

Nuclear Elements). The best studied examples are, respectively, the L1 and *Alu* retrotransposons (Batzer and Deininger 2002; Kazazian 2004; Hedges and Batzer 2005).

I.2.3.1. LINE – L1

L1 is an abundant family of non-long terminal repeat retrotransposons that comprises around 17% of human DNA (Smit 1996). The vast majority (99.8%) of L1s can no longer retrotranspose because they are 5' truncated, internally rearranged, or mutated (Gilbert *et al.* 2002). However, the average human genome is estimated to contain approximately 60-100 retrotransposition-competent L1s (RC-L1s), and around 10% of these elements are classified as highly active or "hot" (Sassaman *et al.* 1997; Brouha *et al.* 2003). The majority of RC-L1s are members of the Ta (Transcribed active) subfamily (Skowronski *et al.* 1988), and many are polymorphic with respect to presence, indicating that they have retrotransposed since the origin of the human species (Boissinot *et al.* 2000; Myers *et al.* 2002).

RC-L1 retrotransposition continues to impact the human genome, for instance, disease-producing *de novo* L1 retrotransposition events have been identified in humans, such as, choroideremia[9] (van den Hurk *et al.* 2007) and pyruvate dehydrogenase complex (PDHc) deficiency[10] (Mine *et al.* 2007; Ostertag and Kazazian 2001). RC-L1s can also mobilize sequences derived from both their 5' and 3' flanks in *cis* by a process termed "L1-mediated transduction" (Pickeral *et al.* 2000). Finally, the RC-L1 encoded proteins also may function in *trans*, resulting in the mobilization of *Alu* elements and the formation of processed pseudogenes, which together comprise ~10% of genomic DNA (Dewannieux *et al.* 2003; Ejima and Yang 2003). Thus, either directly or through the promiscuous mobilization of cellular RNAs, L1 retrotransposition continues to shape the genome.

[9] Choroideremia is an X-linked recessive disease (Xq21.2) that leads to the degeneration of the choriocapillaris, the retinal pigment epithelium, and the photoreceptor of the eye (for revision, please, see MacDonald *et al.* 2004).

[10] PDHc deficiency is an X-linked disease and represents a common cause of congenital lactic acidosis. Most patients with PDH deficiency have a mutation in the α chain of the PDHE1 enzyme. The gene of the α chain is localised to Xp22.1 (for revision, please, see Maj *et al.* 2006).

I.2.3.2. SINE – Alu markers

The name "*Alu* elements" was given to these repeated sequences because members of this family contain a recognition site for the restriction enzyme *Alu*I (Houck *et al.* 1979). Full-length *Alu* elements are ~300 bp long and are commonly found in introns, 3′ untranslated regions of genes and intergenic genomic regions. Initial estimates indicated that these mobile elements were present in the human genome at an extremely high copy number (~500,000 copies; Rubin *et al.* 1980). Recently, a detailed analysis of the draft sequence of the human genome has shown that, out of more than one million copies, *Alu* elements are the most abundant SINEs, which makes them the most abundant of all mobile elements in the human genome (International Human Genome Sequencing Consortium 2001). Because of their high copy number, the *Alu* gene family comprises more than 10% of the mass of the human genome (International Human Genome Sequencing Consortium 2001) and, as they accumulate preferentially in gene-rich regions, *Alu*s are not uniformly distributed in the human genome (Korenberg and Rykoloski 1988). They lack all the machinery necessary to transpose, but studies demonstrated that *Alu* are able to commandeer the requisite mobilization machinery from L1 (Chen *et al.* 2002). *Alu* elements unique to the human genome were initially identified on the basis of a shared high number of diagnostic point mutations, and polymorphic nature respecting their presence or absence in diverse human genomes (Batzer *et al.* 1990; Matera *et al.* 1990). Almost all of the recently integrated human *Alu* elements belong to one of several small and closely related 'young'*Alu* subfamilies, known as Y, Yc1, Yc2, Ya5, Ya5a2, Ya8, Yb8 and Yb9 (Batzer *et al.* 1990; Matera *et al.* 1990; Batzer *et al.* 1995; Carrol *et al.* 2001).

The analysis of human *Alu* insertion polymorphisms has been used to address several questions about human origins and demography (Perna *et al.* 1992; Hammer *et al.* 1994; Batzer *et al.* 1996; Stoneking *et al.* 1997; Comas *et al.* 2000; Jorde *et al.* 2000; Nasidze *et al.* 2001). They have several characteristics that make them unique for the study of human population genetics. Individuals that share *Alu* insertion polymorphisms have inherited the *Alu* elements from a common ancestor, which makes the *Alu* insertion alleles identical by descent (IBD). In addition, there is no evidence for any type of process that specifically removes *Alu* elements from the genome; even when a rare deletion occurs, it leaves behind a molecular signature (Edwards 1992). The ancestral

state of *Alu* insertion polymorphisms is known to be the absence of the *Alu* element at a particular genomic location (Batzer and Deininger 1991; Perna *et al.* 1992). The ancestral state of a genomic polymorphism allows us to draw trees of population relationships without making too many assumptions (Perna *et al.* 1992; Batzer *et al.* 1996; Stoneking *et al.* 1997).

I.2.4. Copy number variation

Genetic variation in the human genome ranges from large, microscopically visible chromosome anomalies to single nucleotide changes. Recently, multiple studies have discovered an abundance of submicroscopic copy number variation of DNA segments ranging from kilobases to megabases in size (Iafrate *et al.* 2004; Sebat *et al.* 2004; Sharp *et al.* 2005; Tuzun *et al.* 2005; McCarroll *et al.* 2006; Redon *et al.* 2006). Deletions, insertions, duplications and complex multisite variants (Fredman *et al.* 2004), collectively termed copy number variations (CNVs) or copy number polymorphisms (CNPs), are found in all humans (Feuk *et al.* 2006a) and other mammals (Freeman *et al.* 2006). CNV is a DNA segment of 1 kb or larger, present at variable copy number in comparison with a reference genome (Feuk *et al.* 2006a). A CNV can be simple in structure, such as, tandem duplication, or may involve complex gains or losses of homologous sequences at multiple sites in the genome. CNVs do not include variants that arise from the insertion/ deletion of transposable elements. Therefore, CNV encompasses previously introduced terms, such as, large-scale copy number variants (LCVs; Iafrate *et al.* 2004), copy number polymorphisms (Sebat *et al.* 2004), and intermediate-sized variants (ISVs; Tuzun *et al.* 2005), but not retroposon insertions. Recently, Iafrate *et al.* (2004) and Sebat *et al.* (2004) reported the widespread presence of copy number variation in normal individuals, and these observations have since been replicated and expanded (e.g. de Vries *et al.* 2005; Sharp *et al.* 2005; Tuzun *et al.* 2005; McCarroll *et al.* 2006; Repping *et al.* 2006).

CNVs influence gene expression, phenotypic variation and adaptation, by disrupting genes and altering gene dosage (McCarroll *et al.* 2006; Repping *et al.* 2006), and can cause disease, as in microdeletion or microduplication disorders (Inoue and Lupski 2002; Shaw-Smith *et al.* 2004), or even confer risk to complex disease traits, such as,

HIV-1 infection and glomerulonephritis[11] (Gonzalez *et al.* 2005; Aitman *et al.* 2006). Furthermore, CNVs can influence gene expression indirectly through position effects, predispose to deleterious genetic changes, or provide substrates for chromosomal change in evolution (Feuk *et al.* 2006a,b; Freeman *et al.* 2006).

Large duplications and deletions have been known for some time to be present in the human genome, initially from cytogenetic observations (e.g. Coco and Penchaszadeh 1982), but their frequency was presumed to be low and for the most part directly related either to tandemly repeated genes or to specific genetic disorders (e.g. Inoue and Lupski 2002). In addition, they were often localized to repeat-rich regions, such as, telomeres, centromeres and heterochromatin (e.g. Giglio *et al.* 2001).

In a recent study, Redon *et al.* (2006) found that 285 out of 1961 (14.5%) genes in the OMIM[12] morbid map overlapped with CNVs. These authors observed numerous examples of possible relevance to both Mendelian and complex diseases. Additionally, CNVs were identified within the regions commonly deleted in contiguous gene syndromes[13], such as, DiGeorge, Smith-Magenis, Williams-Beuren, Prader-Willi and Angelman syndromes, which may be relevant for discriminating uncharacterized or atypical cases.

I.3. Linkage disequilibrium: Insight to the human genome architecture

The knowledge of the human genome architecture significantly contributes to the understanding of disease susceptibility and development. This can be attained by the characterization of the fine-scale structure of LD. LD plays a fundamental role in gene mapping, both as a tool for fine mapping of complex disease genes and in proposed

[11] Glomerulonephritis, also known as glomerular nephritis, is a primary or secondary immune-mediated renal disease characterized by inflammation of the glomeruli. Low copy number of *FCGR3B gene* was associated with glomerulonephritis in the autoimmune disease systemic lupus erythematosus (for revision, please, see Couser 1998).

[12] OMIM - Online Mendelian Inheritance in Man, http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM. This database is a catalog of human genes and genetic disorders authored and edited by Dr. Victor A. McKusick and his colleagues at Johns Hopkins University (http://www.jhu.edu) and elsewhere, and developed for the World Wide Web by NCBI, the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The OMIM database contains textual information and references.

[13] Contiguous gene syndromes are a group of disorders due to deletion of multiple gene *loci* adjacent to one another. They are characterized by multiple, apparently unrelated, clinical features.

genome-wide association studies. LD is also of interest for what it can reveal about evolution of populations. Moreover, studies of LD may enable us to learn more about the biology of recombination (Coop *et al.* 2007; Wang *et al.* 2006). In fact, the HapMap consortium (2005) estimated that around 80% of all recombination has taken place in about 15% of the sequence.

LD is the non-random association of alleles in adjacent *loci*. When a particular allele at one *locus* is found together on the same chromosome with a specific allele at a second *locus*, more often than expected if the *loci* were segregating independently in a population, the *loci* are in disequilibrium. This concept of LD is formalized by one of the earliest measures of disequilibrium to be proposed, D (Lewontin and Kojima 1960). D, in common with most other measures of LD, quantifies disequilibrium as the difference between the observed frequency of a two *loci* haplotype and the frequency it would be expected to show if the alleles are segregating at random. Adopting the standard notation for two adjacent *loci*, A and B, the observed frequency of the haplotype that consists of alleles A and B is represented by P_{AB}. Assuming the independent assortment of alleles at the two *loci*, the expected halotype frequency is calculated as the product of the allele frequency of each of the two alleles, or $P_A \times P_B$, where P_A is the frequency of allele A at the first *locus* and P_B is the frequency of allele B at the second *locus* (Abecasis *et al.* 2005; Jobling *et al.* 2004; Tishkoff and Verrelli 2003; Arcos-Burgos and Muenke 2002; Pritchard and Przeworski 2001). Consequently, one of the simplest measures of disequilibrium is

$$D=P_{AB}-P_A \times P_B$$

LD is created when a new mutation occurs on a chromosome that carries a particular allele at a nearby *locus*, and is gradually eroded by recombination. Recurrent mutations can also lessen the association between alleles at adjacent *loci*.

The extent of LD in populations is expected to decrease with both time (t) and recombinational distance (r, or the recombination fraction) between markers. Theoretically, LD decays with time and distance according to the following formula:

$$D_t=(1-r)^t D_0$$

where D_0 is the extent of disequilibrium at some starting point and D_t, is the extent of disequilibrium t generations later.

A wide variety of statistics, with different strengths depending on the context, have been proposed to measure the amount of LD. Although the measure D has the intuitive concepts of LD, its numerical value is of little use for measuring the strength and comparing levels of LD. This is due to the dependence of D on allele frequencies. The two most common measures are the absolute value of D' and r^2 (Pritchard and Przeworski 2001).

The absolute value of D' is determined by dividing D by its maximum possible value, given the allele frequencies at the two *loci*. The case of D'=1 is known as complete LD. Values of D'<1 indicate that the complete ancestral LD has been disrupted. The magnitude of values of D'<1 has no clear interpretation. Estimates of D' are strongly inflated in small samples. Therefore, statistically significant values of D' that are near one provide a useful indication of minimal historical recombination, but intermediate values should not be used for comparisons of the strength of LD between studies, or to measure the extent of LD (Latini 2004; Varilo 2000, 2003; Angius 2001, 2002).

The measure r^2 is in some ways complementary to D'. The measure r^2 is equal to D^2 divided by the product of the allele frequencies at the two *loci* (Hill and Roberson 1966). Expected levels of LD are a function of recombination. The more recombination between two sites, the more they are shuffled with respect to one another, decreasing LD. Also, LD is a function of N, emphasizing that LD is a property of populations. Another approach for quantifying LD is through the population recombination parameter $4N_ec(\rho)$. This approach avoids reliance on pairwise measures of LD, which differ from marker to marker, and facilitates comparisons between regions.

Mutation and recombination might have the most evident impact on linkage disequilibrium. There are additional contributors to the extent and distribution of disequilibrium. LD can be inflated by demographic factors, including inbreeding, population structure and bottlenecks. Recombination rates are known to vary by more than an order of magnitude across the genome (Jobling *et al.* 2004). Because breakdown of LD is primarily driven by recombination, the extent of LD is expected to vary in inverse relation to the local recombination rate. Some SNPs, such as, those at CpG

dinucleotides, might have high mutation rates and, therefore, show little or no LD with nearby markers, even in the absence of historical recombination. Rapid population growth decreases LD by reducing genetic drift. Population subdivision is likely to have been an important factor in establishing the patterns of LD in humans.

There are two primary routs by which selection can affect the extent of disequilibrium. The first is a hitchhiking effect, in which an entire haplotype that flanks a favoured variant can be rapidly swept to high frequency or even fixation (Jobling *et al.* 2004). Although the effect is generally milder, selection against deleterious variants can also inflate LD, as the deleterious haplotypes are swept from the population. Genetic hitchhiking is expected to affect the frequency distribution of variants at segregating sites such that derived variants will be in higher frequency than expected under a neutral equilibrium model. Genetic hitchhiking is also expected to skew the frequency distribution of variants at segregating site toward rare alleles, resulting in a significantly negative value of Tajima's D[14] (Thornton 2005). It is unknown to what extent this mode of selection increases pairwise LD between high frequency alleles. However, selective sweeps affect sites over a genetic distance on the order of the selection coefficient; consequently, for a single sweep to affect >1 Mb, the advantage of the variant would have to be large (at least 0.01). The second way in which selection can affect LD is through epistatic selection for combinations of alleles at two or more *loci* on the same chromosome. This form of selection leads to the association of particular alleles at different *loci* (Gu *et al.* 2007; Abecasis *et al.* 2005). In a gene conversion event, a short stretch of one copy of a chromosome is transferred to the other copy during meiosis. The effect is equivalent to two very closely spaced recombination events, and can break down LD in a manner similar to recombination or recurrent mutation (Abecasis *et al.* 2005; Pritchard and Przeworski 2001).

LD has been extensively studied in several populations, for example and more recently, Croatia (Vitart *et al.* 2006) and Korea (Lee and Kim 2006). Abbott *et al.* 2006 studied Niue Islanders and report that they are genetically isolated and have a homogeneous southeast Asian ancestry. Moreover, they observe that the Niue population has reduced

[14] The Tajima's D is a widely used test of neutrality in population genetics. It illustrates the allele frequency distribution of nucleotide sequence data and is based on the difference between two estimators of θ (the population mutation rate, 4Neμ). Tajima's estimator uses the average number of pairwise differences between sequences.

autosomal genetic diversity and high levels of linkage disequilibrium that are consistent with the influence of genetic drift mechanisms, such as, a founder effect or bottlenecks. Abbott and collaborators also conclude that high-powered linkage disequilibrium studies, designed to map ancestral polymorphisms that influence complex genetic disease susceptibility, may be feasible in this population. Another study by Vitart *et al.* (2005) analysed 955 unrelated individuals of local ancestry from nine Scottish rural regions and the urban center of Edinburgh, as well as, 96 unrelated individuals from the general UK population. They observed that, despite little overall differentiation on the basis of allele frequencies, there were clear differences among subpopulations in the extent of pairwise LD, measured between a subset of X-linked markers. Vitart and colleagues also reports that there are strategic advantages in studying rural subpopulations, in terms of increased power and reduced cost. They conclude that similar rural-urban contrasts are likely to exist in many other populations with stable rural subpopulations, which could influence the design of genetic association studies and national biobank data collections.

I.3.1. Linkage disequilibrium and the international HapMap project

The completion of the International HapMap Project marks the start of a new phase in human genetics. In order to gain further knowledge in the common patterns of DNA sequence variation, the International HapMap Project was launched in October 2002. This project created a public genome-wide database of common human sequence variation and will provide information to allow indirect association studies to any functional candidate gene, to any region suggested by family-based linkage analysis, or ultimately to whole genome scans of disease risk factors (International HapMap Consortium 2003, 2005). The project shares information rapidly and without restriction on its use. The most important goal of HapMap is to develop a research tool that helps investigators to discover genetic factors that contribute to susceptibility to disease, to protection against illness and to drug response. In its scope and potential consequences, this project has much in common with the Human Genome Project, which sequenced the human genome. Whereas the sequencing project covered the entire genome, including the 99.9% of the genome where humans are all the same, the HapMap

characterizes the common patterns within the 0.1% where humans differ from each other (International HapMap Consortium 2003, 2005).

Phase I of the HapMap Project set as a goal genotyping at least one common SNP every 5 kb across the genome in each of 270 DNA samples. These individuals are 30 mother-father-offspring trios from the Yoruba people of Ibidan Peninsula in Nigeria (referred to as YRI), 30 such trios from the CEPH project in Utah (CEU), 45 unrelated individuals from the Han Chinese population of Beijing (CHB), and 45 unrelated individuals of Japanese ancestry from the Tokyo area (JPT, for many analyses the CHB and JPT samples are combined within a single "analysis panel"). For practicality, and motivated by the allele frequency distribution of variants in the human genome, a minor allele frequency (MAF) of 0.05 or greater was targeted for study (McVean *et al.* 2005). The project has a Phase II, which is attempting genotyping of an additional 4.6 million SNPs in each of the HapMap samples.

Although not designed specifically to enable admixture mapping, the HapMap has helped lay the groundwork for this approach. Admixture mapping requires a map of SNPs that are highly differentiated in frequency across population groups. By typing many SNPs in samples from multiple geographical regions, the data have helped to identify such SNPs for the design of genome-wide admixture mapping panels and can be further used to identify candidate SNPs with large allele frequency differences for follow-up of positive admixture scan results. The advent of genome-wide variation resources, such as the HapMap, opens a new era in population genetics, offering an unprecedented opportunity to investigate the evolutionary forces that have shaped variation in natural populations (International HapMap Consortium 2003, 2005).

The main application of the HapMap data is in the selection of tag single nucleotide polymorphisms (tSNPs) to use in association studies (Montpetit *et al.* 2006). The usefulness of this selection process needs to be verified in populations outside those used for the HapMap project. In addition, it is not known how well the data represent the general population, as only 90-120 chromosomes were used for each population and since the genotyped SNPs were selected so as to have high frequencies. In this study, Montpetit *et al.* (2006) analyzed more than 1000 individuals from Estonia. The population of this northern European country has been influenced by many different waves of migrations from Europe and Russia. These authors genotyped 1536 randomly

selected SNPs from two 500 kb ENCODE regions on chromosome 2. They observed that the tSNPs selected from the CEU HapMap samples captured most of the variation in the Estonia sample. Using the reverse approach, tags selected from the Estonia sample could almost equally well describe the CEU sample. Finally, Montpetit and collaborators observed that the sample size, the allelic frequency, and the SNP density in the dataset used to select the tags each have important effects on the tagging performance. Overall, this study supported the use of HapMap data in other Caucasian populations, but the SNP density and the bias towards high frequency SNPs have to be taken into account when designing association studies.

Another study by Conrad *et al.* (2006) reports haplotype structure across 12 Mb of DNA sequence in 927 individuals representing 52 populations. The geographic distribution of haplotypes reflects human history, with a loss of haplotype diversity as distance increases from Africa. Although the extent of LD varies markedly across populations, considerable sharing of haplotype structure exists, and inferred recombination hotspot locations generally match across groups. To respond to the question: To what extent do the HapMap populations predict patterns of haplotype diversity found in a worldwide set of populations?, Conrad and colleagues (2006) compared their results with the four samples in the International HapMap Project. They observed that the HapMap samples contain the majority of common haplotypes found in most populations: averaging across populations, 83% of common 20 kb haplotypes in a population are also common in the most similar HapMap sample. The authors conclude that, although the portability of tag SNPs based on the HapMap is reduced in low LD Africans, the HapMap will be helpful for the design of genome-wide association mapping studies in nearly all human populations.

Bansal *et al.* (2007) present a statistical method to identify large inversion polymorphisms using unusual LD patterns from high density SNP data. The method is designed to detect chromosomal segments that are inverted (in a majority of the chromosomes) in a population with respect to the reference human genome sequence. These authors demonstrate the power of this method to detect such inversion polymorphisms through simulations done using the HapMap data. Application of this method to the data from the first phase of the International HapMap project resulted in 176 candidate inversions ranging from 200 kb to several megabases in length. Bansal

and collaborators predicted inversions include an 800 kb polymorphic inversion at 7p22, a 1.1 Mb inversion at 16p12, and a novel 1.2 Mb inversion on chromosome 10 that is supported by the presence of two discordant fosmids. Analysis of the genomic sequence around inversion breakpoints showed that 11 predicted inversions are flanked by pairs of highly homologous repeats in the inverted orientation. In addition, for three candidate inversions, the inverted orientation is represented in the Celera genome assembly. Although the power of the method to detect inversions is restricted because of inherently noisy LD patterns in population data, inversions predicted by our method represent strong candidates for experimental validation and analysis.

"…We've discovered the secret of life…"

Francis Crick

CHAPTER II

POPULATION STUDIES:

KNOWING THE PAST TO PREDICT THE FUTURE

II. Population studies: knowing the past to predict the future

Human molecular evolution is based on the concept that patterns of DNA sequence variation determine aspects of human heritage and are shaped by a group of evolutionary influences, such as, genetic drift, selection, mutation and migration. Therefore, genetic variability in the genome reflects both evolutionary adaptive *locus*-specific and population-level processes that affect all components of the genome equally. Genetic research often focuses on distinguishing inconsistencies in patterns of variation between genomic regions to help fill the gap between particular genes and traits (Underhill 2003). By studying the degree of genetic molecular variation, it is possible, in principle, to reconstruct past events, namely, expansions and settlements (Cavalli-Sforza *et al.* 1994). However, since the bulk of common variation in the genome occurs between individuals, the difference between populations is low, making it more challenging to investigate ambiguities concerning affinities and origins of populations. It is the component of inter population variance that best provides insights into the evolution of the extant populations (Cavalli-Sforza and Feldman 2003).

In theory, the evolutionary forces can influence the Hardy-Weinberg equilibrium. Two scientists, Geoffrey Hardy and Wilhelm Weinberg (1908), working independently and based on Mendel's principles of inheritance, developed the concept that is known today as the Hardy-Weinberg Principle, which states: "In a large, randomly breeding (diploid) population, allelic frequencies will remain the same from generation to generation; assuming no unbalanced mutation, gene migration, selection or genetic drift." When a population meets all of the Hardy-Weinberg conditions it is said to be in Hardy-Weinberg equilibrium. If p is the frequency of one allele (A) and q is the frequency of the alternative allele (a) for a biallelic *locus*, then the HWE expected frequency will be p^2 for the AA genotype, $2pq$ for the Aa genotype, and q^2 for the aa genotype. The three genotypic proportions should sum to 1, as should the allele frequencies (Hardy 1908; Weinberg 1908). This equilibrium can be mathematically expressed based on a simple binomial or multinomial distribution of the gene frequencies as:

$$p^2+2pq+q^2=1$$

The most common way to assess HWE is through a goodness-of-fit chi-square (χ^2) test (Weir 1996). The null hypothesis is that alleles are chosen randomly, and the genotypic proportions follow HWE expected proportions (*i.e.* p^2, $2pq$ and q^2). Alternatively, the second allele is dependent on the first allele being selected. This results in the genotypic proportions deviating from the HWE expected proportions (Wittke-Tompson *et al.* 2005; Weir 1996).

HWE predicts how gene frequencies will be transmitted from generation to generation given the specific set of assumptions previously described. Populations in their natural environment can never meet all of the conditions required to achieve HWE, thus, their allele frequencies will change from one generation to the next and the population will evolve. Just how far the population deviates from HWE is an indication of the intensity of the external factors. On the other hand, deviation from Hardy-Weinberg equilibrium has also become an accepted test for genotyping errors (Hosking *et al.* 2004; Leal 2005). However, it is generally considered that testing departures from HWE to detect genotyping error is not sensitive. Cox and Kraft (2006) examined various models of genotyping error, including error caused by neighbouring SNPs that degrade the performance of genotyping assays. They also calculated the power of chi-square goodness-of-fit tests for deviation from HWE to detect such error. They observed that, generally, genotyping error does not generate sufficient deviation from Hardy-Weinberg equilibrium to be detected and genotyping error due to neighbouring SNPs attenuates risk estimates, often drastically.

II.1. Population history, demography and evolutionary forces

The main way to gain insight into past population processes is to analyze and interpret current patterns of genetic variation (von Haeseler 1995). Data on ancient DNA can also help, but they are scarce and will not become abundant in the near future (Cooper and Poinar 2000). One difficulty with modern genes lies in the fact that any given pattern of variation may potentially be explained by several different evolutionary phenomena. A cline or gradient pattern, for example, may reflect adaptation to variable environments, or a population expansion at one moment in time, or continuous gene flow between groups that initially differed in allele frequencies. However, it is possible to discard at least some implausible models by jointly analyzing many *loci* (selection tends to affect

single genes, whereas demographic changes determine similar patterns across the genome), or by exploiting non-genetic information, such as, archaeological and paleobiological data (Barbujani and Bertorelle 2001).

Demographic events can cause an uneven distribution of genetic disorders in different human populations, for example, the occurrence Tay-Sachs disease[15] in Ashkenazi Jews (Risch *et al.* 2003; Weiss 1993), and non-insulin-dependent diabetes mellitus (NIDDM, or diabetes type 2) in Amerindians (Weiss 1993). The interaction between history, demography and genetics is, therefore, of basic importance for the understanding of genetic structure of human populations.

Currently available genetic and archaeological evidence is generally interpreted as supportive of a recent single origin of modern humans in east Africa. However, this is where the near consensus on human settlement history ends, and considerable uncertainty clouds more detailed aspects of human colonization history. Liu *et al.* (2006) using genetic data of 783 autosomal microsatellites in 52 human populations estimated parameters of the expansion of modern humans. Their best estimates suggest an initial expansion of modern humans ~56,000 years ago from a small founding population of ~1000 effective individuals. Their model further points to high growth rates in newly colonized habitats.

The genetic history of a group of populations is usually analyzed by reconstructing a tree of their origins. Reliability of the reconstruction depends on the validity of the hypothesis that genetic differentiation of the populations is mostly due to population fissions followed by independent evolution. Dating the fissions requires comparisons with paleoanthropological and paleontological dates, which are few and uncertain (Cavalli-Sforza 1997). A method of absolute genetic dating uses mutation rates as molecular clocks; it was applied to human evolution using microsatellites, which have a sufficiently high mutation rate. Results agree with a recent expansion of modern humans from Africa. An alternative method of analysis, useful when there is adequate geographic coverage of regions, is the geographic study of frequencies of alleles or

[15] Tay-Sachs disease is an autosomal recessive disorder caused by mutations on the *HEXA* gene (15q23-q24). This gene codes for a subunit of an enzyme called beta-hexosaminidase A. The disease occurs when harmful quantities of a fatty acid derivative called ganglioside accumulate in the brain neurons (for revision, please, see Fernandes Filho and Shapiro 2004).

haplotypes. As in the case of trees, it is necessary to summarize data from many *loci* for conclusions to be acceptable. Results must be independent from the *loci* used. Multivariate analyses like principal components or multidimensional scaling reveal a number of hidden patterns and evaluate their relative importance (Cavalli-Sforza 1997). Most patterns found in the analysis of human living populations are likely to be consequences of demographic expansions, determined by technological developments affecting food availability, transportation or military power. During such expansions, both genes and languages are spread to potentially vast areas. In principle, this tends to create a correlation between the respective evolutionary trees. The correlation is usually positive and often remarkably high. It can be decreased or hidden by phenomena of language replacement and gene replacement, usually partial, due to gene flow (Cavalli-Sforza 1997).

II.1.1. Human population background: paternal and maternal lineages

Explorations into prehistory have been traditionally archaeological; however, additional perspectives have been provided by linguistic and genetic studies. The accumulation of sequence variation in nonrecombining sex-specific *loci* (mitochondrial DNA and Y-chromosome) provides a powerful way to recover genetic prehistory. Nevertheless, the records retained may diverge because of natural selection or differences between male and female behaviours (Underhill 2003).

Since only one mitochondrial DNA (mtDNA) or Y-chromosome lineage can be transmitted by a couple to each of their offspring, compared with four autosomal alleles, the mtDNA and Y-chromosome have a much smaller effective population size – one-quarter that of the autosomes. This makes them much more prone to founder effects during population constrictions. As a result, the mtDNA and Y-chromosome exhibit striking population-specific diversity, which greatly facilitates the identification of founders, aiding in the reconstruction of ancient migrations (Lell and Wallace 2000).

Human mtDNA is a circular double-stranded molecule (Figure II.1), with 16,569 bp in length that codes for 13 subunits of the oxidative phosphorylation system, 2 ribosomal RNAs (rRNAs, ribonucleic acid), and 22 transfer RNAs (tRNAs; Anderson *et al.* 1981; for revison, please see Pakendorf and Stoneking 2005). It is present in hundreds to

thousands of copies in the cell's energy-generating organelles, the mitochondria. mtDNA consists predominantly of coding DNA, with the exception of a ~1100 bp long fragment that has mainly regulatory functions and is, therefore, named the control region. Since the first study of human mtDNA variation (Brown 1980), it has become widely used for studies of human evolution, migration and population history (e.g. Zsurka *et al.* 2007; Weiss and Smith 2007; Olivieri *et al.* 2006; Hebsgaard *et al.* 2007). This widespread use is due to unique features of mtDNA that make it particularly helpful, such as, high copy number, maternal inheritance, lack of recombination and high mutation rate. The high copy number along with its extranuclear cytoplasmic location makes it easier to obtain mtDNA for analysis. Regarding the maternal inheritance, only one case of paternal inheritance of mtDNA is recorded in humans, which was a failure in the normal recognition and elimination of the paternal mtDNA (Schwartz and Vissing 2002). However, this remains an extreemly rare phenomenon.

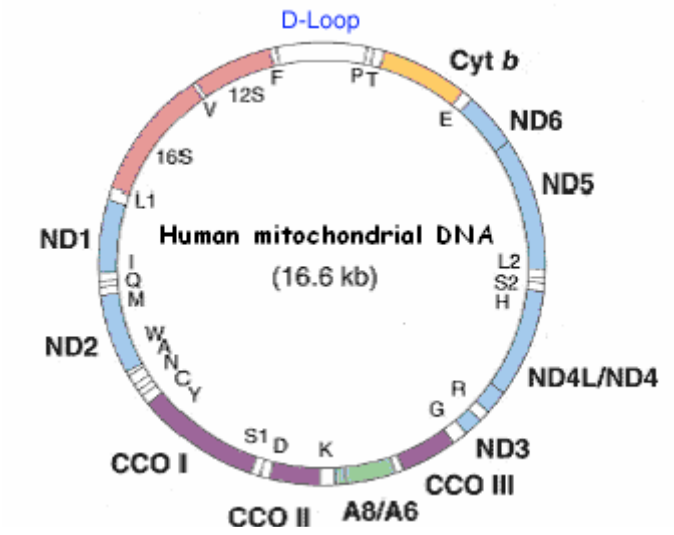


Figure II.1. Human mitochondrial DNA. In D-Loop are located the hypervariable regions (HVRI and HVRII).

Thousands of maternal-offspring comparisons have failed to yield any indication of paternal inheritance (Giles *et al.* 1980; Howell *et al.* 2003). Therefore, at present, maternal inheritance of mtDNA in humans is still regarded as the rule (Schwartz and Vissing 2002). A somatic cell has only two copies of any given nuclear DNA molecule, but hundreds to thousands of copies of mtDNA. Nevertheless, recombination of mtDNA

is a very rare phenomenon. In addition, in the absence of heteroplasmic DNA molecules, any recombination events would result in mtDNAs that do not differ from the original. In terms of mutation rate, the mtDNA presents several orders of magnitude higher than that of nuclear genes, with an estimated rate of $0.017x10^{-6}$ substitutions/ site/ year for the whole genome excluding the control region (Ingman *et al.* 2000). However, in the two hypervariable regions (HVRI and HVRII) of the noncoding control region, the rate is even higher. Phylogenetic comparisons, based on either inter or intraspecific comparisons, yielded estimates of $0.075\text{-}0.165x10^{-6}$ substitutions/ site/ year (Hasegawa *et al.* 2003).

Studies of mtDNA variation in worldwide populations (Figure II.2) have repeatedly found evidence for the "Recent African Origin" hypothesis, with the most recent common ancestor of human mtDNA located in Africa about 100,000-200,000 years ago (Cann *et al.* 1987; Ingman *et al.* 2000). Moreover, direct analyses of mtDNA from fossils of Neanderthals and early modern humans from Europe indicate no contribution of Neanderthal mtDNA to modern humans.

Another insight gained from studies of mtDNA is a better understanding of the migrations that shaped human populations, such as, the peopling of the New World (Kolman *et al.* 1996; Silva *et al.* 2002; Torroni *et al.* 1993) the colonization of the Pacific (Lum and Cann 2000; Murray-McIntosh *et al.* 1998), the initial migration to New Guinea and Australia (Ingman and Gyllensten 2003; Redd *et al.* 2002; van Holst Pellekaan *et al.* 1998), and the settlement of Europe (Richards *et al.* 1996; Simoni *et al.* 2000; Torroni *et al.* 1998). mtDNA is only one *locus* and does not accurately reflect the history of a population because of drift effects or selection. It is, thus, clear that studies of mtDNA variation need to be complemented with data on the male-specific Y-chromosome, and ideally with autosomal data as well (Bamshad *et al.* 2003; Nasidze *et al.* 2004; Shen *et al.* 2004).

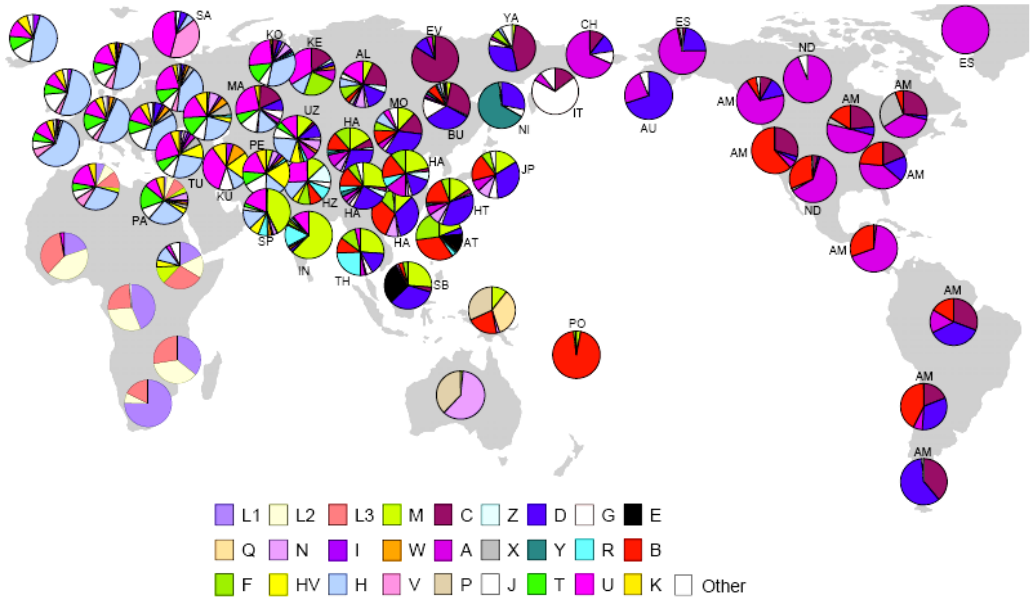


Figure II.2. Worldwide distribution of mtDNA haplogroups. The data in this chart is supposed to represent the situation before the recent European expansion beginning about 1500 YBP (adapted from McDonald 2005).

The haploid Y-chromosome is poor in genes compared to the other nuclear chromosomes. However, the fact that it is largely nonrecombining and presents low effective population size leads to the preservation of haplotypes over evolutionary time scales and to record numerous episodes of population divergence, even on micro-geographic scales (Figure II.3). These properties make it essential in the characterization of population affinity, substructure and history (Underhill 2003 and references therein). The Y-chromosome provides a comparative model for evaluating haplotypes from other regions of the genome. The identification of complex population origin scenarios can be best achieved with an integrative approach, since all evidence should be reflective of an overall history. On the other hand, when different genes yield different haplotype patterns, *locus*-specific forces should be considered. The recent and ongoing progress in deciphering the Y-chromosome structure in contemporary populations (e.g. Walsh *et al.* 2007; Domingues *et al.* 2007; Keyser-Tracqui *et al.* 2006) provides new opportunities to formulate specific testable hypotheses involving human evolutionary population genetics. Although the genetic legacy of *Homo sapiens* remains incomplete, the recent ability to unearth new levels of shared Y-chromosome haplotypic heritage and subsequent diversification provide not only an index of contemporary

population structure, but also a preamble to human prehistory and substantial foundation for comparisons with other genomic regions (Underhill 2003).

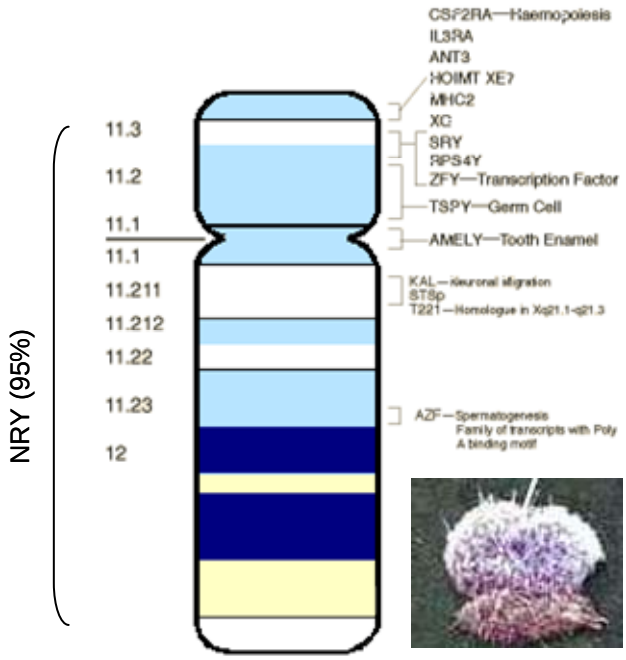


Figure II.3. Human Y-chromosome. The NRY accounts around 95% of the Y-chromosome.

The particular distinctive clinal patterns of NRY (nonrecombining portion of the Y-chromosome) haplotypes (Figure II.4), together with patterns of associated genetic diversification with geography mark trajectories of gene flow and, by inference, the movement of populations (Nonakal *et al.* 2007; Karafet *et al.* 2001). Additionally, the lower effective population size of Y-chromosomes relative to other components of the human genome make this chromosome particularly sensitive to the influences of drift and founder effect. Whatever the causes of this property (e.g. localized natural selection, gender-based differential reproductive success, and/ or migratory behaviour), it is particularly useful since it explains the characteristic high stratification of NRY diversity with geography relative to other genes including mtDNA (Underhill 2003). Currently, over 400 binary polymorphisms (SNPs and *Alu*s) describe the Y-chromosome tree. Several mutually reinforcing binary mutations divide the Y-chromosome haplotype phylogeny into two distinctive components, haplogroup A and the remainder of all other haplogroups, specifically B through R (Y-chromosome

Consortium[16]). The ancestral alleles associated with these ancient polymorphisms are localized exclusively to a minority of both extant north African and subSaharan populations, whereas the majority of other Africans and all non-Africans carry only the derived mutant alleles (Underhill 2003). This mode indicates that almost all modern Y-chromosomes trace their ancestry to a common primogenitor, as expected in a stable genealogy. These Y-chromosome data contradict the possibility that early hominids contributed significantly, if at all, to the gene pool of anatomically modern humans (Capelli *et al.* 2001; Ke *et al.* 2001). This is evidence that all modern human Y-chromosomes trace their ancestry to Africa and that the descendants of the derived lineage left Africa and eventually completely replaced previous archaic human Y-chromosome lineages. A second distinctive monophyletic haplogroup called B, defined by several binary polymorphisms, is also restricted to African populations. Both A and B lineages are diverse and suggest a deeper genealogical heritage than other haplotypes. Representatives of these lineages are distributed across Africa, but generally at low frequencies (Underhill 2003). The phylogenetic position of A and B lineages nearest the root of the Y-tree, their survivorship in isolated populations and accumulated variation are suggestive of an early diversification and dispersal of human populations within Africa, and an early widespread distribution of human populations in that continent. The discovery of *Homo sapiens* fossils in Ethiopia dating to 160,000 years ago is consistent with an African origin of our species (White *et al.* 2003; Underhill 2003).

[16] Y-Chromosome Consortium website: http://ycc.biosci.arizona.edu. This consortium has established a system of defining Y-DNA haplogroups by letters A through R, with further subdivisions.

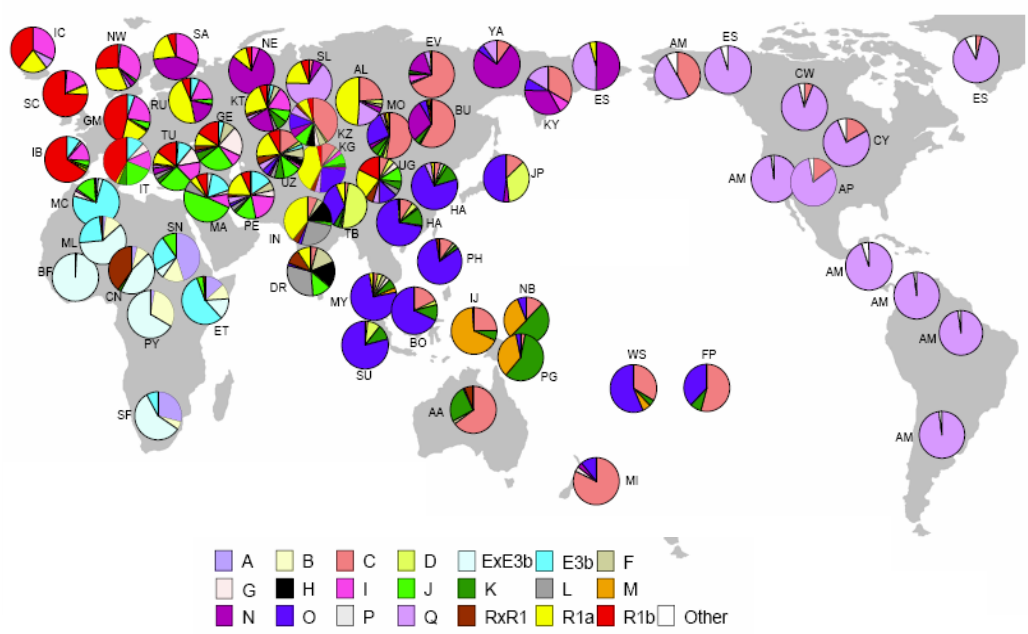


Figure II.4. Worldwide distribution of Y-chromosome haplogroups. The data in this map is supposed to represent the situation before the recent European expansion beginning about 1500 YBP (adapted from McDonald 2005).

Diversity analysis of mtDNA (Chen *et al.* 2000; Quintana-Murci *et al.* 1999) and Y-chromosome (Hammer *et al.* 2001; Semino *et al.* 2002) support a single east African source of migration out of Africa. However, it is possible that there was an earlier migration event from Africa, across southeastern Asia, and into Australo-Melanesia (Thangaraj *et al.* 2003; Kivisild *et al.* 2003). If such an early migration event occurred, it is not clear whether it originated from a population that was genetically differentiated from the population(s) giving rise to subsequent migrations across Eurasia. Both source populations may have originated in northeast Africa from a single common ancestral group. Furthermore, if this earlier migration event did occur, it is likely that the gene pool of modern populations in Australo-Melanesia, which overall are most genetically similar to other non African populations, would reflect admixture between early and later migrants into the region (Tishkoff and Varrelli 2003).

It is generally accepted that the earliest human occupants of Europe arrived during the Paleolithic, on the order of 40,000-50,000 years before the present (YBP), and that agriculture arose in the Near-east during the Neolithic, 10,000 YBP. However, debate has arisen over the mechanism of dispersal of farming within Europe (Lell and Wallace 2000). The demic-diffusion model, proposed by Ammerman and Cavalli-Sforza (1984),

postulates that extensive migrations of Near-eastern farmers during the Neolithic brought agricultural techniques to the European continent. Under this model, the migrant farming populations expanded with little admixture with the Mesolithic European inhabitants, so that a large proportion of the present-day European gene pool should be derived from the Neolithic migrants. Alternatively, others have proposed a cultural-diffusion model (Dennell 1983) in which the transfer of agricultural technology occurred without significant population movement. Under this model, the majority of the genetic diversity within Europe would have its roots in the Paleolithic (Cavalli-Sforza *et al.* 1994; Lell and Wallace 2000).

In contrast to the gradients observed for classic gene frequencies and other nuclear DNA markers, including the Y-chromosome, initial studies of European variation in the maternally inherited mtDNA did not seem to support the demic-diffusion of Neolithic farmers (Lell and Wallace 2000). The mtDNA landscape of Europe appeared very homogeneous, with little geographic clustering of types (Richards *et al.* 1996; Comas *et al.* 1998). In particular, Richards *et al.* (1996) argued that the genetic contributions of Neolithic migrants had been greatly exaggerated and that the major extant lineages of Europe could be traced back to the Upper Paleolithic. This questioning of the demic-diffusion model for the peopling of Europe led to a debate over the interpretations of the genetic studies supporting the competing models (Cavalli-Sforza and Minch 1997; Barbujani *et al.* 1998). In addition to the early Paleolithic and Neolithic expansions into Europe, mtDNA studies in Europe have suggested a Late Upper Paleolithic population expansion from southeastern Europe, as evidenced by clines radiating from Iberia (Torroni *et al.* 1998). Nevertheless, a more recent study has questioned this conclusion (Simoni *et al.* 2000; Lell and Wallace 2000). Simoni *et al.* demonstrated that both a Paleolithic expansion and the Neolithic demic-diffusion of farmers could have determined a longitudinal cline of mtDNA diversity.

II.1.2. Evolutionary forces

The human population is not in equilibrium. Humans occupy such a broad range of environments and respond to environmental changes by evolving in a predominantly cultural rather than biological way. The current patterns of migration and population

growth are similar to those that have predominated over much of human prehistory. These observations raise questions such as: Have human allele frequencies been frozen in time? Are humans still undergoing natural selection? Can we expect any changes to the human phenotype and will humans speciate? (Jobling *et al.* 2004). Some of these questions address the microevolutionary pressures – mutation, genetic drift, migration and selection – operating on modern humans, while other queries focus on the macroevolutionary future – whether speciation is likely or inevitable.

II.1.2.1. Genetic drift

Genetic drift – along with natural selection, mutation and migration – is one of the basic mechanisms of evolution. It describes changes in allele frequency from one generation to the next due to sampling variance. The frequency of an allele in the offspring generation will vary according to the probability distribution of the frequency of the allele in the parent generation. Many aspects of genetic drift depend on the size of the population. This is especially important in small mating populations, where chance fluctuations from generation to generation can be large. Such fluctuations in allele frequency between successive generations may result in some alleles disappearing from the population. For example, two separate populations that begin with the same allele frequency might "drift" by random fluctuation into two divergent populations with different allele sets, for example, alleles that are present in one have been lost in the other (Pardo *et al.* 2005; Arcos-Burgos and Muenke 2002).

In small populations subject to drift, the rate of evolutionary change can be speeded up dramatically, and allele and genotype frequencies can change unpredictably from one generation to the next: the smaller the population, the more extreme these fluctuations tend to be. Like selection, genetic drift is a process of differential reproductive success; nevertheless, the key element of this evolutionary force is that the individuals that survive and reproduce are random, *i.e.* unrelated to their phenotype and genotype (Willi *et al.* 2007; Rudan *et al.* 2006). Because it is a random process, the outcome in any generation is unpredictable; however, certain generalities can be made and reliably predict the cumulative effects of genetic drift. On average it is expected that (*i*) small populations will show large but random fluctuations in allele and genotype frequencies,

i.e. some alleles will be lost over time, reducing the amount of genetic variation in the population, eventually, only one allele will become fixed; and (*ii*) replicate populations will diverge genetically over time and because everything happening within a generation is random, the population will appear to be in Hardy-Weinberg equilibrium at any time. Genetic drift can be observed by the occurrence of two main processes: bottleneck and founder effect (Figure II.5).

A population bottleneck is a significant reduction in the size of a population that causes the extinction of many genetic lineages within that population, thus, decreasing genetic diversity. Several studies have demonstrated the occurrence of bottlenecks in the human population (e.g. Rootsi *et al.* 2007; Battilana *et al.* 2006; Kasperaviciute *et al.* 2004). Schmegner *et al.* (2005) studying the *NF1*[17] gene demonstrated that the recent European population went through a bottleneck during the last 150,000 years of its history. Moreover, considering this timeframe, the bottleneck could either reflect a speciation event which led to the anatomically modern human (AMH), or a severe reduction of the population size during the emigration of AMHs out of Africa or the immigration into Europe.

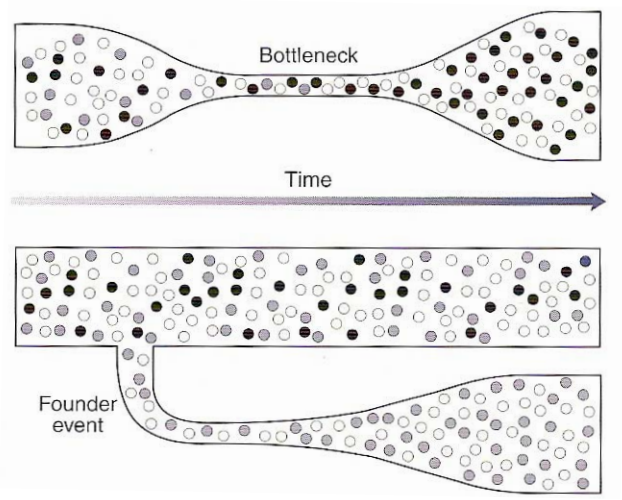


Figure II.5. Bottleneck and founder effects representation. Circles of different colours represent different alleles. Both effects result in loss of allelic diversity (adapted from Jobling *et al.* 2004).

[17] For further knowledge on the *NF1* gene, please, consult Chapter I of the present thesis.

Another study by Behar *et al.* (2004) tested the effects of a maternal bottleneck on the Ashkenazi Jewish population. They analysed mtDNA in 565 Ashkenazi Jews from different parts of Europe. Their results showed that while several Ashkenazi Jewish mtDNA haplogroup appear to derive from the Near-east, there is also evidence for a low level of introgression from host European non-Jewish populations. The diversity patterns obtained provide evidence for a prolonged period of low effective size in the history of the Ashkenazi population. Overall, the data best fit a model of an early bottleneck (~100 generations ago), perhaps corresponding to initial migrations of ancestral Ashkenazi in the Near-east or to Europe. Behar *et al.* (2004) conclude that a genetic bottleneck followed by the recent phenomenon of rapid population growth are likely to have produced the conditions that led to the high frequency of many genetic disease alleles in the Ashkenazi population. Another study by Kasperaviciute *et al.* (2004) analysed the genetic composition of the Lithuanian population through mtDNA and Y-chromosome markers. Significant differences between Lithuanian and Estonian Y-chromosome STR haplotypes indicated that these populations have had different demographic histories. Kasperaviciute *et al.* (2004) suggest that the observed pattern of Y-chromosome diversity in Lithuanians may be explained by a population bottleneck associated with Indo-European contact. Different Y-chromosome STR distributions in Lithuanians and Estonians might be explained by different origins or, alternatively, be the result of some period of isolation and genetic drift after the population split.

More recently and to explore the evolutionary forces that might have morphed human genome architecture, Gherman *et al*. (2007) investigated the origin, composition, and functional potential of "numts" (nuclear mitochondrial pseudogenes), partial copies of the mitochondrial genome found abundantly in chromosomal DNA. Their data indicate that these elements are unlikely to be advantageous, since they possess no gross positional, transcriptional, or translational features that might indicate beneficial functionality subsequent to integration. Using sequence analysis and fossil dating. These authors also show a probable burst of integration of "numts" in the primate lineage that centers on the prosimian–anthropoid split, mimics closely the temporal distribution of *Alu* and processed pseudogene acquisition, and coincides with the major climatic change at the Paleocene–Eocene boundary. Gherman and collaborators propose a model according to which the gross architecture and repeat distribution of the human genome can be largely accounted for by a population bottleneck early in the anthropoid

lineage and subsequent effectively neutral fixation of repetitive DNA, rather than positive selection or unusual insertion pressures.

The founder effect can be defined as what happens when a small group of individuals leaves a larger population and establishes a new one. Hence, chance plays a important role in determining which alleles are represented in the new population. The particular alleles may not be representative of the larger population. As the new population grows, the allele frequencies will usually continue to reflect the original small group (Zlotogora 2007; Jobling *et al.* 2004).

Founder populations have been the subject of complex disease studies because of their decreased genetic heterogeneity, increased linkage disequilibrium and more homogeneous environmental exposures. However, it is possible that disease alleles identified in founder populations may not contribute significantly to susceptibility in outbred populations (Zlotogora 2007; Laberge *et al.* 2005). Newman *et al.* (2004) examined the Hutterites, a founder population of European descent, for 103 polymorphisms in 66 genes that are candidates for cardiovascular or inflammatory diseases. The data revealed that this founder population is informative and offers considerable advantages for genetic studies of common complex diseases. Hamet *et al.* (2005) studied 120 extended families with at least one sib-pair affected with early-onset hypertension and/ or dyslipidemia in the Saguenay-Lac-Saint-Jean (Quebec). They observed founder effect over several generations and classes of living individuals. Other studies (Rootsi *et al.* 2007; Kalaydjieva *et al.* 2005) demonstrated evidences of the influence of founder effects in the genetic signature of the populations. For example, Nebel *et al.* (2005) studying the Y-chromosome of Ashkenazi Jews demonstrated that of the 495 Y-chromosomes, 57 (11.5%) were found to belong to R-M17[18]. The haplotype structure, diversity and geographic distribution suggested a founder effect for this haplogroup, introduced at an early stage into the evolving Ashkenazi community in Europe. R-M17 chromosomes in Ashkenazi may represent vestiges of the mysterious Khazars. In summary, the study of founder effects allows that traits transmitted through generational lineage may be determined quantitatively within population subsets, thus, accelerating the uncovering of causal haplotypes in complex diseases (Hamet *et al.* 2005).

[18] According to the Y-Chromosome Consortium (http://ycc.biosci.arizona.edu) nomenclature the mutation R-M17 corresponds to R1a1* lineage.

II.1.2.2. Selection

Natural selection, as defined by Darwin and elaborated by Fisher, is the differential reproduction of genotypes in succeeding generations. Genotypic variation produces individuals with varying capacities to survive and reproduce in different environments. Selection can occur at any stage from the formation of a genotype at fertilization to the individual generating viable progeny. Overall, it may include the (*i*) survival in reproductive age, that is, viability and mortality; (*ii*) success in attracting a mate, *i.e.* sexual selection; (*iii*) ability to fertilize, this is, fertility and gamete selection; and, finally, (*iv*) number of progeny, *i.e.* fecundity (Nielsen 2005; Jobling *et al.* 2004). The overall sum of these is the ability of an individual genotype to survive and reproduce, its fitness, which is partly dependent on the environment. Relative fitness of a genotype compared to other genotypes competing for the same resources is an important factor when measuring selection. A selection coefficient of 0.1 represents a 10% decrease in fitness of the genotype compared to the fittest one (Gilad *et al.* 2006; Jobling *et al.* 2004).

Simply, mutations that increase fitness undergo positive selection whereas mutations that reduce the fitness are subject to negative selection, also known as purifying selection. Positive selection has undoubtedly played a critical role in the evolution of *Homo sapiens*. Of the many phenotypic traits that define our species, for example, the enlarged brain, the advanced cognitive abilities, the complex vocal organs, bipedalism and opposable thumbs, most are likely the product of strong positive selection. Comparative genetics and genomics studies in recent years have uncovered a growing list of genes that might have experienced positive selection during the evolution of human and/ or primates (Wang *et al.* 2006; Voight *et al.* 2006; Sabeti *et al.* 2006). These genes offer valuable insights into understanding the biological processes specific to humans, and the evolutionary forces that gave rise to them.

However, to understand the dynamics of selection at diploid *loci* it should be considered the impact of mutants on the fitness of the genotypes, and not on the individual alleles. The two alleles within a diploid genotype can interact to determine the phenotypic fitness of an organism in different ways. This in turn affects the efficiency of natural selection in fixing or eliminating novel alleles (Nielsen 2005). For example, in codominant selection, a novel deleterious allele will be eliminated more rapidly from

the population if it reduces the fitness of the heterozygote, as well as, the homozygote. Alternatively, in overdominant selection, a new allele may increase the fitness of an heterozygote relative to that of both homozygotes. The two homozygous genotypes may exhibit different reductions in fitness creating a balanced polymorphism. On contrary, underdominant selection operates where new alleles reduce the fitness of the heterozygote alone. Other processes, besides overdominant selection, can create a balancing selection, for example, frequency-dependent selection, where the frequency of a genotype determines its fitness. If a genotype has higher fitness at low frequencies but lower fitness at higher frequencies, an intermediate equilibrium value will be reached over time (Charlesworth 2006; Krawczak and Zschocke 2003). The major histocompatibility complex (MHC) *locus* has been suggested to be under both frequency-dependent and overdorninant selection (Muller-Hilke and Mitchison 2006).

Other classic examples of balanced polymorphisms in humans are those that protect against malaria[19] when heterozygous, but have a reduced fitness compared to wild-type when homozygous. A number of these types of balanced polymorphisms have arisen in different areas of malarial endemicity (Polley *et al.* 2007; Verra *et al.* 2006).

Changes in genetic regulation contribute to adaptations in natural populations and influence susceptibility to human diseases. Despite their potential phenotypic importance, the selective pressures acting on regulatory processes and gene expression are largely unknown. Studies in model organisms suggest that the expression levels of most genes evolve under stabilizing selection, although a few are consistent with adaptive evolution (Gilad *et al.* 2006). Nonetheless, it has been proposed that gene expression levels in primates evolve largely in the absence of selective constraints. Gilad *et al.* (2006) demonstrated that stabilizing selection is likely to be the dominant mode of gene expression evolution. An important implication is that mutations affecting gene expression will often be deleterious and might underlie many human diseases.

Tishkoff *et al.* (2007) conducted a genotype-phenotype association study in 470 Tanzanians, Kenyans and Sudanese and identified three SNPs (G/C-14010, T/G-13915

[19] Malaria is a potentially deadly tropical disease characterized by cyclical bouts of fever with muscle stiffness, shaking and sweating. It is caused by a parasite of the *Plasmodium* genus that is transmitted by the female mosquito of *Anopheles* genus (for review, please, see Conway 2007).

and C/G-13907) that are associated with lactase persistence[20]. These SNPs also show derived alleles that significantly enhance transcription from the lactase (*LCT*) promoter *in vitro*. Genotyping across a 3 Mb region demonstrated haplotype homozygosity extending >2.0 Mb on chromosomes carrying C-14010, consistent with a selective sweep over the past, approximately 7,000 years. Overall, they conclude that these data provide a marked example of convergent evolution due to strong selective pressure resulting from shared cultural traits, animal domestication and adult milk consumption.

The identification of signals of very recent positive selection provides information about the adaptation of modern humans to local conditions (Voight *et al.* 2006). Voight and colleagues (2006) report a genome-wide scan for signals of very recent positive selection in favor of variants that have not yet reached fixation. They observed in three continental groups widespread signals of recent positive selection. Most signals are region-specific, though a significant excess are shared across groups. Contrary to some earlier studies that suggested a paucity of recent selection in subSaharan Africans, they found that the strongest signals of selection were from the Yoruba population. Finally, these authors conclude that since the signals suggest the existence of genetic variants that have substantially different fitnesses, it must indicate *loci* that are the source of significant phenotypic variation. Though the relevant phenotypes are generally not known, such *loci* should be of particular interest in mapping studies of complex traits.

II.1.2.3. Mutation and recombination

Mutation is the process generating new alleles. It provides the raw material on which selection and the other forces of evolution can act. There are a broad variety of mutational changes, and these occur at varying rates. Each mutation is a single change occurring in a single cell. Evolutionary consequences only follow from those changes that occur in the germline, and not those in somatic tissues, as somatic mutations are not

[20] The enzyme lactase, located in the villus enterocytes of the small intestine, is responsible for digestion of lactose in milk. Lactase activity is high and vital during infancy, but in most mammals, including most humans, lactase activity declines after the weaning phase. In other healthy humans, lactase activity persists at a high level throughout adult life, enabling them to digest lactose as adults. This dominantly inherited genetic trait is known as lactase persistence. The distribution of these different lactase phenotypes in human populations is highly variable and is controlled by a polymorphic element *cis*-acting of the *lactase* gene (2q21, for revision, please, see Sibley 2004).

heritable. The dynamics of many types of mutations vary between the soma and the germline. Because of the fidelity of DNA polymerases and the operation of DNA repair mechanisms, germline mutations occur at low rates for individual nucleotides, although they are inevitable in every replication cycle. It has been estimated that every human carries, on average, 128 new mutations (Giannelli *et al.* 1999).

In the absence of other processes, an allele will decrease in frequency as it accumulates mutations, a phenomenon known as mutation pressure. By knowing the mutation rate for the whole gene (μ), the initial allele (P_0) frequency, assuming no back mutation and ignoring stochastic processes, the allele's frequency (p_t) t generations later is calculated by:

$$p_t = p_0 e^{-\mu t}$$

At low mutation rates, mutation pressure is a weak force that can only have appreciable impact over long time scales. After 1000 generations, the wild-type allele of a gene 1 kb in size with a *per* generation nucleotide mutation rate of $2x10^{-9}$ will only decrease in frequency from 1.0 to 0.998 (Jobling *et al.* 2004). However, the possibility of back mutations and recurrent mutations was not analysed. If we consider a gene 1 kb in length then the number of possible alleles is enormous, 4^{1000}. The probability of back mutations and recurrent mutations is correspondingly small. This model is known as the infinite alleles model (IAM; Crow and Kimura 1970). On the other hand, considering the evolution of a polymorphic microsatellite, oscillating in size by number of repeats, the opportunity for back mutation and recurrent mutation is much higher than for SNPs. Thus, the IAM does not always appear to be a close approximation of biological reality. Therefore, it is necessary different models for different types of mutation. The stepwise mutation model (Ohta and Kimura 1973) provides a better fit to microsatellite evolution. According to this model, mutation increase and decrease allele length by one unit with equal probability (Hartl and Clark 1997; Jobling *et al.* 2004).

Initially, the SMM considered single-step changes only, but there is good empirical evidence for a lower rate for multiple step mutations which the model can account for (Di Rienzo *et al.* 1994). There are, however, other known aspects of microsatellite evolution not incorporated within the SMM model, for example, the (*i*) positive correlation between allele length and mutability; (*ii*) lower length threshold under which mutation rate becomes undetectable; (*iii*) possible small bias towards expansions of

short alleles, resulting in an increase in size of the microsatellite; (*iv*) possible preference for deletions rather than expansions in longer alleles, producing an equilibrium in allele length; and (*v*) massive expansions in triplet repeat diseases and consequent negative selection. Other types of mutations, such as, rearrangements and GC-rich minisatellites, do not fit any of the above models. Considering other aspects of sequence evolution it is necessary to suppose that several changes might have occurred at the same site, then more complex models of mutation must be developed. For example, it could be necessary to consider the probability that an A will mutate to a C and then subsequently back from a C to an A again. These models come into play when considering sequence evolution over long time scales, where back mutations result in the observed sequence divergence being an underestimate of the real number of mutational changes. In the simplest model all nucleotide substitutions occur at the same rate, while the most complex model allows a different rate for each nucleotide change. These models can be represented as a substitution scheme and as a probability matrix. The simplest example is known as the Jukes-Cantor model (JC, Jukes and Cantor 1969), and one of the more complex models is the general reversible model (REV). There are a number of intermediate models that contain some, but not all, of the complexity of the REV model (Jobling *et al.* 2004; Hartl and Clark 1997). The frequency of each nucleotide clearly influences the probability of nucleotide changes averaged over an entire sequence. For example, an A to G transition may have the same rate as a C to T transition, but if there are twice as many Cs in a sequence then the probability of an A to G occurring within the sequence is not the same as that of a C to T. The JC model does not take potential bias in base composition into account, but the REV model does (Jobling *et al.* 2004; Hartl and Clark 1997).

Another process generating diversity is meiotic recombination which is a consequence of sexual reproduction, and enhances the ability of populations to adapt to their environment through the combining of advantageous alleles at different *loci*. Recombination generates new combinations of alleles on the same DNA molecule, known as haplotypes and in this way increases haplotype diversity. Consequently, recombination is capable of breaking up advantageous allelic combinations. Theoretically, by increasing the likelihood of disrupting a beneficial haplotype, outbreeding can result in a drop in fitness known as outbreeding depression. While alleles at *loci* on different chromosomes are randomly segregated during meiosis,

alleles at *loci* closely linked on the same chromosome are not, as recombination between them occurs infrequently (Jobling *et al.* 2004; Hartl and Clark 1997).

In comparison to mutation models, recombination models have traditionally been relatively simple. The simplest model is that the rate of recombination is uniform. In other words, the probability of a crossover occurring between a pair of markers is determined only by the physical distance that separates them. The products of this type of recombination event are two new haplotypes containing contiguous stretches of alleles from each ancestral haplotype. Empirical studies of recombination in humans and model organisms have revealed two biological properties of recombination that conflict with the simplest model of recombination, this is, not every recombination event results in a crossover (Jobling *et al.* 2004; Hartl and Clark 1997; Hellenthal and Stephens 2006; Spencer *et al.* 2006).

Recombination rates are not uniform along a segment of DNA. Crossovers appear to be concentrated in hotspots between which are regions recombinationally inert. At larger scales, recombination rates vary along the chromosome in ways that are only now being elucidated, but are often low near centromeres and high near telomeres (Jobling *et al.* 2004; Hartl and Clark 1997; Hellenthal and Stephens 2006). In humans, the rate of recombination, as measured on the megabase scale, is positively associated with the level of genetic variation, as measured at the gene scale. Despite considerable debate, it is not clear whether these factors are causally linked or, if they are, whether this is driven by the repeated action of adaptive evolution or molecular processes, such as, double-strand break formation and mismatch repair (Spencer *et al.* 2006). Spencer and colleagues (2006) introduced three innovations to the analysis of recombination and diversity: (*i*) fine-scale genetic maps estimated from genotype experiments that identify recombination hotspots at the kilobase scale, (*ii*) analysis of an entire human chromosome, and (*iii*) the use of wavelet techniques to identify correlations acting at different scales. They show that recombination influences genetic diversity only at the level of recombination hotspots. Hotspots are also associated with local increases in GC-content and the relative frequency of GC increasing mutations but have no effect on substitution rates. Broad-scale association between recombination and diversity is explained through covariance of both factors with base composition. These results

evidence a direct and local influence of recombination hotspots on genetic variation and the fate of individual mutations (Lindsay *et al.* 2007).

II.1.2.4. Migration or gene flow

Migration, often used as a synonym for gene flow, is probably the most powerful microevolutionary factor leading to the uniformity of populations characteristics. Theory predicts that whenever the relative weight of gene flow exceeds that of drift, as is to be expected in modern human populations (Morton 1982), the frequencies of a neutral allele at equilibrium will be distributed unimodally (Wright 1921). However, the equilibrium distribution is established slowly and, as a consequence, sharper genetic change may be expected in the regions in which past gene flow has been inefficient to eliminate pre-existing genetic differences (Sokal *et al.* 1989; Barbujani *et al.* 1989).

Gene flow has, therefore, relied upon indirect methods that relate measures of population subdivision to gene flow via a model for the population structure. Unlike genetic drift, mutation and selection, migration cannot change species allele frequencies, but it is capable of changing allele frequencies within a subpopulation. In general, gene flow is the outcome when a migrant contributes to the next generation in their new location, and migration is the movement from one occupied area to another. Thus, to observe gene flow directly it is necessary to monitor the movement of migrants and their reproductive success.

The simplest model of gene flow is the island model devised by Sewall Wright. A metapopulation is split into "islands" of equal size N, which exchange genes at the same rate, *m*, *per* generation. The assumptions of the island model include: (*i*) no geographical substructure apart from the division into islands – all islands are equivalent, (*ii*) each population persists indefinitely, (*iii*) no mutation, (*iv*) no selection, (*v*) each population has reached equilibrium between mutation and genetic drift, and (*vi*) the migrants are a random sample from the source "island".

The stepping-stone model (Kimura and Weiss 1964) removes from the "island" one the lack of geographic substructure. The stepping-stone introduces the idea of geographical distance by only allowing the exchange of genes between adjacent discrete

subpopulations. This model also assumes equal rates of migration between subpopulations. Both models have been used to show that even very low rates of migration between subpopulations are capable of retarding their genetic differentiation (Jobling *et al.* 2004; Hartl and Clark 1997).

Migration can be modelled as occurring within a continuous population, rather than discrete subpopulations, by considering that mating choices are limited by distance, and that these distances are typically less than the overall range of the population. This is the basis for isolation by distance model (IBDM, Wright 1943; Malécot 1950). Within such model, genetic similarity develops in neighbourhoods as a function of dispersal distances, for example, parent birthplaces. Neighbouring populations frequently exchange individuals by an ongoing process of bi-directional migration. However, a third, hybrid population does not usually result from this kind of exchange. The term admixture is often reserved for the formation of a hybrid population from the mixing of ancestral populations that have previously been in relative isolation from one another. The range expansion of one population into a region inhabited by a previously isolated population is one of such scenario. Therefore, admixture can be thought as being initiated at a specific point in time, when the populations first came into contact. When we examine modern populations, we detect not simply the proportions of admixture established when the populations first met, but the summation of cumulative gene flow from when they first met to the present-day (Price *et al.* 2007; Mao *et al.* 2007). Thus, the consequences of admixture and gene flow may be difficult to distinguish. Naturally, the imprint of past admixture in modern populations has also been modified by drift, selection and mutation processes.

The isolation and expansion that result in subsequent admixture can be driven by environmental changes. During the recent ice ages, the environment at more northerly latitudes became uninhabitable. Humans and other plant and animal species found refuge in more hospitable climate, known as “glacial refugia”. These refugia were often isolated from one another. For example, it is known three major European glacial refugia: the Iberian Peninsula, Italy and the Balkans. After the end of the last ice age, many species started the long process of re-colonizing the more northerly latitudes from these refugia. During this period, many previously isolated populations were in contact

with each other, therefore, the genetic consequences can be analyzed through admixture (Rootsi *et al.* 2004; Iriondo *et al.* 2003; Torroni *et al.* 2001; Jobling *et al.* 2004).

Admixture shapes genetic diversity in a number of different ways. Our ability to detect admixture depends in part on how differentiated the source populations were from one another, the more different the ancestral populations were, the easier it is to detect admixture. There are some problems with the assessment of admixture in a single genome, some alleles may have their ancestry in one parental population while other alleles have their ancestry in another. This is a consequence of sexual reproduction and diploidy. In fact, it is highly unlikely that any individual in an admixed population will be able to trace all their genes to a single source population; different genomes within an admixed population are likely to exhibit differing amounts of admixture. Thus, an estimate of population admixture can only be an average of the admixture among the individual genomes within it (Jobling *et al.* 2004; Price *et al.* 2007; Mao *et al.* 2007).

All generic admixtures will lead to a variety of phenotypic effects. Any quantitative trait that is generically encoded and well differentiated between populations will be altered in admixed populations. In societies where surnames follow clear lines of inheritance, they have often been used for population genetic analyses as mentioned in Chapter I of the present thesis. Admixture studies are no exception. Patterns of surname introgression have been clearly shown to be correlated with levels of admixture in a number of different populations (Chakraborty 1986; McEvoy *et al.* 2006). These conclusions have subsequently been reinforced by genetic typing. Nevertheless, surname analysis is useful when admixture has occurred within the timeframe of surname usage, which varies greatly from population to population, and may be very recent. However, if records are detailed enough, surname analysis can reveal how admixture processes may have changed over time (Jobling *et al.* 2004).

Disease prevalences are often clearly different between ancestral populations. An obvious medical consequence of admixture is that the hybrid population is expected to have disease prevalence's for Mendelian disorders that are intermediate between those of the ancestral populations. When the most frequent diseases differ between the populations, this can lead to an overall lowering of the disease burden through a reduction in the probability of having two parents carrying the same deleterious recessive allele (Jobling *et al.* 2004; Alegre *et al.* 2007).

II.2. Genetic distance and population structure

II.2.1. Genetic distance measures

Measures of genetic distance are statistics that allow us to compare the relatedness of populations or molecules (Jobling *et al.* 2004). The greater the evolutionary distance between them, the greater the value of the statistic. If a measure is greater between population A and B than between C and D, we can say that C and D are more closely related than are A and B. Such measures allow the exploration of population structure and molecular diversity in greater detail, by pairwise comparisons, rather than by averaging over all populations or molecules. Additionally, it is possible to convert distance measures to an evolutionary time scale (Jobling *et al.* 2004), and observations, such as, C and D share a more recent common ancestor than A and B are probable. Conversely, genetic distance measures also allow the construction of phylogenies of populations or molecules (e.g. Kumar *et al.* 2007; Khan *et al.* 2007).

There are a number of commonly used measures of genetic distances between populations. Despite the abundance of measures, which arose in response to different data types and different expectations about evolutionary processes. For example, diversity data from markers with a high mutation rate may be analyzed with a genetic distance measure that emphasizes the contribution of mutational processes to population divergence (Jobling *et al.* 2004). If we consider two populations X and Y with the frequency of the *ith* allele being x_i and y_i, respectively, the simplest measure of genetic distance between them sums the difference between the allele frequencies, $\Sigma(x_i-y_i)$. This needs to be squared to avoid differences in sign, $\Sigma(x_i-y_i)^2$. However, sufficient weight to alleles with frequencies close to 0% or 100% is not given. Two commonly used classical measures of genetic distance are F_{ST} and Nei's standard genetic distance, D (Nei 1973). Both of these vary between 0 (for identical populations), and 1 (for populations that share no alleles). For use as a genetic distance, F_{ST} is specifically formulated for two populations and can be defined as:

$$F_{ST}=V_p/p(1-p)$$

where p and V_p are the mean and variance of gene frequencies between the two populations, respectively. This is just a weighted form of the simple measure considered above, that increases the influence given to alleles that are almost fixed (p~100%) or

barely polymorphic (p~0%). Nevertheless, there are a variety of different methods for estimating F_{ST}. Nei's standard genetic distance, D, relates the probability of drawing two identical alleles from the two different populations (which is $\Sigma x_i y_i$) to the probability of drawing identical alleles from the same population $\Sigma(x_i^2$ and $y_i^2)$ by the following equation:

$$D=-\ln(\Sigma x_i y_i/\Sigma x_i^2(y_i^2)^{1/2})$$

By making assumptions about the processes that are driving the divergence of populations, we can relate distance measures to absolute time. This relationship can then be used to generate a corrected version of the statistic that can be shown, under certain assumptions, to be linear with respect to evolutionary time (Khaitovich *et al.* 2005; Ayub *et al.* 2003). However, bottlenecks and migration can disrupt the linear relationship between a given genetic distance measure and time. Linearity of the genetic distance measure is a useful property especially when constructing phylogenies. The other major property that affects the usefulness of a measure is its variance: the lower the variance of the statistic, the higher the confidence (Jobling *et al.* 2004). Whatever measure of genetic distance between populations is used, its significance must be tested, *i.e.* determine if the distance is significantly different from zero. This is especially important for human populations, which are often closely related (Jobling *et al.* 2004).

II.2.2. Population structure and inbreeding

Genetic subdivision or structure affects both the evolution and the persistence of populations. For instance, subdivision has been shown to have an important effect on the probability of fixation of beneficial and deleterious alleles, the evolution of mating systems or the probability of population extinction. One of the reasons of this influence is that subdivision changes the way in which the different evolutionary processes (selection, genetic drift, mutation and migration) act on allele frequency, compared to a continuous population. As a result, the population's genetic load (*i.e.* the decline in fitness due to accumulation of deleterious alleles) can be strongly determined by population structure (Glémin *et al.* 2003).

Subdivision can vary in several ways, including the size and the number of the subpopulations and the rate of migration between subpopulations. Changes in these parameters can significantly modify the balance between drift and selection within

subpopulations and, thus, genetic load: (*i*) for slightly deleterious and partially recessive alleles, subpopulation size determines both the response to selection and the strength of genetic drift; larger subpopulations should be associated with lower frequencies of deleterious alleles; (*ii*) migration between subpopulations restores genetic variability within subpopulations, enhancing selection; and (*iii*) the number of subpopulations influences population genetic variance. Increasing the number of subpopulations should result in a higher genetic differentiation between them and, thus, a higher potential for fitness to be restored by migration. It is also interesting to note that subdivision can have variable effects according to the characteristics of deleterious mutations. For instance, genetic variance within subpopulations could decrease for nearly additive alleles but it can increase for highly recessive alleles.

Population subdivision results in the loss of genetic variation within subpopulations due to evolutionary forces. This means that population subdivision would result in decreased heterozygosity relative to the expected heterozygosity under random mating as if the whole population was a single breeding unit. Wright developed three fixation indexes to evaluate population subdivision: F_{IS} (Individual within the Subpopulation), F_{ST} (Subpopulation within the Total population) and F_{IT} (Individual within the Total population). F_{IS} is a measure of the deviation of genotypic frequencies from panmictic frequencies in terms of heterozygous deficiency or excess. It is what is known as the inbreeding coefficient, which is conventionally defined as the probability that two alleles in an individual are identical by descent. The technical description is the correlation of uniting gametes relative to gametes drawn at random from within a subpopulation averaged over subpopulations. It is calculated in a single population as:

$$F_{IS}=H_{EXP}-H_{OBS}/H_{EXP}$$

where H_{OBS} is the observed heterozygosity and H_{EXP} is the expected heterozygosity calculated on the assumption of random mating (Hartl and Clark 1997). It shows the degree to which heterozygosity is reduced below the expectation. Compared with HWE expectations, the value of F_{IS} ranges between -1 and +1. Negative F_{IS} values indicate heterozygote excess (outbreeding), and positive values indicate heterozygote deficiency (inbreeding). Additionally, F_{ST} measures the reduction in heterozygosity in a subpopulation. F_{ST} is the most inclusive measure of population substructure and the most useful for examining the overall genetic divergence among subpopulations. Also

called coancestry coefficient (Weir and Cockerham 1984) or "fixation index", it is defined as correlation of gametes within subpopulations relative to gametes drawn at random from the entire population. Its calculation is performed by using the subpopulation average heterozygosity and total population expected heterozygosity. F_{ST} is always positive; it ranges between 0 (panmixia: no subdivision, random mating and no genetic divergence within the population) and 1 (complete isolation: extreme subdivision). F_{ST} values up to 0.05 indicate negligible genetic differentiation, whereas >0.25 means very great genetic differentiation within the population analyzed (Hartl and Clark 1997). F_{ST} is usually calculated for different genes, and then averaged across all *loci* and all populations. Using the F_{ST} values, less differentiation is seen between human populations within continents than between continents, which is consistent with simple isolation by distance. This highly versatile parameter is also used as a genetic distance measure between two populations instead of a fixation index among many populations (Weir 1996). F_{IT} is rarely used. It is the overall inbreeding coefficient of an individual relative to the total population.

One process that contributes to population subdivision is inbreeding. Inbreeding and assortative mating are deviations from the Hardy-Weinberg assumption of random mating. It results from mating between relatives, and is probably the most common deviation from the Hardy-Weinberg model (Jobling *et al.* 2004; Hartl and Clark 1997). Assortative mating, like inbreeding, leads to non-random patterns of mating; however, the basis for assortative mating is not relatedness but phenotypic similarity or dissimilarity. Both processes sort existing variation, altering genotypic frequencies within populations. Except in extreme cases, inbreeding and assortative mating do not dramatically alter allele frequencies. Nevertheless, their consequences for the evolution of populations can be highly significant. True inbreeding is the deviation from random mating within an individual population. Because inbreeding involves disproportionate mating between relatives, its effect is to increase homozygosity across all *loci*. One observable consequence of inbreeding is that the proportion of heterozygotes is significantly lower than expected under the HWE model across multiple *loci* (Jobling *et al.* 2004; Hartl and Clark 1997). Population size can also greatly impact the extent and rate of loss of heterozygosity. In large populations, most individuals are effectively unrelated, so the effect of inbreeding decreases rapidly as average relatedness among individuals decreases (Jobling *et al.* 2004; Hartl and Clark 1997).

Inbreeding can be calculated most directly through pedigree analysis, though this is often not possible in natural populations. Alternatively, we can estimate it indirectly from the observed alleles and genotypic frequencies, as the frequency of heterozygotes observed relative to that expected under HWE ($2pq$). In this way, then, inbreeding is a measure of the fractional reduction in heterozygosity relative to a panmictic population with the same allele frequencies.

"The human genome underlies the fundamental unity of all members of the human family, as well as the recognition of their inherent dignity and diversity. In a symbolic sense, it is the heritage of humanity."

Universal Declaration on the Human Genome and Human Rights

CHAPTER III

GENETIC ISOLATES *VERSUS* OUTBRED POPULATIONS

III. Genetic isolates *versus* outbred populations

The question "Are genetic isolated populations more useful for the mapping of genes than outbred populations?" is still a subject of large discussion in the scientific community[21]. Some researchers have argued that small isolated populations are valuable for linkage and LD mapping, whereas others have argued that populations are only ideal when they have maintained constant size throughout much of their history and others find no advantage in isolated populations. It is clear that the appearance of biotechnologies that allow the genome-wide genotyping of large quantities of markers, at a relatively low cost *per* sample, played an evident role in the decrease in the importance of human genetic isolates.

Generally, genetic isolates are subpopulations resulting from the founder effect of a small number of individuals as a consequence of bottleneck. These populations exist in geographical, cultural or geographical and cultural context over many generations without genetic interchange from other subpopulations. In recent years, there has been success in mapping genes causing several diseases, mainly those exhibiting rare classical Mendelian recessive models of inheritance, essencially through linkage analysis. The initial successes, which came by studying isolated populations, such as, the Finnish and the Old Order Amish, have exponentially increased the interest in these kinds of populations (Arcos-Burgos and Muenke 2002 and references therein). Some of these successfully mapped diseases include, for example, gyrate atrophy of choroids and retina[22] (HOGA; Mitchell *et al.* 1988), retinoschisis[23] (Alitalo *et al.* 1987) and Uscher syndrome type III[24] (Sankila *et al.* 1995) in the Finnish population and bipolar disorder

[21] Since 2003, with a two year interval, an international meeting entitled "Genetic of complex diseases and isolated populations" occurs to discuss the use of isolated populations in human genetics. In 2007, it was held, in the city of Turim, Italy, the 3rd meeting. For further information, please, consult the website: http://www.fobiotech.org/geneticisolates2007/home.html.

[22] Gyrate atrophy of the choroid and retina is an autosomal recessive chorioretinal dystrophy that begins in childhood and leads to blindness in the fourth to seventh decade of life. The primary defect is deficiency of ornithine-delta-amino-transferase (10q26), which results in accumulation of ornithine (for revision, please, see Hasanoğlu *et al.* 1996).

[23] Retinoschisis is an recessive X-linked genetic disease characterized by intraretinal splitting due to degeneration. The abnormality may not be clinically manifest until middle life. The retinoschisis gene (*RS1*; Xp22.2) encodes for a protein called retinoschisin (for revision, please, see Sikkink *et al.* 2007).

[24] Usher syndrome type III is autosomal recessive disorder characterized by postlingual, progressive hearing loss, variable vestibular dysfunction, and onset of retinitis pigmentosa symptoms. Mutations in at least two genes are responsible for Usher syndrome type III; however, *CLRN1* (3q25) is the only gene that has been identified. This gene codes for clarin 1 protein (for revision, please, see Roux 2005).

in the Old Order Amish (Ginn *et al.* 1996). In Table III.1 there are some examples of studies performed in isolated populations.

Scientists who agree on the use of genetic isolates argue that these populations offer many advantages for genome-wide mapping (Table III.2). Firstly, most of them arise as the result of a founder effect that in conjunction with the high degree of inbreeding produces high incidence of recessive disorders.

Table III.1. Examples of genome scans in isolated populations (adapted from Varilo and Peltonen 2004).

Population	**Age pop. (years)**	**Reported genome scans**	**Study sample**	***Loci* showing linkage**
Amish	~250	Bipolar disease	1 extended pedigree with 207 individuals	Chr 6,13,15
Hutteries	~100	Allergic asthma	653 individuals	Suggestive: Chr 1, 3p, 5q, 13q
Mennonite	~200	Hirschsprung disease	1 family for linkage, 28 families	Chr 13q22
Pima Indians	>10,000	Type II diabetes (DM), body mass index (BMI)	264 nuclear famílies, 966 siblings	BMI and DM, Chr 11, DM Chr 1
Bedouins	200	Nonsyndromic deafness	1 extended pedigree, 55 individuals	Chr 13q12
Finland, late settlement	330	Schizophrenia, Asthma	21 families, 233 individuals, 253 families, 443 individuals	Chr 1q
Finland, early settlement	2000	Multiple sclerosis	21 familias, 191 individuals	Chr 6p, 17q22
Finland	-	Familial combined hyperlipidemia	35 families, 168 individuals	Chr 6p
lceland	~1000	Schizophrenia	5 families, 91 individuals	Chr 1q21
North Sweeden	350	Familial prostate cancer	28 families, 366 individuals	Chr 1q21

Another main features associated with the genetic isolates power is the existence of multigenerational and extended pedigrees, where most of individuals are descendents of a small number of founders in a short number of generations (Peltonen *et al.* 2000). In addition, homogeneous and carefully delineated phenotypes are key components in making these communities useful for genetic analysis. A further advantage is that isolates with a small effective number of unrelated founders frequently show a smaller number of disease susceptibility variants within the current population compared with outbred populations. On the other hand, outbred populations offer the benefit of large cohorts of affected individuals. Nevertheless, the genetic background of outbred

populations is generally less uniform and, therefore, higher variance in disease genotypes is observed (Sheffield *et al.* 1998; Shifman and Darvasi 2001).

Table III.2. Benefits of isolated and outbred populations (adapted from Peltonen *et al.* 2000).

Benefits of population isolates	**Benefits of outbred populations**
Higher prevalence of some diseases	More affected people
More inbreeding - opportunity to map recessive genes	More opportunity for replication
More uniform genetic background	Markers more polymorphic
Good genealogical records	Genes mapped pertinent to more of humanity
Easier to standardize phenotype definitions	
Wider intervals of linkage disequilibrium	
Closer to Hardy-Weinberg equilibrium	
Less migration and more intact families	
More uniform environment	

Population isolates can have different demographic histories. Some lack reliable information on their initial genetic makeup, their total number of founders, and the extent and duration of their isolation (Varilo and Peltonen 2004). However, isolates, such as, Iceland, northern Sweden or Finland, have easily accessible genealogical records. Nevertheless, because isolates vary in their demography, genetic background and environment, different populations request different study designs – especially for complex traits. In consequence, replication of results in other outbred populations are more difficult. Alternatively, mapping in large outbred populations has been also unsatisfactory. Many *loci* for common diseases have been mapped, but few have been narrowed to smaller chromosomal intervals (Table III.1). Several of the mapped *loci* are statistical "ghosts" that appear in some studies and disappear in others. Possible reasons for these inconsistencies include (*i*) genetic heterogeneity, both at the allelic and *locus* levels; (*ii*) insufficient sample size; (*iii*) imperfect statistical analysis; (*vi*) diagnostic and genotyping errors; and (*v*) pooling of diverse phenotypes into the same diagnostic classes (Peltonen *et al.* 2000 and references therin). Population isolates are especially valuable for isolation of rare high-impact genes because the founder effect and/ or bottlenecks have dramatically restricted the number of alleles, making the genetic background closely resemble that of any monogenic disease (Varilo and Peltonen 2004 and references therin).

Small constant size populations can be expected to exhibit LD over large genomic regions and greatly reduced allelic and haplotype diversity, both due to genetic drift. Consequently, such populations may be especially powerful for the initial phase of mapping common trait *loci*, when adequate study samples are available (Peltonen *et al.* 2000; Kere 2001). In fine mapping studies a substantial advantage is gained by accessing multiple populations with divergent demographic histories, despite practical limitations. The long-range LD needed for coarse, genome-wide mapping of complex traits can be found in carefully selected subpopulations, within an otherwise expanded population (Shifman and Darvasi 2001).

Although there is some discordance in the scientific community, one fact that cannot be neglected is that in some culturally and genetically isolated populations, it is possible to monitor their similar environment, social customs and eating habits and, by reducing the environmental "noise", facilitate the detection of causative genetic and/ or environmental factors. Moreover, the better the characteristics of the populations and their history, the better are the opportunities to design the optimal strategy for disease gene identification.

In the present chapter only a relatively brief description of human isolated populations that are well characterized and constitute case-studies in human genetic isolates, including the Finnish, the Sardinian, the Old Order Amish, the Hutterites and, finally, the Saguenay-Lac-Saint-Jean population. It is not the intention to exemplify all isolated populations reported in the literature and around the world.

III.1. The Finnish population

The demographic history of Finland is similar to many isolates, that is, a small number of original founders followed by subsequent isolation, rapid expansion and major bottlenecks have allowed genetic drift to shape its gene pool. Both Y-chromosomal haplotypes and mitochondrial sequences show low genetic diversity among Finns compared with other European populations and confirm the long-standing isolation of Finland. The vast majority of Finns descend from two immigration waves occurring about 4,000 and 2,000 years ago. The earlier wave involved eastern Uralic speakers and the later Indo-European speakers from the south. The size of the founding population(s)

is unknown, but as late as the twelfth century, the population of Finland was only about 50,000. It reached 400,000 by the mid-seventeenth century, only to experience the great famine of 1696-1698, where one-third of the population perished. Since then, the Finnish population has grown relatively rapidly (de la Chapelle *et al.* 1998; Kere 2001).

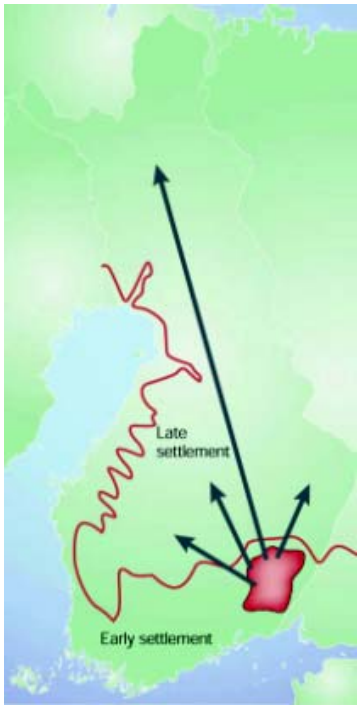


Figure III.1. Map of Finland demonstrating the settlement waves.

In Finland, internal migrations created regional subisolates (Figure III.1). The population spread from the early settlement region on the southern and western coastline towards the east and north. The subisolates in the late settlement region were established for the majority groups of farmers originating from a small area of south Savo in southeastern Finland. They moved to the central, then western, and finally northern parts of the country. Within a century, the inhabited land area of Finland doubled. Until the Second World War, many of these northeastern settlements grew rapidly without further immigration to supplement the descendants of their 40-60 founding families (Peltonen *et al.* 1999; Norio 2003a).

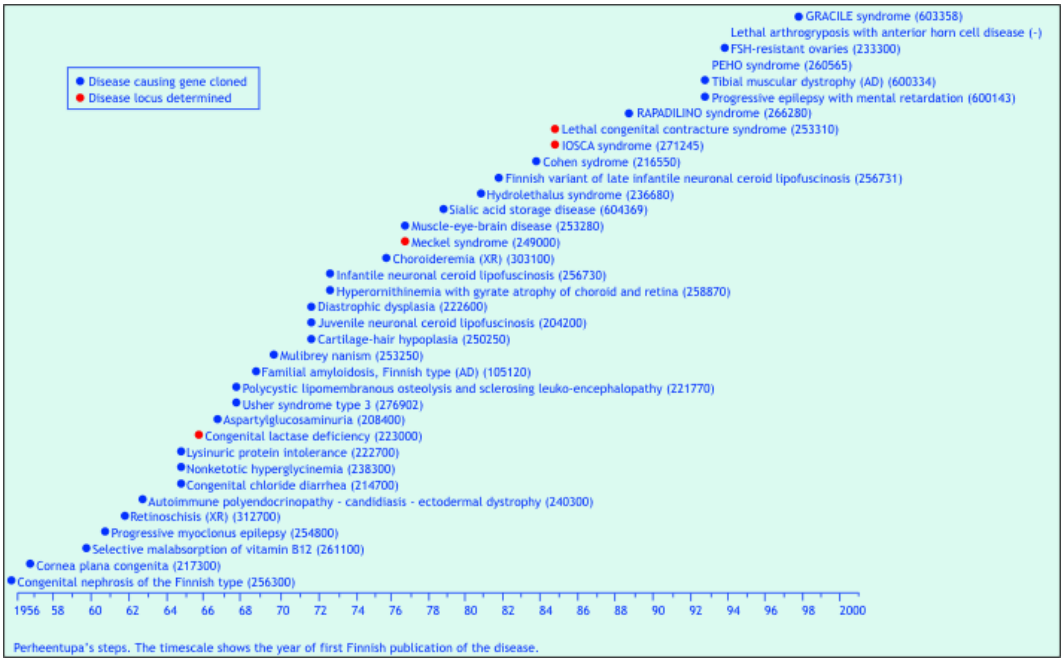


Figure III.2. The timescale of the year of first Finnish publication of some diseases.

Finland's demographic history has led to a unique catalogue of genetic diseases. Around 30, mostly recessive diseases, are highly enriched in Finland (Figure III.2). Other diseases, such as, phenylketonuria[25] and cystic fibrosis[26], are almost nonexistent. Molecular studies have exposed one major mutation (78-98% alleles) in most Finnish Mendelian diseases and have revealed long genetic intervals of linkage disequilibrium (LD) flanking the disease gene, with the length of the LD interval reflecting the age of the mutation (Norio 2003b).

The population history of Finland has led to an uneven regional distribution of the disease alleles. Internal movement in the last few decades has somewhat reduced this

[25] Phenylketonuria (PKU) is an autosomal recessive disorder caused by a deficiency of hepatic phenylalanine hydroxylase (*PAH*; 12q23.2). Left untreated, this condition can cause problems with brain development, leading to progressive mental retardation and seizures. However, PKU is one of the few genetic diseases that can be controlled by diet (for revision, please, see Zschocke 2003). There is a PKU mutation database (http://www.pahdb.mcgill.ca/), where it is reported all mutations found in this gene.

[26] Cystic fibrosis is an autosomal recessive disorder that mainly affects the lungs and digestive system, causing progressive disability and early death. It is caused by a mutation in a gene called the cystic fibrosis transmembrane conductance regulator (*CFTR*; 7q31.2; for revision, please, see Jaffé and Bush 2001). There is a Cystic fibrosis mutation database (http://www.genet.sickkids.on.ca/cftr/app), where it is reported all mutations found in this gene.

effect, but birthplaces of the patients' grandparents represent a typical regional clustering (Norio 2003c). Several studies have been performed to characterize the Finnish genetic heritage. One of the most recent works studies the genetic association between insulin degrading enzyme and the development of Alzheimer's disease (Vepsäläinen *et al.* 2007). Insulin degrading enzyme (IDE) on chromosome 10q24 has been previously proposed as candidate gene for late-onset Alzheimer's disease (AD), based on its amyloid beta-protein degrading activity. These authors genotyped SNPs in the *IDE* gene among Finnish AD patients (n=370) and control subjects (n=454). Their results revealed SNPs rs4646953 and rs4646955 to be associated with AD conferring an approximately two-fold increased risk. Single *locus* findings were corroborated by the results obtained from haplotype analyses. This suggests that genetic alterations in or near the *IDE* gene may increase the risk for developing AD.

III.2. The Sardinian population

Sardinia is the second largest island of the Mediterranean sea (Figure III.3). Located just south of Corsica, it is one of the autonomous regions with special statute under the Italian Constitution. This population has a very rich history, with influences of several peoples, such as, Phoenicians, Spanish, Egyptians, among others, who can have contributed to their genetic background. Recent studies indicate that, whereas the Sardinian population as a whole is comparable to outbred populations for LD mapping of common variants (Eaves *et al.* 2000; Taillon-Miller *et al.* 2000), LD in Sardinian subisolates is more extended, making these populations particularly suitable for this approach. To evaluate the extent of LD, Angius *et al.* (2002) compared different subpopulations within Sardinia selected on the basis of their geographical position and isolation: two small isolated villages (Talana, Urzulei), two larger but remote areas (Ogliastra, Nuoro province), and a cohort of samples representing the wider Sardinian population. LD analysis was carried out by using six microsatellite markers located on Xq13.3, that have been extensively studied in different populations. The results indicate different extents and patterns of LD in the subpopulation samples depending on their degree of isolation and demographic history. All LD measurements and haplotype analyses indicate that there is a decreasing trend from Talana (the most inbred

Figure III.3. Map of Sardinia.

population, LD up to 9.5-11.5 Mb) to the more outbred Sardinian population (LD only for intervals <2 Mb). In one village (Talana), five haplotype classes accounting for 80% of the entire sample perfectly matched five Ogliastra clusters, supporting the origin of the village from the Ogliastra genetic pool. In contrast, the other isolated village (Urzulei) showed a different pattern of haplotypes with a closer relationship to the Nuoro region subpopulation. LD analyses therefore show that even neighbouring isolate villages may differ in their genetic background. These authors highlight the importance of selecting appropriate populations and/ or subpopulations for the analysis of complex traits. Isolated subpopulations showing different extents of LD can provide a powerful method for mapping complex traits by LD scanning at relatively low marker density.

More recently, studies on the thiopurine S-methyltransferase (TPMT), which is an enzyme involved in the normal metabolic inactivation of thiopurine drugs, demonstrated that the Sardinians come out as outliers when compared with other European populations, an observation consistent with previous genetic inferences that Sardinia has

features of a genetic isolate (Rossino *et al.* 2006). Patients with intermediate or no TPMT activity are at risk of toxicity after receiving standard doses of thiopurine drugs and it was shown that inter-individual differences in response to these drugs is largely determined by genetic variation at the *TPMT locus*. This study was designed to investigate in the Sardinian population the frequency distribution of four of the most common variants accounting for TPMT deficiency and to conduct comparative analyses with other populations, in order to obtain insights into the main factors that have shaped diversity at the *TPMT locus* in Sardinia. The results obtained from 259 Sardinians genotyped show that 6.95% were found to be heterozygous for one of four *TPMT* variants screened; for each variant the frequency estimate was 1.74%, 0.58%, 0.39% and 0.77% for TPMT*2, TPMT*3A, TPMT*3B and TPMT*3C, respectively. The authors conclude that although Sardinia does not show reduced diversity at the *TPMT locus*, the spectrum of *TPMT* allele frequencies affords evidence of remarkable influence of genetic drift and founder effects throughout its population history.

III.3. The Old Order Amish population

The Old Order Amish (OOA) of Lancaster County, Pennsylvania (Figure III.4), represent a genetically closed homogeneous Caucasian population of Central European

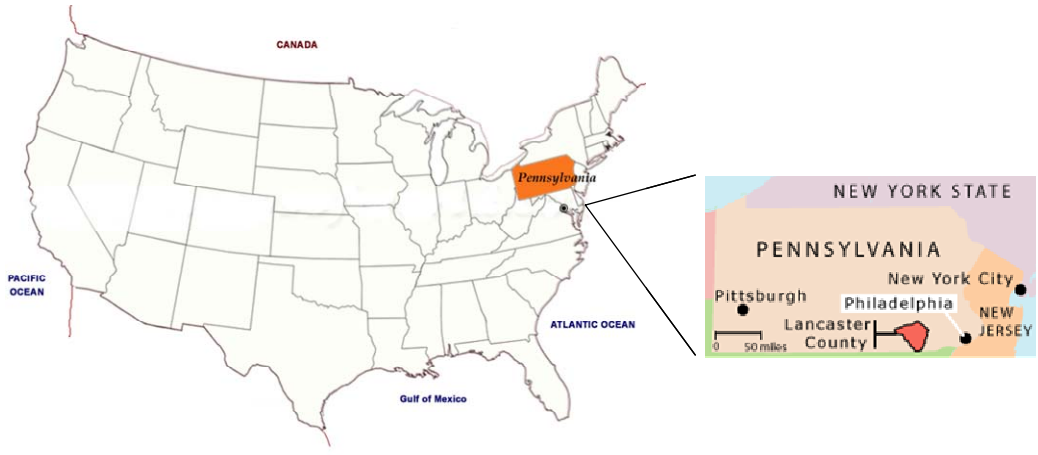


Figure III.4. Map of Lancaster county.

ancestry ideal for recruitment of large multiplex pedigrees and sib-pairs for genetic studies. Religious persecution prompted the earliest Amish migration to the USA. In the mid 1700s the original group was composed of about 200 individuals. Today OOA are composed of over 30,000. They have excellent family records which include dates of birth and death of all Amish dating back to the early 1700s. This population has a fairly uniform standard of living and lifestyle, which reduces non-genetic variability and boosts the power to discern determinants of genetically inherited traits. Additionally, they have low migration rates, and do not practice birth control. Families are large, averaging seven siblings and extended families live either in the same household or nearby. Two-thirds of the family members can be traced to a single founder. All of these factors facilitate the collection of multigenerational extended pedigrees with several long-lived members. Furthermore, the large sib-ship sizes provide the unparalleled opportunity to reconstruct genotypes of deceased long-lived pedigree members by genotyping their living offspring (Sorkin *et al.* 2005).

Recently, van der Walt and collaborators (2005) described the maternal lineages and Alzheimer disease risk in the Old Order Amish. The consequences of genetic isolation and inbreeding within this group are evident by increased frequencies of many monogenic diseases and several complex disorders. Conversely, the prevalence of Alzheimer disease is lower in the Amish than in the general American population. Since mitochondrial dysfunction has been proposed as an underlying cause of AD and a specific haplogroup was found to affect AD susceptibility in Caucasians, they investigated whether inherited mitochondrial haplogroups affect risk of developing AD dementia in Ohio and Indiana Amish communities. Ninety-five independent matrilines were observed across six large pedigrees and three small pedigrees then classified into seven major European haplogroups. Haplogroup T is the most frequent haplogroup represented overall in these maternal lines (35.4%), while observed in only 10.6% in outbred American and European populations. Furthermore, haplogroups J and K are less frequent (1.0%) than in the outbred data set (9.4-11.2%). Affected case matrilines and unaffected control lines were chosen from pedigrees to test whether specific haplogroups and their defining SNPs confer risk of AD. Van der Walt and colleagues did not observe frequency differences between AD cases compared to controls overall or when stratified by sex. Therefore, they suggest that the genetic effect responsible for

AD dementia in the affected Amish pedigrees is unlikely to be of mitochondrial origin and may be caused by nuclear genetic factors.

III.4. The Hutterites population

The Hutterites are a religious sect that originated in the Tyrolean Alps in the 1500's. Between the mid 1700's and mid 1800's, during their occupancy in Russia, the population grew in size from approximately 120 to over 1000 members (Hostetler 1974). In the 1870's, approximately 900 of these members migrated to south Dakota and roughly half settled on three communal farms (Figure III.5). Due to a high natural fertility rate and the proscription of contraception among communal Hutterites (Sheps 1965), the population expanded dramatically since migrating to the United States. Today there are >35,000 Hutterites living on >350 communal farms (called colonies) in the northern United States and western Canada. Genealogical records trace all extant Hutterites to fewer than 90 ancestors who lived in the early 1700's to the early 1800's (Martin 1970). The relationships between these ancestors are unknown, but some of them may have been related. The three original south Dakota colonies have given rise to the three major subdivisions of Hutterite population structure, called the Schmiedeleut (S-leut), Dariusleut (D-leut) and Leherleut (L-leut); the members of each "leut" have remained reproductively isolated from each other since 1910 (Bleibtreu 1964).

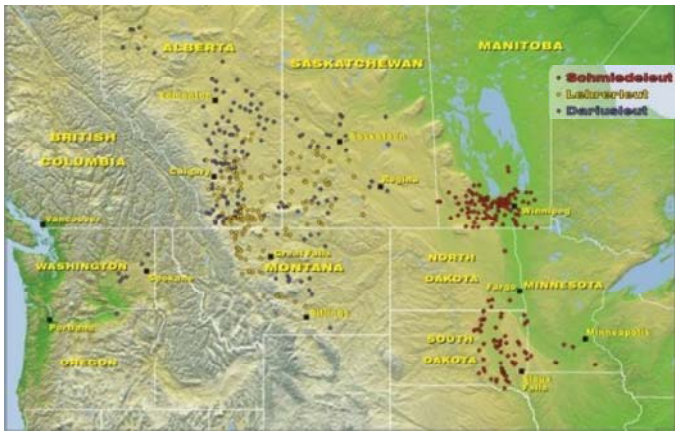


Figure III.5. The Huterites geographical location.

In 2004, Newman and collaborators questioned if common disease susceptibility alleles are the same in outbred and founder populations. Founder populations have been the subjects of complex disease studies because of their decreased genetic heterogeneity, increased linkage disequilibrium and more homogeneous environmental exposures. However, it is possible that disease alleles identified in founder populations may not contribute significantly to susceptibility in outbred populations. In this study these authors examine the Hutterites for 103 polymorphisms in 66 genes that are candidates for cardiovascular or inflammatory diseases. Newman *et al.* (2004) compare the frequencies of alleles at these *loci* in the Hutterites to their frequencies in outbred European-American populations and test for associations with cardiovascular disease-associated phenotypes in the Hutterites. Their results show that alleles at these *loci* are found at similar frequencies in the Hutterites and in outbred populations. In addition, they report associations between 39 alleles or haplotypes and cardiovascular disease phenotypes ($p<0.05$), with five *loci* remaining significant after adjusting for multiple comparisons. These data indicate that this founder population offers considerable advantages for genetic studies of common complex diseases.

III.5. The Saguenay-Lac-Saint-Jean population

Saguenay-Lac-Saint-Jean (SLSJ) is a geographically isolated region located 125 miles northeast of Quebec City (Figure III.6). It is usually divided into three subregions, Bas Saguenay, Haut Saguenay and Lac-St-Jean. From 1838 to 1911, almost 75% of the 28,656 immigrants came from Charlevoix, a region situated east of Quebec City, whereas the remaining 25% came mostly from other eastern regions of the province. The immigration has considerably diversified since 1911. Although the migration balance has been negative since 1870, the population, 98% of whom are French speaking, has risen from 5,000 inhabitants in 1852 to 50,000 in 1911 to ~300,000 today. Several dominant and recessive autosomal disorders (e.g. myotonic dystrophy and cystic fibrosis) have a higher prevalence, while others (e.g. spastic ataxia Charlevoix-Saguenay type and polyneuropathy with or without agenesis of the corpus

callosum[27]), frequently found in the SLSJ region and Charlevoix, are almost nonexistent elsewhere (De Braekeleer 1988).

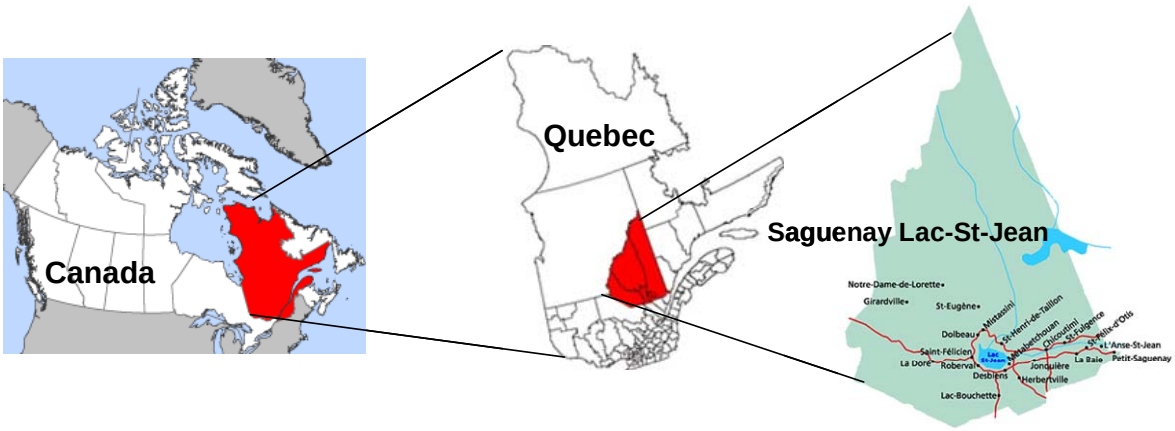


Figure III.6. Map of Sanguenay-Lac-Saint-Jean.

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a clinically homogeneous form of early-onset familial spastic ataxia with prominent myelinated retinal nerve fibers. More than 300 patients have been identified, and most of their families originated in the Charlevoix-Saguenay region of northeastern Quebec, where the carrier prevalence has been estimated to be 1/22. Consistent with the hypothesis of a founder effect, Richter *et al.* (1999) observed excess shared homozygosity at 13q11, among patients in a genome-wide scan of 12 families. Analysis of 19 pedigrees demonstrated very tight linkage between the ARSACS *locus* and an intragenic polymorphism of the *gamma-sarcoglycan* (*SGCG*) gene, but genomic DNA sequence analysis of all eight exons of SGCG revealed no disease-causing mutation. On the basis of haplotypes composed of seven marker *loci* that spanned 11.1 cM, the most likely position of the ARSACS *locus* was 0.42 cM distal to the *SGCG* polymorphism. Two groups of ARSACS-associated haplotypes were identified: a large group that carries a common *SGCG* allele and a small group that carries a rare *SGCG* allele. The haplotype groups do not appear to be closely related. Therefore, although chromosomes within each haplotype group may harbor a single ARSACS mutation identical by descent, the two mutations could have independent origins.

[27] Peripheral neuropathy with or without agenesis of the corpus callosum is an autosomal recessive disease characterised by progressive sensorimotor neuropathy, mental retardation, dysmorphic features and complete or partial agenesis of the corpus callosum. It is caused by mutations in the *SLC12A6* gene (sodium/ chloride transporter; 15q13-q14; for revision, please, see Dupre *et al.* 2003).

Dominantly transmitted myotonic dystrophy (DM1) is highly prevalent in SLSJ where its carrier rate reaches 1/550, compared with 1/5,000 to 1/50,000 elsewhere. To shed light on the origin of DM1 in Saguenay-Lac-Saint-Jean, Yotoya *et al.* (2005) screened 50 nuclear DM1 families and studied the genetic variation in a 2.05 Mb (2.9 cM) segment spanning the site of the expansion mutation. The markers analyzed included 22 biallelic SNPs and two microsatellites. Among 50 independent DM1 chromosomes, these authors distinguished ten DM1-associated haplotypes and grouped them into three haplotype families – A, B and C –, based on the relevant extent of allele sharing between them. To test whether the data were consistent with a single entry of the mutation into SLSJ, Yotoya and collaborators evaluated the age of the founder effect from the proportion of recombinant haplotypes. Taking the prevalent haplotype A1_21 (58%) as ancestral to all the disease-associated haplotypes in this study, the estimated age of the founder effect was 19 generations, long predating the colonization of Nouvelle-France. In contrast, considering A1_21 as ancestral to the haplotype family A only, yielded the estimated founder age of nine generations, consistent with the settlement of Charlevoix at the turn of 17th century and subsequent colonization of SLSJ. These authors conclude that it was the carrier of haplotype A (present-day carrier rate of 1/730) that was a "driver" of the founder effect, while minor haplotypes B and C, with corresponding carrier rates of 1/3,000 and 1/10,000, respectively, contribute DM1 to the prevalence level known in other populations.

"There were, however, Portuguese, Spanish, Italians, English, Flemish, French, Scottish, Germans, Jews, and Moors then living who would voyage to the islands, willingly or unwillingly, to become the root stock of an island people eventually proud to be known as Azoreans…"

Guill 1993

CHAPTER IV

THE AZORES

IV. The Azores

IV.1. Geographic location and demographic characterization

The Azores, the largest Portuguese archipelago, is located in the north Atlantic Ocean between parallels 36º 55'N and 39º 45'N and meridians 24º 45'W and 31º 17'W. It is composed of nine volcanic islands unevenly distributed by three geographic groups: the Eastern group with two islands – São Miguel and Santa Maria –, the Central which includes five islands – Terceira, Pico, Faial, São Jorge and Graciosa –, and the Western group with Flores and Corvo (Figure IV.1).

Figure IV.1. Map of Azores Islands.

The Azores archipelago has a total area of 2332.74 km^2, unevenly distributed by the nine islands, varying from São Miguel, the largest, with a total area of 746.82 km^2 to Corvo with 17.13 km^2 (Table IV.1). The present-day population is composed of 241,763 inhabitants (National Institute of Statistics – Portugal, 2001 Census), derived from about 27 generations. The majority of the population lives on São Miguel (54.4%). The remainder is unevenly dispersed throughout the other eight islands; for example, Corvo, the smallest island, has only 425 individuals (Figure IV.2). From the total Azorean population, 41.4% are living in the Central group; however, Terceira represents 55.7% of the Central islands population.

Table IV.1. Demography data of the Azores Islands.

Islands	Area (km^2)	Population size	Population density ($Inh./km^2$)
São Miguel	746.82	131,609	176.23
Santa Maria	97.1	5578	57.46
Terceira	402.2	55,833	139.65
Pico	447	14,806	32.85
Faial	172.43	15,063	88.64
São Jorge	237.59	9674	39.39
Graciosa	62	4780	78.44
Flores	142	3995	28.19
Corvo	17.13	425	24.82

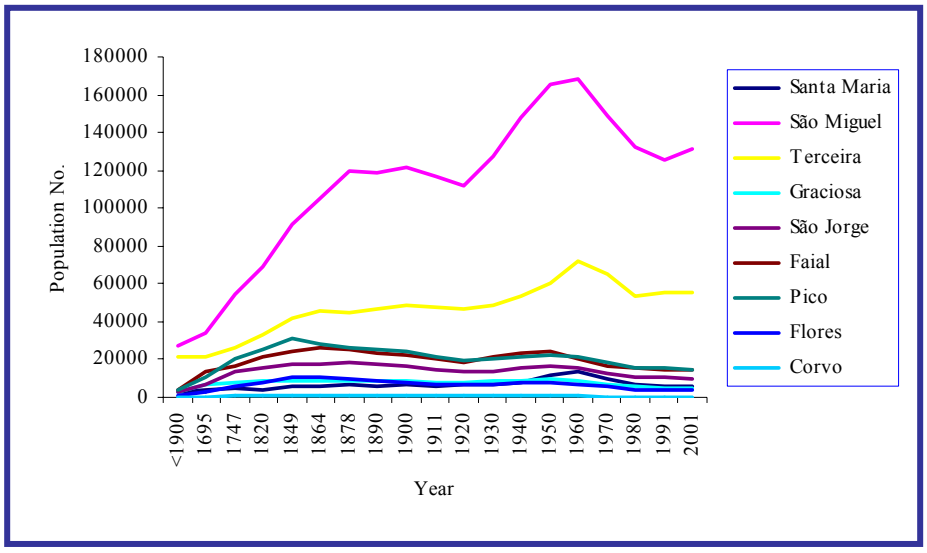


Figure IV.2. Demographic evolution of the Azores Islands population.

Until late 1800s, the Azorean population increased to a considerable rate, being the islands of São Miguel and Terceira those who displayed the greater population increase (Figure IV.2). The fluctuations observed are not derived from massive death related to diseases or famine. They are mainly due to migratory movements. People searched better living conditions in other places. The first news of exit of Azoreans occurs in the first half of the 17th century (Mendonça 1996). Along the 18th century, the emigration

towards Brazil becomes more regular, but it is during the 19th century that the migratory phenomenon reaches unknown proportions, a total of 22,397 individuals migrated between 1881-1885. In the 1960s, the population decreases considerably, once again by migration, mainly towards the United States (US), Canada and Bermuda. In a ten year period (1960-1970), 148,005 Azoreans emigrated to the United States, Canada and Bermuda (Direcção Regional das Comunidades; Mendonça 1996), because the US government changed its emigration policies, allowing the entrance of Azoreans in the country.

IV.2. Discovery and settlement

The discovery of the Azorean archipelago is a controversial historical subject. Much has been written, sometimes with nationalistic passion. One uncontroversial fact is that the Azores was uninhabited when discovered. A Portuguese royal letter "Carta Regia" dated 2 July 1439 is the first document that recognizes the existence of the Azores Islands. This letter enumerates seven islands, and reveals that sheep had already been loosed on the islands at the direction of Prince Henry of Portugal. The Carta Regia further gives royal license to Henry to populate the islands, which lay, according to subsequent documents, 260 leagues (832 nautical miles) into the Ocean. A second reference to the existence of the Azores, a Majorcan map drawn by Gabriel de Valseca in 1439, showing the Atlantic coast of Africa until south of the Canary Islands appeared. It illustrates the position of the Canaries, Madeira, Porto Santo, and seven islands in the proximate location of the Azores (Guill 1993, Marques 1991).

Two versions of the Azores discovery emerge more constantly in the literature (Arruda 1932), there are those who support the hypothesis that the geographical appearance of the archipelago was in the 14th in the reign of Afonso V, and those who defend that the discovery occurred in the first half of the 15th century (Mendonça 1996; Matos 1989; Pires 1983). The first hypothesis is based on the presence of nine islands displayed in the orientation north-south near the Iberian peninsula. However, the fact that there is a poor representation of the geographic location of the islands and that there is no evolution in term of map representation, similar to what happened with the Canary and Madeira Islands, historians are more leaned to the second hypothesis, being the

discovery of the Azores Islands in the first half of the 15th century (Marques 1991). According to Marques (1991) the discovery occurred in 1427 by Diogo de Silves, pilot of Henry. On August 15 of 1432, Gonçalo Cabral arrived to Santa Maria, the easternmost island of the Azorean archipelago. It was the feast day of the Assumption of Our Blessed Mother, or Santa Maria and, consequently, the island was named after her. The island had forests, water fluxes and birdlife. Apparently, there were many birds in flight, thought to be goshawks, and, hence, the islands got the Portuguese name "Açor" or hawk (Guill 1993).

The discovery of the last two islands – Flores and Corvo – is also controversial. However, it is known that it occurred after all other islands, probably in 1452 by Diogo de Teive and his son, João de Teive (Mendonça 1996; Matos 1989; Marques 1991). After the discovery of the Azores, Henry received in 1439 the king's authorization to populate the islands. To fulfil this task Gonçalo Velho initiates the peopling by the Eastern group, Santa Maria and São Miguel (Matos 1989). Peopling was a slow and difficult process. Someone wrote "…The Azorean settlement was done with people from the interior of mainland Portugal, those who could not swim nor build boats, making impossible the abandonment of the islands…" Historical data report that the Portuguese crown was compelled to give out land and privileges in order to attract people to the islands (Guill 1993). Gonçalo Velho gathered settlers from the mainland and Madeira. To increase his labour force, he requested Henry to release to his guard small time criminals, known as "degredados" (persons convicted of lesser crimes and were serving time in prison or in designated periods of servitude). These "degredados" were identified from other settlers by a ring piercing the left ear lobe. Velho added to his work force some Moorish prisoners, captured in the Moroccan wars and not yet ransomed by Moslem families or authorities. Velho called on the vicar of Tomar to send priests and specialists in the construction of religious structures. Sugar production on Madeira had been established with high profits. Therefore, Henry contracted António Corvelo and his two sons, Francisco and Genero, to establish sugar cane plantations and to build sugar-processing facilities (Guill 1993; Marques 1991).

The island of Santa Maria was the first of the archipelago being populated. In 1439, Gonçalo Velho, its first captain-donatary, accompanied by two nephews and a group of settlers, in their majority from Algarve, settled in the coast of this island (Matos 1989).

Later, João Soares de Albergaria, nephew of Gonçalo Velho, gives a new impulse on peopling of Santa Maria (Matos 1989). The beginning of settlement of the São Miguel Island in 1444 is essentially contemporary to Santa Maria. According to Matos (1989), the group of initial settlers that came to the São Miguel Island was composed of Portuguese, black slaves and moors. Some authors, based on toponimic data, refer the influence of native individuals of the French Bretagne in the island of São Miguel (Matos 1989). The frequent marine relations between the region of Algarve and Azores, especially with São Miguel, aside from allowing commercial interchanges, fomented a change of residence and a narrowing of relations between the populations of mainland and islands. With the death of Gonçalo Velho, the captainship of São Miguel is sold to a member of the donatary family of Madeira Island, having begun (in 1474) the flow of Madeiran families to Azores (Matos 1989).

The good political relations between Portugal and Flanders (reinforced by marriage unions between the Portuguese royal family and the ducat of Burgandy) led to the Flemish participation in the Azorean settlement. Van der Hagen (with the Portuguese name Guilherme Silveira) was the first to transport Flemish to the Azores to supplement the activities of Gonçalo Velho. Van der Hagen was born in Bruges, grandson of John the Fearless. He took his first Flemish settlers to the third island Terceira, since Velho concentrated his attention on Santa Maria and São Miguel. He landed his settlers on the north coast of Terceira, in the area now known as Quatro Ribeiras. In 1450, when Henry elected another Flemish nobleman, Jácome de Bruges, who had also been in his service for some time, as captain-donatary of Terceira, van der Hagen returned to Flanders and brought new settlers. He moved to the island of Faial, near a location now called Praia do Norte. In addition to the Flemish, the first settlers of the Terceira Island were native from mainland Portugal and Madeira. Some of them were "noble" families from both places. However, these settlers, in a low number, also participated in the peopling of other islands, mainly Flores and Corvo (Matos 1989).

The first settlers of the Pico Island, that initially was used to shepherd the cattle, came from Faial, the nearest and most flemish island (Matos 1989). In the middle of 15th century, the Graciosa Island already had settlers, namely Vasco Gil Sodré, natural of Montemor-o-Velho (center of mainland Portugal), accompanied by its family and

servants. They were the pioneers in the settlement of the island. The influence of the Terceira Island seems to have been decisive in the settlement and development of the Graciosa Island (Ferreira 1987). The first attempt of peopling of the Flores Island, by Guilherme Silveira, was not successful. The definitive settlement took place at the end of the first decade of the 16th century, promoted by Antão Vaz, who arrived with a group of native settlers of Terceira and Madeira (Matos 1989). Later arrived people from the rest of the islands, essentially São Miguel and mainland Portugal (Matos 1989). The Corvo Island just starts being peopled in 1548. The presence of slaves in the island is explained by the fact that Gonçalo de Sousa sent slaves of their confidence to the island, with the mission to cultivate the earth and to take care of cattle (Matos 1989). Meanwhile, the geographic proximity between the two islands foments exchanges of individuals from one island towards the other (Matos 1989). There are also reports of the presence of Jews in all islands. Since people were needed to settle the islands, the persecutions were left aside, and Jews, often called New Christians, were allowed to live in the islands (Marques 1991). In the following centuries, the Azores, with a strategic position, became very important in the commercial trades (India, Africa and America), as well as, in the production of goods that were sent to mainland Portugal. This emerging economy attracted people of different nationalities, such as, French, English and Spanish, among others, contributing to the genetic pool of the Azorean people.

IV.3. Genetic studies on the Azorean population

Along the years the Azores population has progressively been studied. Nevertheless, when this PhD thesis started, there was lack of knowledge in the population genetic structure of the Azoreans. However, during the time between the beginning and the end of this thesis, further works, namely Service *et al.* 2006; Santos *et al.* 2005; Fernando *et al.* 2005; Spinola *et al.* 2005; Santos *et al.* 2005; Montiel *et al.* 2005; Santos *et al.* 2004; Santos *et al.* 2003, were published. Therefore, these and other papers will be thoroughly discussed in the present manuscript.

Some of the research subjects in Azores population vary from heart (Bettencourt *et al.* 2006; Cymbron *et al.* 2006; Shneider *et al.* 1995; de Sa *et al.* 1994), psychiatric (Pato *et*

al. 2005; Coutinho *et al.* 2004; Sklar *et al.* 2004) and ataxia (Gonzalez *et al.* 2004; Lima *et al.* 2001; St George-Hyslop *et al.* 1994; Friedman 1988; Romanul *et al.* 1977) diseases, to forensic genetics (Corte-Real *et al.* 1999; Velosa *et al.* 2002) and genetic population structure (Bruges-Armas *et al.* 1999; Smith *et al.* 1992). In the next paragraphs, a brief description of some important studies in the Azorean population are presented (for other publications on this population, please, see Appendix IX.5)

Congenital malformations of the heart and great vessels are among the most frequent of all clinically significant birth defects, having a major contribution on paediatric morbidity, mortality, and healthcare costs. Population based epidemiologic studies indicate a prevalence of congenital heart disease (CHD) ranging from 3.23 to 12.23 *per* 1000 live births (Macmahon *et al.* 1953; Robida *et al.* 1997). This wide variation in the reported values is mainly due to the difference in the methodologies used, but a number of other factors, such as, consanguinity (Becker *et al.* 2001, Nabulsi *et al.* 2003), ethnic background (Botto *et al.* 2001), environmental pollutants (Cedergren *et al.* 2002, Grech 1999) and access to health care also contribute to this variation (for revision, please see Weismann and Gelb 2007). Cymbron *et al.* (2006) carried out the first study performed in the Azorean population to characterize the prevalence of CHD in children born alive in São Miguel island from January 1992 to December 2001. Based on the Azorean Registry of CHD, which includes complete clinical and personal information, 189 patients were diagnosed. The results obtained by Cymbron *et al.* show that during this 10-year period, the average prevalence of CHD is 9.16 *per* 1000 live births (range 4.77-12.75). The most frequent cardiac malformations found were: ventricular septal defect (38.1%), atrial septal defect (12.2%) and patent ductus arteriosus (11.6%). This study detected four familial clusters, representing a total of 13 patients. Until now, Cabral *et al.* (2007) identified 44 familial clusters corresponding to 109 patients. This study reveals evidence for familial aggregation, which is of great interest for understanding the genes involved in these complex pathologies.

Schizophrenia is a common psychiatric disorder characterized by psychosis, cognitive dysfunction and negative symptoms, whose etiology involves interactions between both genetic and environmental factors (Austin 2005). Its incidence shows prominent worldwide variation (up to fivefold) and is about 40% greater in men than in women. Schizophrenia is a common complex disorder. Furthermore, epidemiological studies

have shown that the incidence is higher among those who grow up in urban areas and among migrants. To understand the genetic basis of this disease in Azores Islands populations, Sklar *et al.* (2004) performed a genome-wide scan of 29 families with schizophrenia, which identified a single region on 5q31-5q35 with strong linkage (non-parametric linkage, NPL=3.09, p=0.0012 at D5S820). Empirical simulations set a genome-wide threshold of NPL=3.10 for significant linkage. Additional support for this *locus* in schizophrenia comes from higher-density mapping and mapping of 11 additional families. The combined set of 40 families had a peak NPL=3.28 (p=0.00066) at markers D5S2112-D5S820. These data and previous linkage findings from other investigators provide strong and consistent evidence for this genomic region as a susceptibility *locus* for schizophrenia. Exploratory analyses of a novel phenotype, psychosis, in families with schizophrenia and bipolar disorder, detected evidence for linkage to the same markers as found in schizophrenia (peak NPL=3.03, p=0.0012 at D5S820), suggesting that this *locus* may be responsible for the psychotic symptoms observed in both diseases.

Autism Spectrum Disorder (ASD) is a syndrome with a wide clinical phenotype, characterized by impairments in social interaction and reciprocal communication and by patterns of stereotyped behaviours. The ASD term is used here to define a broad concept of autism, manifested as a spectrum of behavioural, cognitive and linguistic problems that include autistic disorder, Asperger syndrome and a pervasive developmental disorder not otherwise specified. ASD is a chronic and severe neurodevelopmental disorder, with a significant social impact. Oliveira *et al.* (2007) estimated the prevalence of ASD in a pediatric population from Portugal, its clinical characterization and the identification of associated medical conditions. For this purpose, they performed a survey in elementary schools, targeting 332,808 school age children in the mainland and 10,910 the Azores, asking teachers to identify children in their classes with an autistic profile. Clinical history was collected and a broad laboratory investigation for the identification of associated medical conditions was applied. In parallel, a systematic search of autistic children in educational, social and health registries was carried out in a restricted geographic region, targeting 56,325 children. The global prevalence of ASD was 9.2 *per* 10,000 in mainland Portugal, with intriguing regional differences, and 15.6 *per* 10,000 in the Azores. A high diversity of associated medical conditions was documented in 20,0% of the children, with an

unexpectedly high rate of mitochondrial respiratory chain disorder cases opening new perspectives for the investigation of ASD etiology.

Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder characterized by a wide range of clinical features, among which gait ataxia and limitation of eye movements are generally present (Lima *et al.* 2001). The name, Machado-Joseph, comes from two families of Portuguese/ Azorean descent who were among the first families described with the unique symptoms of the disease in the 1970s. Recently, researchers have identified MJD in several family groups not of obvious Portuguese descent, including an African-American family from north Carolina, an Italian-American family, and several Japanese families. On a worldwide basis, MJD is the most prevalent autosomal dominant inherited form of ataxia (for review, please, see Paulson 2007). Disease manifestations usually arise during adult life[28]. The mean age at onset is 40.2 years, although extremes of 6 years and 70 years have been reported (Sequeiros and Coutinho 1993). The *MJD locus* was assigned to the long arm of chromosome 14 (Takiyama *et al.*1993) and is associated with the expansion of a CAG trinucleotide repeat in a gene on 14q32.1 (Kawaguchi *et al.* 1994). In the Azores Islands (Portugal), MJD reaches the highest prevalence reported worldwide. It has been postulated that it is highly represented in the Azorean population as a result of a founder effect. To test this hypothesis, Lima *et al.* (1998) reconstructed the ascending genealogies of 32 Azorean families presently identified as harboring the disease (103 patients), using parish records as the main source of data. These patients were originally from the islands of São Miguel, Terceira, Graciosa and Flores. The genealogies of the two main Azorean-American families (Machado and Joseph) were also reconstructed. To identify the links between the MJD families, these authors calculated the kinship coefficient between the proponents of these genealogies. The family from Terceira was linked to three different MJD families from Flores through common ancestors. No kinship was observed between the MJD families from São Miguel and families from any other island. Links between the two Azorean-American families and Azorean MJD families were found. The founders present in more than one ascendance were identified.

[28] The types of MJD are distinguished by the age of onset and range of symptoms. Type I is characterized by onset between 10 and 30 years of age, fast progression, and severe dystonia and rigidity. Type II generally begins between the ages of 20 and 50 years, has an intermediate progression, and causes symptoms that include spasticity, spastic gait, and exaggerated reflex responses. Type III patients have an onset between 40 and 70 years of age, a relatively slow progression, and some muscle twitching, muscle atrophy, and unpleasant sensations such as, numbness, tingling, cramps, and pain in the hands, feet, and limbs.

Their chronological and geographic distribution indicates that more than one *MJD* haplotype was introduced in the Azores, probably by settlers coming from the Portuguese mainland. Two distinct haplotypes have been identifyed, one on the island of São Miguel and the other on Flores (Gaspar *et al.* 2001).

IV.4. Objectives of the scientific research

The global knowledge of the neutral variation of a population is an essential part in the understanding of the disease related variation, since it has also been subject to evolutionary forces, such as, genetic drift, mutation, selection and migration. Moreover, the comprehension of our "roots" and genetic signature has several implications in society's own knowledge, in the design of future genetic studies, as well as, in the health care system.

The location of the Azorean population in the middle of the Atlantic, its geography, namely, nine islands dispersed through three groups, its socio-cultural characteristics and, finally, the same environmental conditions, make *a priori* this population a good model to perform genetic studies of complex diseases, which will probably have a good reproductivity in other expanded populations.

The present PhD thesis had as main objective the overall characterization of the neutral variation of the Azorean population, through information retrieved from surnames, autosomal markers, as well as, Y-chromosome lineages. More generally, it was our purpose to:

- complement the settlement data and, consequently, validate the genetic origin of this population;
- understand the genetic diversity patterns of each Azorean island population and of the whole population;
- identify gene flow patterns between each island, as well as, with other European and African populations;
- compare the genetic background of the Azoreans with mainland Portugal and other well described populations;
- assess the population subdivision and, therefore, its genetic structure;
- estimate how inbreeding may play a role in the genetic makeup of this population;
- determine the extent of linkage disequilibrium and its implications in genetic mapping of complex diseases in the Azorean population.

"…I don't want to argue that the isonymy method is one of great accuracy or wide applicability. It has two advantages: One is that it is cheap and easy to use, requiring data that are often readily available in public records. The second is that it supplies a way of estimating the effects of inbreeding during the early periods before there are pedigree records. A rough and ready answer may be quite useful for many purposes, and the isonymy method can sometimes supply it with minimum effort…".

J.F. Crow

CHAPTER V

STRUCTURE OF AZOREAN POPULATION: VIEW FROM SURNAMES

Population Structure of São Miguel Island, Azores: A surname Study

Published in Hum Biol, 2003

Surnames in Azores: Analysis of the isonymy structure

Published in Hum Biol, 2005

Geography of surnames in Azores: specificity and spatial distribution analysis

Published in Am J Hum Biol, 2005

V.1. Population Structure of São Miguel Island, Azores: A surname Study

V.1.1. Summary

The knowledge of population structure may constitute a powerful tool for mapping genes underlying susceptibility to Mendelian and complex diseases. To obtain a better understanding of the population structure of São Miguel Island (Azorean archipelago, Portugal), we carried out a surname survey using the surnames listed in the most recent telephone book (2001). We identified 1315 different surnames in a total of 27,621 subscribers. The frequency of the different surnames was used to calculate the following parameters: isonymy (I), random component of inbreeding (F_{ST}), genetic diversity according to Fisher (α), migration rate according to Karlin-McGregor (ν), and Nei's genetic distance. Eleven localities were selected, due to population size and geographic distribution, for analysis using the parameters above. Our results show that 51% of Salga's population and 52% of Sete Cidades's population are represented by 6 and 8 surnames, respectively. This demonstrates the effective isolation of these two small places, which are located in opposite extremes of São Miguel Island. Salga, Achada and Sete Cidades present the lowest values of Fisher's α, indicating less genetic diversity. In contrast, the capital Ponta Delgada presents the highest value of α (78.13), indicating more genetic diversity. Our data indicate that the clustering of the localities corresponds to the geographic features of the island, where localities close together tend to share similar surnames.

V.1.2. Introduction

Surnames are useful, simple and cost effective when used as a tool for examining the genetic structure of human populations. They are not evenly distributed among ethnic groups or geographic areas, and, thus, the study of surname frequencies allows the inference of how gene frequency helped to shape population structure (Lasker 1985). Here, we describe a study of the population structure of São Miguel Island, using isonymy parameters based on the surnames present in the 2001 telephone book. São

Miguel presents a particular orography where the distribution of genes may have been influenced by geographic barriers. It was our main objective to understand the distribution of surnames, the effect of the geographical isolation within the island, and the relations established between the different localities of São Miguel Island.

V.1.3. Material and Methods

V.1.3.1. Localities

In the present study, we chose a group of eleven localities scattered throughout the island of São Miguel (Figure V.1). The selected group was constituted by one urban locality – the capital Ponta Delgada – and ten rural localities: Achada, Bretanha, Furnas, Ginetes, Maia, Nordeste, Rabo-de-Peixe, Povoação, Salga and Sete Cidades. The choice of these localities was based on population size, demographic characteristics and geographic isolation. Salga and Sete Cidades were chosen because of their relative geographical isolation, small population size and opposite location in relation to the east-west axis (Figure V.1). Bretanha, Rabo-de-Peixe and Maia were selected because of their location in the northern part of the island, whereas Ginetes, Ponta Delgada and Povoação by their location in the south. The inclusion of Nordeste and Achada was based on their difference in population size and their distance from the capital, Ponta Delgada. Furnas was included in this study because of its attraction as a touristic site.

V.1.3.2. Surnames

In Azores, as in mainland Portugal, each individual inherits two surnames, one from the mother (mid surname) and one from the father (last surname). The mid surname is the last surname of the mother's father. Generally, the last surname of a name (father's) is passed to the next generation. Although, we do not exclude the possibility that some surnames may have been created in the Azores, the majority of surnames arrived with the Portuguese settlers.

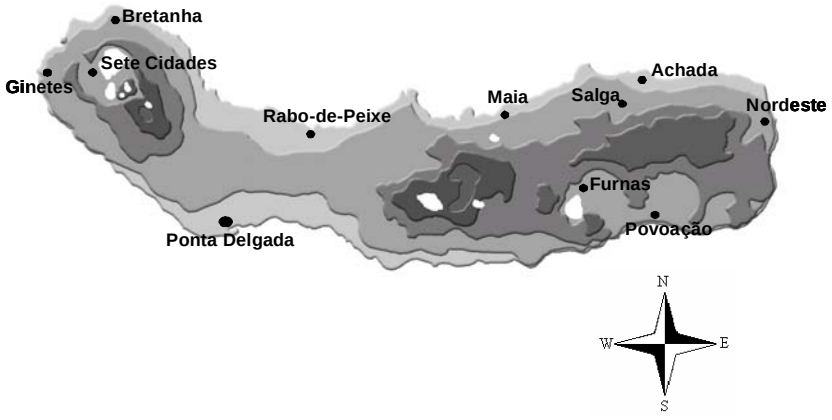


Figure V.1. Map of São Miguel Island (Azores). Displayed are the eleven localities, including the capital Ponta Delgada, selected in this study. For Salga, Sete Cidades, Achada, Nordeste, Ginetes, Maia, Furnas, Bretanha, Povoação, Rabo-de-Peixe and Ponta Delgada, the number of inhabitants is 548; 853; 587; 1381; 1266; 1091; 1544; 1325; 2424; 7041 and 20,091, respectively. White spots in the map denote existing lakes.

We used the 2001 telephone book, which is alphabetically ordered using the last surname, to calculate the frequency distribution of surnames for all localities. We only considered the last surname, because it was not possible to get the mid surnames for all individuals. All different surnames were considered as new entries regardless of similarity of spelling. Surnames with the same phonetics, such as, Batista and Baptista, may have simultaneous temporal origin, but they may not always derive from the same individual. In such cases we considered them as two entries. No differentiation of sex was made and the commercial surnames were excluded from the list.

V.1.3.3. Mathematical methods

The distribution of surnames for the whole island of São Miguel was studied fitting a regression line to $\log_2$-$\log_2$ transformation of the number of surnames, *S*, which are represented *k* times (Barrai *et al.* 1987). Unbiased random isonymy within the locality was calculated according to Rodriguez-Larralde *et al.* (1993) by the formula:

$$I_{ii}=\Sigma_k(p_{ik})^2-1/N_i$$

where p_{ik} is the relative frequency of surname k in the *ith* locality, and N_i is the sample size (number of private telephone users) of the same locality. The random isonymy between localities i and j was estimated as

$$I_{ij}=\Sigma p_{ki}p_{kj}$$

where p_{ki} and p_{kj} are the relative frequencies of surname k in the localities i and j, respectively (Relethford 1988). The random component of inbreeding (F_{ST}) within the locality was obtained from the formula:

$$F_{ST}=I_{ii}/4$$

The calculation of F_{ST} for the whole island was based on the formula suggested by Relethford (1988)

$$F_{ST}=\Sigma w_i\varphi_{ii}$$

where φ_{ii} is the random component of inbreeding ($I_{ii}/4$) of the *ith* locality, and w_i is the weight due to sample size, N_i/N_t, being N_t the sample size of the whole island. For each locality we calculated Fisher's α based on Barrai *et al.* (1992)

$$\alpha=1/I_{ii}$$

The determination of the Karlin-McGregor's ν was based on the formula proposed by Zei *et al.* (1983)

$$\nu=\alpha/(N_i+\alpha)$$

establishing the relationship between Fisher's α, Karlin-McGregor's ν and population size. To obtain Nei's distance, we estimated the standardized isonymy (R_{ij}) proposed by Chen and Cavalli-Sforza (1983)

$$R_{ij}=I_{ij}/(I_iI_j)^{1/2}$$

in which I_{ij} is the isonymy between localities, and I_i and I_j are the isonymies within the localities. Nei's distance (Nei 1973) was computed by

$$D_{ij}=-\ln R_{ij}$$

A dendogram was constructed from the matrix of Nei's distance using the unweighted pair group method with arithmetic mean (UPGMA) for a graphical representation of the surname relationship between the different localities. The calculation of the geographic distance between all localities was performed using the UTM (Universal Transverse Mercator) coordinates

$$D=[(m_i-m_j)^2+(p_i-p_j)^2]^{1/2}$$

where m_i and p_i are the UTM coordinates for the *ith* locality, and m_j and p_j are the UTM coordinates for the *jth* locality.

V.1.4. Results

V.1.4. 1. Surname distribution

The population structure of São Miguel Island was analysed by computing the frequency distribution of surnames obtained from the 2001 telephone book. The total number of subscribers found in that list was 27,621. This represented approximately 21% of the total population of the island which is 131,609 inhabitants (National Institute of Statistics – Portugal, 2001 Census). These 27,621 subscribers bear 1315 different surnames.

In order to obtain a graphical overview of the shape of the surnames distribution, we calculated how many surnames display the same absolute frequency. This allowed the logarithmic computation relating the number of different surnames and the number of times that they appear in the list (Figure V.2). The data show that there is an excess of surnames that appear only once. In fact, 598 of the 1315 different surnames have an absolute frequency of one. Moreover, as expected, the most abundant surnames in the population are fewer in the distribution. For instance, only one surname has the absolute frequency of 1415.

Table V.1 summarizes the frequency and distribution obtained for the selected localities of São Miguel Island. We first observed that the ratio of the number of subscribers over the size of the population for each locality remains fairly constant (around 1/3, Table V.1). Salga is the smallest locality with a sample size of 123 subscribers and only 37 different surnames. In contrast, Ponta Delgada contains 5677 phone subscribers and 610 different surnames. The biggest rural locality is Rabo-de-Peixe with 936 phone subscribers and 181 different surnames. The surname distribution obtained in terms of relative frequency revealed that the most frequent surname in São Miguel Island is Medeiros with a frequency of 5.1% of total subscribers. Sousa is the second most common surname with 3.5%, followed by Silva (3.2%) and Melo (2.7%). When

comparing the distribution of surnames within each of the rural localities, we observed that approximately half of the subscribers are represented by a small number of surnames. About 50% of the subscribers of Salga, Sete Cidades and Achada are represented by 6, 8 and 7 surnames, respectively. Moreover, the most frequent surnames in each of these localities (Melo, Medeiros and Sousa) differ from each other, but are also very frequent in the island, as shown above.

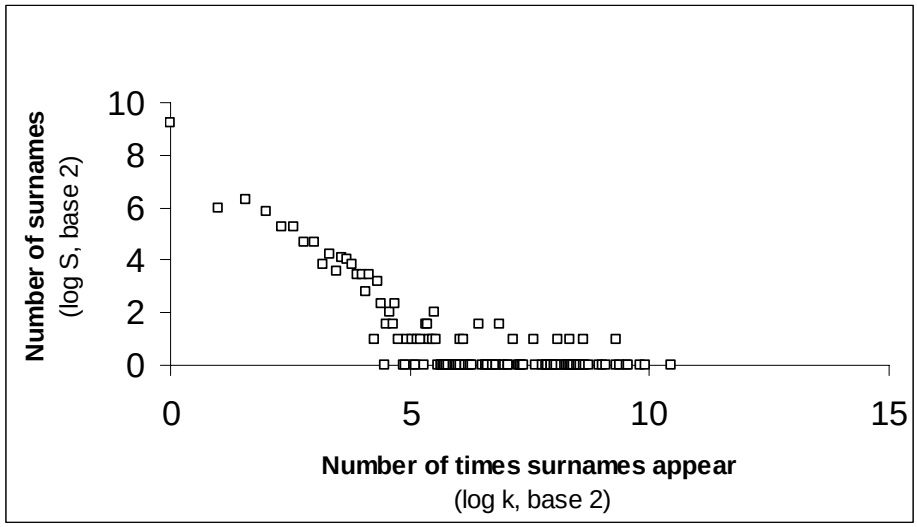


Figure V.2. Relationship between the number of surnames and the number of times they appear in the 2001 telephone book in São Miguel Island. Note that there is an excess of surnames that appear only once (dot on top of the Y axis ($\log_2 1=0$, $\log_2 598=9.22$).

V.1.4. 2. Isonymy analysis

The results obtained for the isonymy parameters are described in Table V.2. The calculation of isonymy was based on surname frequency. The highest value of isonymy (I=0.0576) is found in Salga followed by Achada (I=0.0456). In contrast, Ponta Delgada shows the lowest value, I=0.0128. Among the ten rural localities, Furnas is the one with the lowest value of isonymy (I=0.0176).

Table V.1. Surnames frequency and distribution in São Miguel Island localities.

Localities	Phone subscribers	No. subscribers/ No. inhabitants	Most frequent surname	No. different surnames	Distribution Subscribers (%) / most frequent surnames (No.)
Salga	123	1/4	Melo	37	51 / 6
Sete Cidades	148	1/6	Medeiros	40	52 / 8
Achada	162	1/3	Sousa	52	50 / 7
Nordeste	310	1/4	Medeiros	80	50 / 14
Ginetes	346	1/3	Medeiros	91	51 / 15
Maia	372	1/5	Pacheco	91	50 / 14
Furnas	398	1/4	Melo/Costa	117	50 / 17
Bretanha	519	1/3	Pavão	116	50 / 15
Povoação	804	1/3	Medeiros	142	50 / 17
Rabo-de-Peixe	936	1/8	Andrade/Vieira	181	50 / 19
Ponta Delgada (capital)	5677	1/3	Medeiros	610	50 / 29
São Miguel Island	**27,621**	**1/5**	**Medeiros**	**1315**	**50 / 26**

Table V.2. Results obtained in the calculation of isonymy (I), inbreeding coefficient (F_{ST}), Fisher's α (α) and Karlin-McGregor ν (ν) for each locality in São Miguel Island.

Localities	**I**	F_{ST}	**α**	**ν**
Salga	0.0576	0.0144	17.36	0.123
Sete Cidades	0.0450	0.0112	22.22	0.130
Achada	0.0456	0.0114	21.93	0.119
Nordeste	0.0294	0.0073	34.01	0.099
Ginetes	0.0275	0.0069	36.36	0.095
Maia	0.0249	0.0062	40.16	0.097
Furnas	0.0176	0.0044	56.49	0.124
Bretanha	0.0232	0.0058	43.10	0.077
Povoação	0.0240	0.0060	41.67	0.049
Rabo-de-Peixe	0.0185	0.0046	54.05	0.054
Ponta Delgada (capital)	0.0128	0.0032	78.12	0.013
São Miguel Island	**0.0133**	**0.0016**	**75.19**	**0.0027**

In order to determine possible population subdivisions and, consequently, differentiation, we estimated the random component of inbreeding (F_{ST}). Salga is the locality with the highest value of F_{ST} and Ponta Delgada has the lowest (Table V.2). The magnitude difference between both is 4.5 fold. Excluding the capital (Ponta Delgada) and the three smallest localities (Salga, Sete Cidades and Achada), we observe no major differences between the values of F_{ST}.

To evaluate and quantify the diversity of surnames within each locality we calculated Fisher's α. The smallest locality, Salga, and the largest one, Ponta Delgada, have the extreme values of α, 17.36 and 78.12, respectively. Furnas possesses one of the highest values (α=56.49), indicating that although it is a small place it contains a high degree of surname diversity. In close relation with the Fisher's α is the degree of migration based on Karlin-McGregor's ν parameter. Once more, the smallest localities – Salga and Sete Cidades – possess higher values of ν (0.123 and 0.130, respectively) when compared to the city of Ponta Delgada (ν=0.013). Surprisingly, Furnas shows a high value of ν

(0.124) when compared with other localities with approximately the same number of subscribers.

In order to investigate the degree of similarity between the different localities, a dendogram was constructed using Nei's distance matrix, which is based on isonymy data (Figure V.3). Overall, geographic distance determines the similarity between localities. For instance, Sete Cidades, Bretanha and Ginetes all located in the western tip of the island branch together, whereas Salga and Achada form a second group.

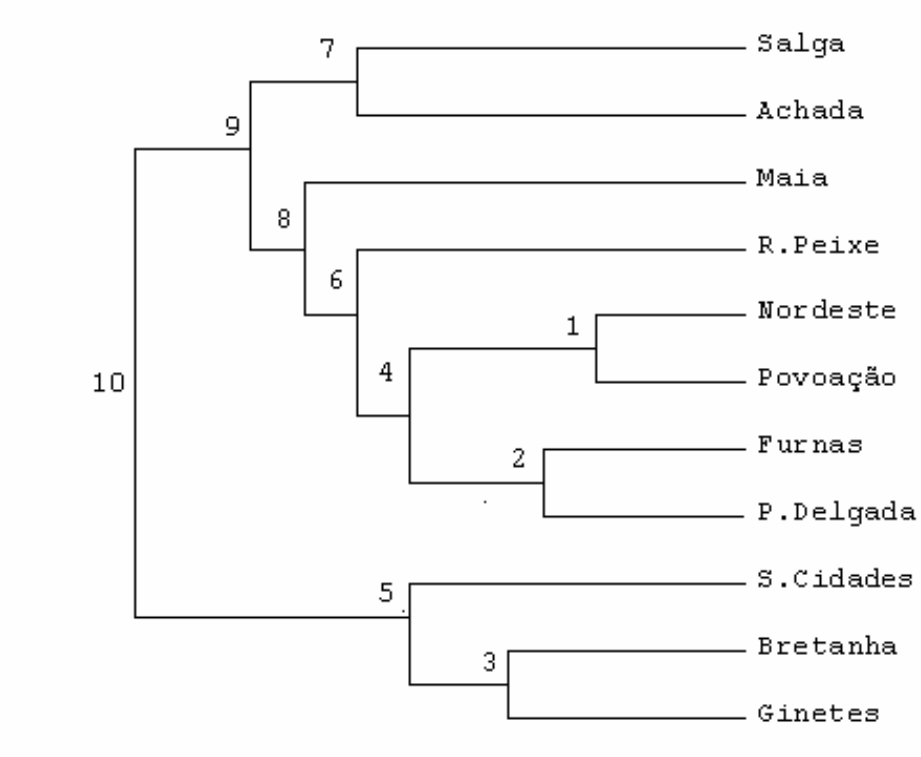


Figure V.3. Dendogram obtained from the matrix of Nei's distance between the eleven localities of São Miguel Island.

V.1.5. Discussion

The log-log model system, proposed by Barrai *et al.* (1987), is useful as a quick method of exploring the distribution of surnames and may allow, depending on the goodness of the fit, the estimation of genetic parameters from surname distributions. Here we used

this method to demonstrate that the population structure of São Miguel Island can be studied using isonymy data.

The settlement of the Azorean archipelago began in the early 15th century, mainly by Portuguese people from north and central mainland Portugal. Indeed, the historical registers suggest that the surnames with the highest frequency in São Miguel population today – Medeiros, Sousa and Silva – came originally from northern Portugal (Sousa 2001). According to Rodriguez-Larralde *et al.* (1994) frequent surnames correspond to the portion of the population which settled in the locality earlier, and, thus, has had the opportunity to spread surnames through its descendants. A branch of the Medeiros family settled in São Miguel Island during the early 15th century, suggesting that a large fraction of the Medeiros today may have a common genetic origin. In contrast, around 45% of the surnames present in the telephone list appear only once, suggesting recent entries in São Miguel. Indeed, 25% of those are of foreign origin, mainly from northern Europe.

To gain a further understanding of the population structure of São Miguel, we studied eleven localities using the following indicators: isonymy (I), Fisher's α (α) and Karlin-McGregor's ν (ν). The data show that the smallest localities of Salga, Achada and Sete Cidades have the highest values of isonymy and a high concentration of very few surnames, suggesting sedentarism (Table V.2). On the other hand, the high values of Karlin-McGregor's ν may indicate migration of people to other localities, leading to a diminution of the diversity of surnames. The estimation of Fisher's α permits the assessment of the richness of surnames present in each locality – low values of α imply less genetic variation. Again, Salga, Sete Cidades and Achada, with lower values of α, display less genetic diversity. As expected, Ponta Delgada has the highest value of Fisher's α and the lowest value of migration rate (ν). Some authors (Rodriguez-Larralde *et al.* 1994; Barrai *et al.* 1996) consider that the localities with higher values of migration rate are genetically more diverse. If that is the case, Ponta Delgada would have low genetic diversity, which is not confirmed by the values of α obtained in this study. Possibly, the discrepancy of these values is due to the large variation of the

number of subscribers observed in the small localities, Salga, Achada and Sete Cidades, compared to the city, Ponta Delgada (Table V.1).

In order to describe the effect of population structure on the degree of inbreeding at a given population subdivision, Wright (1921) created the fixation index. From this model evolved the concept of F_{ST}, defined as an indicator of genetic differentiation and random inbreeding. Our results show that Salga, Sete Cidades and Achada are highly inbred, thus, confirming the bigger differentiation and effective isolation of these localities when compared to the others. According to the classification proposed by Wright (1984), the value of F_{ST} for the whole island reveals little genetic differentiation ($F_{ST}<0.05$). However, small values of F_{ST} may still be significant when analysing very young populations (10-20 generations), such as, the Azorean population (~27 generations).

High correlations between genetic and geographic distances reflect a significant effect of the latter on the genetic variation between populations (Relethford 1982). In addition, there is a tendency to observe low correlation between geographic and surname distance in recently founded populations, while high correlations are detected in well established groups. This reflects the accumulation of the effect of isolation by distance over time (Jorde 1989). Although the population of São Miguel is young, the multivariate cluster analysis may indicate moderate correlation between geographic and genetic distances (Pearson's r=0.37, $p<0.01$), where the closer the distance of localities, the higher is the chance of clustering. Furnas represents an exception, since it clusters with Ponta Delgada (Figure V.3). This is explained by the fact that Furnas is a touristic location and many people, mainly from Ponta Delgada, have summer houses there. In addition, Salga and Achada, which belong to the political subdivision of Nordeste, form a single cluster, apart from Nordeste. This may be due to geographic barriers, which hinder communication between Nordeste and Salga/ Achada, as opposed to Nordeste and Povoação (Figure V.1). The cluster formed by Ginetes, Bretanha and Sete Cidades branch off from the rest of the tree, implying a greater isolation. Natural barriers, such as, mountains, have kept certain areas isolated. We point out that no phylogenetic relationship between localities is implied from the data.

V. 2. Surnames in Azores: Analysis of the isonymy structure

V.2.1. Summary

Geographic isolation is a significant factor to consider when characterizing human populations. The knowledge of the genetic structure of isolated populations has been of great importance to disease *locus* positioning and gene identification. In order to investigate the genetic structure of the Azorean population, we conducted a survey based on the frequencies of surnames listed in the 2001 telephone book. We calculated the following parameters: Isonymy (I), random component of inbreeding (F_{ST}), genetic diversity according to Fisher (α), Karlin-McGregor's migration rate (ν) and Nei's distance. In a total of 1271 subscribers and 163 different surnames, Graciosa Island presents the lowest value of abundance of surnames (α=15.75), suggesting great genetic isolation when compared to the other eight islands. Migration, based on the diversity of surnames within islands, ranges from 0.2747 (Corvo Island) to 0.0026 (São Miguel Island), indicating that people migrate preferentially towards the economically more developed islands. The value of the random component of inbreeding obtained for the whole population (F_{ST}=0.0039) indicates little genetic differentiation (Wright's F_{ST}<0.05). Moreover, isonymy similarity revealed by UPGMA method shows three subclusters corresponding to the geographic distribution of the islands.

V.2.2. Introduction

In societies where surnames run through paternal line, surnames may simulate neutral alleles transmitted only by the Y-chromosome. This aspect of surnames, in addition to their easy access and manipulation, makes them useful to study population structure (Pettener *et al.* 1998). Recently, we used surnames to characterize the population structure of the biggest island of the Azores, São Miguel (Branco and Mota-Vieira 2003). The value of random component of inbreeding (F_{ST}=0.0016) obtained for São Miguel´s population indicated little genetic differentiation. Here we extended our analysis of surnames to include the whole archipelago, using data obtained on the 2001

telephone book. We focus our analysis on surname distribution among the islands, taking into account the geographic feature of the archipelago.

V.2.3. Material and Methods

Azores is composed of nine islands divided into three groups designated according to their geographical location: (*i*) Western group, Corvo and Flores; (*ii*) Central group, Terceira, Graciosa, Pico, Faial and São Jorge; and (*iii*) Eastern group, São Miguel and Santa Maria (see map on Figure V.5). We based our study on surnames listed in the 2001 Azorean telephone book, which is alphabetically ordered by subscriber's last surname. This corresponds to the father's last surname, which is the only surname considered in this study. We first determined the total number of subscribers to produce a list of unique surnames for each island. We also computed a list of different surnames for the whole archipelago. We considered surnames with the same phonetics (e.g. Ataíde and Athayde) as different, because they may have simultaneous temporal origin, but may not derivate from the same individual. In addition, we did not consider commercial surnames.

Surname distribution was studied fitting a regression line to $\log_2$-$\log_2$ transformation of the number of surnames, *S*, which are represented *k* times (Barrai *et al.* 1987). The frequency of surnames was used to calculate the following parameters: Isonymy (I), random component of inbreeding (F_{ST}), Fisher's α (α), Karlin-McGregor's ν (ν) and Nei's genetic distance, according to methods described in Branco and Mota-Vieira (2003).

V.2.4. Results and Discussion

V.2.4.1. Surname distribution in Azorean population

The population studied here contains 57,387 subscribers, representing 23.7% of the population and about 80% of the total number of Azorean families. Overall, we

computed 2451 different surnames. The discrepancy between the numbers of subscribers among the islands reflects the difference in population size (Table V.4). In addition, the islands with the highest level of economic development, São Miguel, Terceira and Faial, have the highest number of different surnames, 1315, 1198 and 480, respectively (Table V.4). The most common surnames overall are Silva (5.1% of the total subscribers), Sousa (3.3%) and Medeiros (2.9%), names that come originally from northern Portugal, (Sousa 2001). Interestingly, the most frequent surnames in Flores and Corvo are not in the group of 20th most frequent surnames in Azores, although they are common in the archipelago.

Figure V.4 shows the graph relating the number of times that a surname appears, k, with the number of surnames that have an equal absolute frequency, S. According to Barrai *et al.* (1987) surnames distribution that are almost exactly linearized by a log-log transformation, fit the Karlin-McGregor model and allow the estimation of genetic parameters. Our distribution meets the above condition, therefore, we carried on our surname analysis using several isonymy parameters.

V.2.4.2. Isonymy parameters

The genetic structure of the Azorean population was studied using the following isonymy parameters: Isonymy (I), Fisher's α (α) and Karlin-McGregor's ν (ν). Table V.4 summarizes the data obtained. The values of isonymy are similar in all islands, with the exception of Graciosa, which shows the highest value of isonymy (0.0635). This result, in addition to a low value of migration rate (0.0122), suggests that people in Graciosa have become sedentary.

A high isonymy in Graciosa reflects diminished genetic diversity, indicated by a very low value of α (15.75). In contrast, Terceira has the highest value of Fisher's α, 90.91, a result of an increase in foreign surnames due to the American air base stationed on that

Table V.4. Summary of surnames distribution and isonymy parameters for the Azorean islands. The islands are listed according to the number of subscribers.

Islands	P[a]	N[b]	M[c]	S[d]	I	Fst	α	ν
Corvo	425	106	Pimentel	51	0.0249	0.0062	40.16	0.2747
Flores	3995	1059	Freitas	223	0.0154	0.0038	64.93	0.0578
Graciosa	4780	1271	Silva	163	0.0635	0.0158	15.75	0.0122
Santa Maria	5578	1781	Sousa	242	0.0257	0.0064	38.91	0.0214
São Jorge	9674	2617	Silveira	301	0.0227	0.0056	44.05	0.0165
Faial	15,063	4139	Silva	480	0.0226	0.0056	44.25	0.0106
Pico	14,806	4228	Silva	367	0.0193	0.0048	51.81	0.0121
Terceira	55,833	14,565	Silva	1198	0.0110	0.0027	90.91	0.0062
São Miguel	131,609	27,621	Medeiros	1315	0.0134	0.0033	74.63	0.0026
Azores	**241,763**	**57,387**	**Silva**	**2451**	**0.0243**	**0.0039**	**41.19**	**0.0007**

[a] P = Population size, [b] N = Number of phone subscribers, [c] M = Most frequent surname, [d] S = Number of different surnames

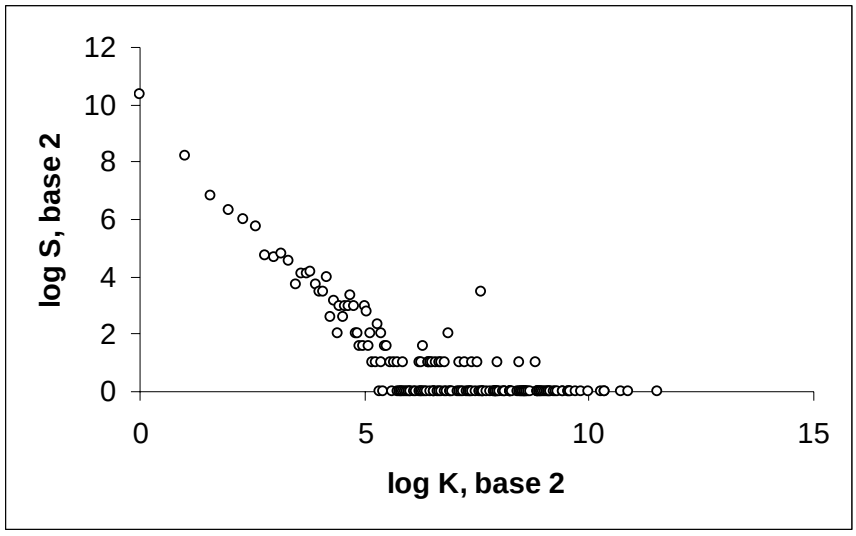


Figure V.4. Logarithmic distribution of surnames in Azores. *S* represents the number of surnames and *k* the number of times they appear.

island. Comparing the values of α between Azores (41.19) and the two major islands – São Miguel (74.63) and Terceira (90.91) – we conclude that the Azorean population presents a very low value of diversity[29].

Human migration may affect the genetic diversity because new alleles may be lost or introduced into the population. To estimate the degree of migration we computed Karlin-McGregor ν (Table V.4). Rodriguez-Larralde *et al.* (1994) and Barrai *et al.* (1996) suggested that the higher the migration rate (ν), the higher is the genetic diversity (α). However, this is not observed in our population, where São Miguel and Terceira, with the lowest value of migration, have the highest level of diversity (Table V.4). In addition, Corvo and Flores show the highest values of ν, 0.2747 and 0.0578, respectively, indicating that people emigrate toward the more developed islands.

Inbreeding, which is based on isonymy values, allows inferences about the degree of genetic differentiation (Rodriguez-Larralde *et al.* 1993). Among Azores islands, the

[29] Although surname analysis results reveal that the Azorean population presents very little genetic diversity, microsatellite data demonstrated a high genetic diversity for this population. These results will be thoroughly discussed in Chapter VIII, General Discussion, of the present thesis.

values of F_{ST} are comparable, indicating a certain degree of population homogeneity. Interestingly, Graciosa, with the lowest value of surname diversity (α), now displays the highest value of inbreeding, suggesting higher genetic differentiation and isolation from other islands. We compared our data with that of two other very young and isolated populations, Kings County in New York (Christensen 2000), and Bedford County in Pennsylvania (Christensen 1999), and we observed that Azores presents a higher value of F_{ST}, thus, a higher inbreeding. This supports the results obtained by Pacheco *et al.* (2003), showing relatively higher rates of consanguineous marriages in Azores compared to Madeira archipelago and mainland Portugal. On the other hand, according to the classification proposed by Wright (1984), the value of F_{ST} for the Azores archipelago (F_{ST}=0.0039) reveals little genetic differentiation. This value is in agreement with previous observation for the island of São Miguel (Branco and Mota-Vieira 2003), where low value of differentiation is also observed.

To estimate the degree of similarity between the islands we constructed a dendogram based on a matrix of Nei's genetic distance (Figure V.5). The data shows two major clusters separating the Eastern group, São Miguel and Santa Maria, from the other 7 islands. São Miguel and Santa Maria were the first islands to be settled, and lately the initial population dispersed, contributing to the settlement of the other islands. The dendogram also shows a second division separating the Central group from Flores and Corvo (Figure V.5). This is compatible with the geographic feature of the archipelago, and the ease with which the population migrates within groups of islands. As expected, Pico and Faial display close surname similarity, because there are regular boat connections between both islands, facilitating interaction between individuals. In addition, our data show that geographic distances are correlated with genetic distances ($r=0.726$, $p<0.0001$), and that the closer the distance between the islands, the higher is the chance of clustering.

V.2.5. Conclusions

Genetically isolated populations offer many advantages for mapping inherited traits. Indeed, in cases of environmental and population homogeneity the dissection of such

traits is considerably facilitated (Arcos-Burgos and Muenke 2002). Our analysis is based on a population sample of 57,387 individuals, which represents 80% of the overall Azorean families. We used surnames as the means to assess the genetic structure of the Azorean population. The data shows that there is a strong correlation between geographic distances and genetic distances. For instance, Pico and Faial connected by year-round daily boat trips, display high similarity of surnames (dendogram on Figure V.5). The dendogram also shows that Santa Maria and São Miguel, the first two islands to be settled in the east part of the archipelago, share a similar pattern of surnames. As expected, genetic diversity is higher in more developed islands (e.g. São Miguel and Terceira), a phenomenon that is further increased by a recent immigration of foreigners.

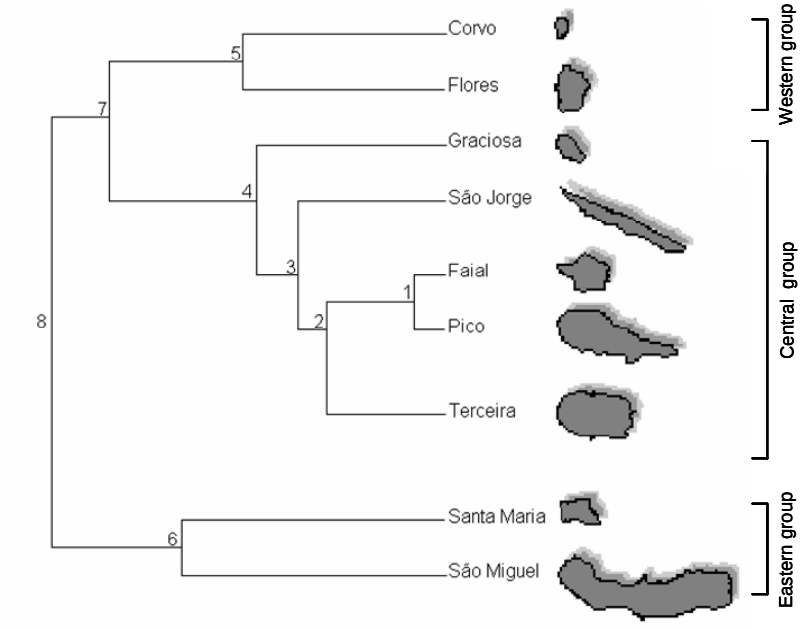


Figure V.5. Cluster analysis based on the matrix of Nei's distance for the Azorean population.

In contrast, Graciosa is the most inbred, probably a result of a long fixation of early settlers. Finally, inbreeding analysis reveals that the population displays little genetic differentiation (Table V.4, F_{ST}=0.0039). In conclusion, our data reveals the influence of the geography of the archipelago over the distribution of surnames among the islands, and demonstrates that isonymy analysis is a powerful method to characterize genetic structure in small populations.

V.3. Geography of surnames in Azores: Specificity and spatial distribution analysis

V.3.1. Summary

In order to obtain a better understanding of the genetic structure of the Azorean population, a specificity and spatial distribution analysis was performed based on 2454 different surnames present in the Azorean telephone directory (2002). We considered as specific surnames those with an absolute frequency ratio equal or higher than 50%. The results revealed 51 specific surnames in the whole archipelago. The smallest island presents the only surname with 100% of specificity (Pedras). In addition, São Miguel Island, which contains 54.4% of the Azorean population, has the highest number of specific surnames (25 specific surnames). The spatial distribution analysis was used to detect genetic similarity between municipalities through the calculation of spatial autocorrelation (Moran's *I* coefficient). Of the 240 surnames included in the analysis, 113 showed statistically significant patterns. Five different patterns were obtained, of which the most relevant is isolation by distance and depression (41.6%). However, 43.4% had no defined pattern. The overall correlogram shows a majority of positive values for distances lower than 49 km and between 269-309 km, indicating high similarity between closer municipalities and between distant municipalities whose populations show historic and socio-cultural affinities. In conclusion, our data are in agreement with the historical background of the Azorean population.

V.3.2. Introduction

Azores (Portugal) constitutes an interesting model for studying internal processes of differentiation; it has a particular orography, which confers a relative geographic and cultural isolation (Branco and Mota-Vieira 2003, 2005). In the present work we carried out further investigation into the genetics of the Azores, through analysis of specificity and spatial distribution of surnames. Our main goal is to understand the geography of surnames in the archipelago: mobility between the municipalities and between the islands; and know the patterns of dispersion of individuals and genes.

V.3.3. Material and Methods

V.3.3.1. Dataset

Dataset includes all surnames transcribed from the 2002 telephone directory. The only surname considered was the father's last surname, since it is passed to the next generation. Surnames with similar spelling or writing, such as, Cimbron and Cymbron, were considered different. They may have simultaneous temporal origin, but may not always derive from the same individual. Double subscriber registration, identified by online service of PT communications[30], was eliminated. This dataset excludes headings of firms, organizations, hotels, etc. In addition, users were not distinguished by sex.

V.3.3.2. Specificity Analysis

Azores is composed of nine islands divided into three groups designated according to their geographical location: (*i*) Western group, Corvo (Cor) and Flores (Flo); (*ii*) Central group, Terceira (Ter), Graciosa (Gra), Pico (Pic), Faial (Fai) and São Jorge (Jor); and (*iii*) Eastern group, São Miguel (Mig) and Santa Maria (Mar; Figure V.6). The specificity analysis was performed using the 30 most frequent surnames present in each island, because these surnames probably arrived with the first settlers. Surnames with higher frequency in an island have, possibly, smaller frequency on the others, so they will be specific of that island. We used their correspondent absolute frequency in the island and in the archipelago. We then calculated the ratio island/ Azores for each surname and ordered them accordingly. We only considered as specific surnames those with a ratio equal or higher than 50%.

V.3.3.3. Spatial Autocorrelation Analysis

In the present study, we chose the total number (19) of municipalities (administrative divisions) existing in the Azores archipelago (Figure V.6), because the autocorrelation

[30] Online service of Portugal telecommunications - www.118.pt.

analysis needs a minimal number of populations – 15 to 25 (or more). Santa Maria, Graciosa, Faial and Corvo islands have only one municipality each; Flores, Terceira and São Jorge have two municipalities each; Pico has three municipalities; and São Miguel has six.

Spatial autocorrelation summarizes the genetic similarity between populations in relation to their geographical proximity. In particular, spatial autocorrelation helps to focus on the similarity of values of a variable, *i.e.* the frequency of a surname, between pairs of populations within arbitrary classes of distance (Caravello and Tasso 1999). This method allows estimation of the spatial distribution of surnames in the considered territory, in order to emphasize the specific processes of diffusion of the individuals. To evaluate spatial autocorrelation we used Moran's *I* coefficient (Moran 1950) applied to a database of 240 surnames obtained from the total number of surnames present in the archipelago. These surnames were chosen according to their absolute frequency in the archipelago. Therefore, to obtain the maximum dispersion patterns, surnames with a frequency higher than 23 were selected. The remaining 2214 different surnames show low relative frequency in the archipelago; thus, not justifying their analysis.

The following formula permits an estimate of this autocorrelation coefficient:

$$I=\frac{n\sum_{i=1}^{n}\sum_{j=1}^{n}w_{ij}(p_i-p)(p_j-p)}{W\sum_{i=1}^{n}(p_i-p)^2}$$

where p_i and p_j are the relative frequency of surnames at the *i*th and *j*th locality, p is the mean across the n municipalities, w_{ij} is equal to 1 for all the pairs of municipalities falling in the studied distance class and equal to 0 for all the other pairs, and W is the sum of all w_{ij} values in that distance class.

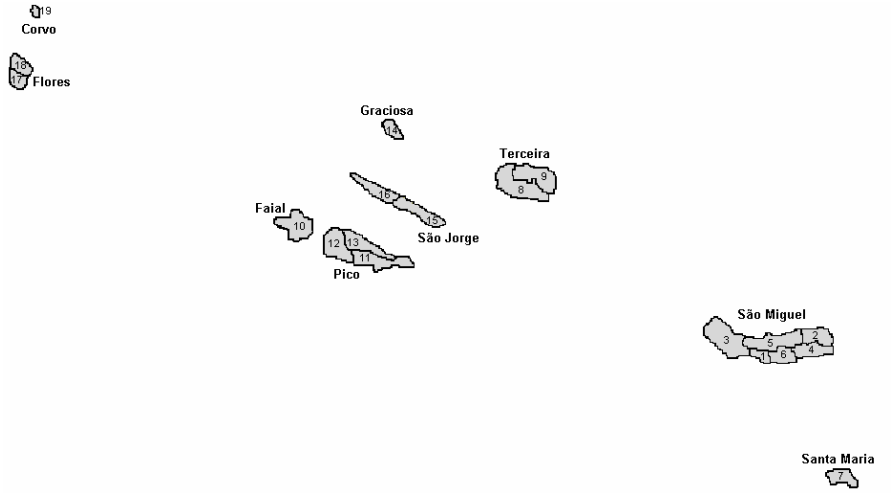


Figure V.6. Map of the Azores archipelago denoting the 19 municipalities (38ºN, 27ºW). The continuous line indicates the administrative divisions, marked with numbers: 1-Lagoa, 2-Nordeste, 3-Ponta Delgada, 4-Povoação, 5-Ribeira Grande, 6-Vila Franca do Campo, 7-Vila do Porto, 8-Angra do Heroísmo, 9-Praia da Vitória,10-Horta, 11-Lajes, 12-Madalena, 13-São Roque, 14-Santa Cruz, 15-Calheta, 16-Velas, 17-Lajes, 18-Santa Cruz and 19-Corvo.

Geographic distance between municipalities is important to assess the limits of the different distance classes. For each surname, Moran's *I* coefficient was computed in five arbitrary distance classes, with the following upper limits: 49 km, 195 km, 269 km, 309 km and 605 km. The boundaries of these distance classes were chosen to yield intervals with equal number of point pairs, *i.e.* locality pairs in each class. The calculation of the distance was performed using the UTM (Universal Transverse Mercator) coordinates (Branco and Mota-Vieira 2003).

In large samples Moran's *I* coefficient varies between -1 to +1, where positive significant values ($I>0$) indicate similar surname frequencies and negative significant values ($I<0$) indicate dissimilarity (Barbujani *et al.* 1992). The overall significance of the 240 correlograms was assessed by Bonferroni test[31] (Oden 1984; Sokal and Thomson 1998). Only significant ($p\leq0.05$) correlograms, 113 out of the 240, were

[31] A very simple method due to Bonferroni (1936) is to divide the test-wise significance level by the number of tests: $\alpha\beta=\alpha/k$ (for example, with $k=10$ and $\alpha=0.05$, therefore, $\alpha\beta=0.005$). So the significance level will be 0.005 to each of the ten tests. This leads to only a 5% chance that any of the tests will be declared significant under the null hypothesis.

accepted for analysis. The patterns of autocorrelation coefficients were schematically classified according to the spatial distributions, into: Isolation by distance and depression (IBD+D), isolation by distance and double depression (IBD+DDP), depression (D), intrusion (I) and long-distance differentiation (LDD; Barbujani 2000; Barbujani and Sokal 1991).

In almost all cases, autocorrelation tends to be significant and positive at short distances. This is likely the consequence of isolation by distance, when neighbouring localities share a common gene pool (Barbujani 1987). The isolation by distance patterns are usually associated with a depression, *i.e.* a decrease in surname similarity, generally in long distance classes. However, simple depressions may also characterize the mobility of a given surname. Long-distance differentiation patterns are described by a positive autocorrelation in the first two distance classes. This will define regions of homogenous gene frequencies. Moreover, autocorrelation is negative at large distances; but the absolute values of Moran's *I* are all small. Finally, the intrusion pattern reveals a maximum similarity at one peak, indicating an entrance of a surname on that distance, and negative autocorrelation is observed at both shorter and larger distances.

V.3.4. Results

V.3.4.1. Surname distribution

In this study, the population structure of Azores Islands was analyzed through the computation of the frequency distribution of surnames from the telephone directory. In Azores the use of the telephone is so widespread that directories include nearly all resident families. Our dataset includes 55,528 subscribers, representing approximately 23% of the total population (Table V.5). We first calculated the surnames absolute frequency for all municipalities. Out of the 2454 different surnames, 2038 (83%) have absolute frequency lower than 10, but these correspond to only 3894 subscribers. The remaining 51,634 subscribers correspond to 416 surnames that have an absolute frequency greater than 10. This result demonstrates that a large fraction of the Azorean population share few surnames.

In Table V.5 we summarize the distribution of the total surnames over the municipalities. In this table we present some data relevant in the present study, as the number of families and the number of subscribers with the 240 surnames studied by autocorrelation analysis for the 19 municipalities. Note that the ratio of the number of subscribers over the number of families shows the representation of our dataset (77%). Vila Nova do Corvo is the smallest municipality with a sample size of 105 subscribers distributed by 51 different surnames. In contrast, Ponta Delgada contains 14,436 subscribers and 948 different surnames. The most frequent surname in the archipelago is Silva with a frequency of 5.1%, Sousa is the second most common surname with 3.3%, followed by Medeiros (3.0%), Melo (2.3%) and Costa (2.3%).

The absolute frequency of the surnames differs from one municipality to another, and contiguous municipalities tend to have similar frequencies, a result of a possible past diffusion effect. For example, in Pico Island, Silva is evenly distributed over the three municipalities (131 subscribers in Madalena, 123 in Lajes and 81 in São Roque).

V.3.4.2. Specificity analysis

The influence of geographic discontinuity on surname diversity was studied through a surname specificity analysis. Specific surnames may correspond to the portion of the population that first settled in or may represent recent entries (Barrai *et al.* 1996). The São Miguel Island shows the highest number of specific or autochthonous surnames, being the most relevants: Cabral (with a ratio equal to 80%), Pacheco (81%), Medeiros (83%), Cordeiro (87%), Rego (87%), Arruda (88%), Botelho (89%), Ponte (90%), Raposo (90%) and Carreiro (91%; Table V.6). Islands Pico and São Jorge only have one specific surname: Jorge and Brasil, respectively, both with a ratio equal to 54%. The island of Santa Maria showed five specific surnames: Moura (52%), Figueiredo (59%), Chaves (59%), Bairos (80%) and Leandres (93%). Corvo Island is the only one that has a surname with 100% of specificity (Pedras), but this includes only two subscribers. On average there are six specific surnames *per* island (Table V.6).

Table V.5. Azores: Geographic, demographic and telephone subscribers data.

Name of geographic group, Azorean island and administrative division	Population density (Inh./ Km^2)[a]	Population size[a]	No. of families[a]	No. of subscribers[b]	No. of surnames[b]	Subscribers with the 240 studied surnames[c]	
						No.	%
Eastern group							
São Miguel	176.23	131,609	36,600	26,613	1308	23,398	87.92
Lagoa	310.05	14,126	3862	2426	386	2049	84.46
Nordeste	52.12	5291	1754	1265	150	1188	93.91
Ponta Delgada	283.95	65,854	18,595	14,436	948	12,814	88.76
Povoação	60.98	6726	1979	1527	234	1348	88.28
Ribeira Grande	158.56	28,462	7533	4957	450	4364	88.04
Vila Franca do	142.95	11,150	2877	2002	281	1635	81.67
Santa Maria	57.46	5578	1814	1701	244	1543	90.71
Vila do Porto	57.46	5578	1814	1701	244	1543	90.71
Central group							
Terceira	139.65	55,833	17,271	14,038	1223	12,015	85.59
Angra do Heroísmo	149.80	35,581	10,957	8509	675	7449	87.54
Praia da Vitória	124.79	20,252	6314	5529	855	4566	82.58
Faial	88.64	15,063	4788	4021	484	3534	87.89
Horta	88.64	15,063	4788	4021	484	3534	87.89
Pico	32.85	14,806	4829	4222	376	3887	92.07
Lajes	32.04	5041	1582	1489	211	1358	91.20
Madalena	41.16	6136	2057	1667	214	1553	93.16
São Roque	25.15	3629	1190	1066	196	976	91.56
Graciosa	78.44	4780	1760	1242	161	1148	92.43
Santa Cruz	78.44	4780	1760	1242	161	1148	92.43
São Jorge	39.39	9674	3237	2556	298	2337	91.43
Calheta	32.16	4069	1352	1151	178	1067	92.70
Velas	47.07	5605	1885	1405	224	1270	90.39
Western group							
Flores	28.19	3995	1392	1030	222	868	84.27
Lajes	21.58	1502	556	392	125	331	84.44
Santa Cruz	34.57	2493	836	638	168	537	84.17
Corvo	24.82	425	155	105	51	78	74.29
Vila Nova do Corvo	24.82	425	155	105	51	78	74.29
Azores	**103.77**	**241,763**	**71,846**	**55,528**	**2454**	**48,808**	**87.90**

[a] Data from 2001 census.
[b] Data from 2002 telephone directory.
[c] Surnames studied in the autocorrelation analysis.

V.3.4.3. Spatial autocorrelation analysis (Moran's I coefficient)

Spatial autocorrelation refers to the genetic similarity between populations in relation to their geographical proximity. In our dataset, this analysis reveals that 113 surnames have a statistically significant pattern, of which 41.6% show IBD+D pattern, 9.7% have an intrusion pattern, 2.7% contain a LDD pattern, 1.8% corresponds to a depression pattern, 0.9% encloses an IBD+DDP pattern, and 43.4% have no defined pattern (Table V.7). Out of the 565 data points, which correspond to the individual autocorrelation coefficients, 249 (44%) are significant (Table V.7). The majority of individual coefficients were smaller than 0.20, revealing low similarity of surnames between the five different distance classes. The highest Moran's *I* coefficient at class 1 (0-49 km) is 0.71 for Pacheco, followed by Alvernaz with 0.63.

The 113 Bonferroni significant correlograms were superimposed according to distinct classes and plotted (Figure V.7). Positive autocorrelation is higher at distances up to 49 km, but maintains relatively positive until distances up to 142 km, changing to negative autocorrelation at greater distances. It increases again to positive values in distance class 4 (269-309 km), switching back to negative in the last distance class. The patterns of autocorrelation indicate that after 50 km surname similarity is sharply reduced (Figure V.7). Similar correlograms were averaged to provide summary information of each of the 5 main patterns (Figure V.8).

Table V.6. Specific surnames for each Azorean Island (see Figure V.6 for island location). The ordering is based on the surname specificity.

Surname per island	Absolute frequency of surname		*Surname* Specificity[a]	*Surname per* island	Absolute frequency of surname		*Surname* Specificity[a]
	Island	*Azores*			*Island*	*Azores*	
São Miguel				***Santa Maria***			
Costa	638	1263	0.5051	Leandres	13	14	0.9286
Sousa	933	1813	0.5146	*Terceira*			
Pereira	622	1205	0.5162	Coelho	137	235	0.5830
Rodrigues	326	611	0.5336	Leal	132	212	0.6226
Oliveira	522	955	0.5466	Lourenço	136	196	0.6939
Melo	725	1267	0.5722	Rocha	342	485	0.7052
Ferreira	495	808	0.6126	Mendes	217	273	0.7949
Pimentel	280	431	0.6497	Fagundes	123	144	0.8542
Correia	367	535	0.6860	Meneses	209	243	0.8601
Furtado	320	445	0.7191	Barcelos	131	144	0.9097
Almeida	338	467	0.7238	Toste	224	229	0.9782
Tavares	346	458	0.7555	***Faial***			
Carvalho	260	344	0.7558	Vargas	58	90	0.6444
Amaral	410	542	0.7565	Escobar	50	57	0.8772
Moniz	395	498	0.7932	***Pico***			
Cabral	700	876	0.7991	Jorge	55	101	0.5446
Pacheco	607	745	0.8148	***Graciosa***			
Medeiros	1376	1654	0.8319	Veiga	17	31	0.5484
Cordeiro	330	379	0.8707	Picanço	70	94	0.7447
Rego	304	349	0.8711	Ortins	9	12	0.7500
Arruda	330	376	0.8777	***São Jorge***			
Botelho	386	434	0.8894	Brasil	111	207	0.5362
Ponte	307	340	0.9029	***Flores***			
Raposo	475	526	0.9030	Armas	9	15	0.6000
Carreiro	304	333	0.9129	Noia	15	23	0.6522
Santa Maria				Estácio	9	12	0.7500
Moura	61	117	0.5214	***Corvo***[b]			
Figueiredo	65	110	0.5909	Emílio	2	3	0.6667
Chaves	79	133	0.5940	Pedras	2	2	1.0000
Bairos	49	61	0.8033				

[a] Surname specificity is estimated by the proportion of the surname in island/ Azores.
[b] Only 22 surnames were studied.

Table V.7 Autocorrelation coefficients (Moran's *I*) for the considered surnames in the Azorean population. Only significant patterns are reported.

	Distance Class					Overall	
Surnames	**1**	**2**	**3**	**4**	**5**	**Significance**	**Classification**
Alexandre	0.12	-0.01	-0.31 **	0.08	-0.15	0.047	DF
Almeida	0.10 *	0.01	-0.35 **	0.11 *	-0.14	0.003	DF
Alvernaz	0.63 **	-0.17	-0.30 *	-0.46 **	0.02	0.000	IBD + D
Amaral	0.51 **	-0.12	-0.53 **	0.13	-0.27 *	0.000	DF
Andrade	0.13	0.16 *	-0.53 **	0.46 **	-0.47 **	0.001	DF
Andre	-0.06	-0.10	-0.28	0.28 **	-0.11	0.040	I
Araujo	0.43 **	-0.01	-0.46 **	-0.01	-0.23	0.000	IBD + D
Areias	0.21 **	-0.47 **	-0.04	0.08 *	-0.06	0.000	DF
Arruda	0.19 **	-0.04	-0.33 **	0.05	-0.14	0.001	IBD + D
Avila	0.44 **	-0.10	-0.38 **	-0.16	-0.08	0.001	IBD + D
Azevedo	0.33 **	0.13	-0.48 **	-0.30 *	0.04	0.010	IBD + D
Baptista	-0.02	0.08	-0.24	0.35 **	-0.44 **	0.004	DF
Barbosa	0.40 **	0.01	-0.48 **	0.04	-0.24	0.001	IBD + D
Barcelos	0.27 **	-0.50 **	-0.05	0.06	-0.07	0.000	IBD + D
Barros	0.10	-0.25	-0.13	0.29 **	-0.28 *	0.034	DF
Benevides	0.11 *	-0.04	-0.29 **	0.03	-0.09	0.004	IBD + D
Bento	0.27 **	-0.09	-0.28 *	-0.02	-0.15	0.009	IBD + D
Bettencourt	0.33 **	0.31 **	-0.38 *	-0.58 **	0.04	0.001	IBD + D
Borba	0.26 **	-0.32 *	-0.19	0.01	-0.04	0.044	IBD + D
Borges	0.27 **	-0.19	-0.27	0.18 *	-0.26 *	0.042	DF
Botelho	0.09 *	-0.04	-0.28 **	0.06	-0.10	0.006	D
Braga	0.11 *	0.21 **	-0.11	-0.02	-0.46 **	0.000	DF
Branco	0.31 **	0.05	-0.49 **	0.12	-0.27 *	0.001	DF
Brilhante	-0.03	-0.02	-0.22 **	0.06 *	-0.07	0.023	I
Brito	0.26 **	-0.40 **	-0.13	0.14 *	-0.14	0.005	DF
Bulhoes	0.24 **	-0.07	-0.31 **	-0.02	-0.12	0.011	IBD + D
Cabral	0.17 **	0.05	-0.30 **	0.02	-0.20	0.007	IBD + D
Camara	0.12 *	-0.02	-0.32 **	0.02	-0.08	0.004	IBD + D
Carneiro	0.23 **	-0.07	-0.37 **	0.01	-0.08	0.001	IBD + D
Carreiro	0.31 **	-0.04	-0.37 **	-0.00	-0.17	0.000	IBD + D
Carvalho	0.01	0.02	-0.27 **	0.09 *	-0.12	0.002	DF
Chaves	0.04	0.01	-0.03	0.00	-0.29 **	0.039	LDD
Coelho	0.33 **	-0.49 **	-0.08	0.21 *	-0.23	0.000	DF
Conceiçao	-0.06	0.00	-0.32 *	0.32 **	-0.21	0.007	I
Cordeiro	0.04	-0.05	-0.21 **	0.04	-0.09	0.034	D
Correia	0.33 **	-0.03	-0.51 **	0.22 *	-0.29 *	0.000	DF
Couto	0.43 **	0.04	-0.44 **	-0.00	-0.30 *	0.001	IBD + D
Dinis	0.29 **	-0.45 **	-0.12	0.11	-0.10	0.001	DF

(Continued)

Table V.7. Continuation.

Surnames	Distance Class 1	2	3	4	5	Overall Significance	Classification
Duarte	-0.10	-0.03	-0.34 *	0.34 **	-0.14	0.003	I
Dutra	0.29 **	0.03	-0.30 *	-0.21	-0.08	0.021	IBD + D
Enes	0.31 **	-0.46 **	-0.12	0.06	-0.06	0.001	IBD + D
Estrela	0.15 *	-0.01	-0.37 **	0.10	-0.15	0.018	DF
Fagundes	0.34 **	-0.57 **	-0.08	0.07	-0.05	0.000	DF
Faria	-0.07	0.01	-0.46 **	0.46 **	-0.21	0.001	I
Farias	-0.03	-0.05	-0.19 **	0.04	-0.06	0.024	I
Ferraz	0.29 **	-0.31 *	-0.18	0.08	-0.16	0.022	DF
Figueiredo	0.05	-0.09	0.04	0.06	-0.33 **	0.014	LDD
Franco	0.34 **	-0.03	-0.34 **	-0.04	-0.20	0.000	IBD + D
Frias	0.30 **	-0.07	-0.51 **	0.23 *	-0.22	0.002	DF
Furtado	0.32 **	-0.08	-0.48 **	0.21 *	-0.24	0.001	DF
Gil	0.18 *	-0.33 **	-0.07	0.03	-0.09	0.034	IBD + D
Godinho	0.36 **	-0.59 **	-0.05	0.09	-0.08	0.000	DF
Goulart	0.36 **	-0.09	-0.25	-0.30 *	-0.01	0.002	IBD + D
Gouveia	0.28 **	0.05	-0.46 **	0.05	-0.19	0.005	IBD + D
Homem	0.29 **	-0.40 **	-0.15	0.09	-0.11	0.006	DF
Jorge	0.26 **	-0.17	-0.03	-0.32 *	-0.02	0.032	IBD + DDP
Junior	0.10	0.09	-0.16	0.09	-0.38 **	0.022	DF
Leal	0.33 **	-0.32 *	-0.18	0.01	-0.11	0.005	IBD + D
Leite	0.40 **	-0.11	-0.36 *	0.01	-0.21	0.002	IBD + D
Leonardo	0.23 **	-0.56 **	0.04	0.14 *	-0.13	0.000	DF
Linhares	0.23 *	-0.60 **	0.22 *	0.05	-0.18	0.000	DF
Lourenço	0.17 **	-0.38 **	-0.08	0.04	-0.03	0.005	IBD + D
Luz	0.43 **	0.01	-0.18	-0.09	-0.43 **	0.001	IBD + D
Maciel	0.29 **	0.05	-0.20	-0.43 **	0.00	0.012	IBD + D
Maia	0.06	-0.03	-0.29 **	0.12 *	-0.14	0.036	DF
Medeiros	0.26 **	-0.07	-0.39 **	0.09	-0.16	0.000	DF
Mendes	0.30 **	-0.48 **	-0.10	0.08	-0.08	0.000	DF
Meneses	0.26 **	-0.45 **	-0.09	0.10	-0.08	0.001	DF
Miguel	-0.02	-0.03	-0.31 **	0.20 **	-0.11	0.042	I
Moniz	0.34 **	-0.06	-0.43 **	0.09	-0.22	0.004	DF
Monteiro	0.17 *	-0.33 **	0.12	0.23 *	-0.45 **	0.002	DF
Morgado	-0.05	0.04	-0.21 **	0.05	-0.10	0.022	DF
Mota	0.60 **	-0.14	-0.59 **	0.10	-0.24	0.000	DF
Moura	0.03	0.08	-0.08	0.08	-0.37 **	0.006	LDD
Nogueira	0.33 **	-0.50 **	-0.09	0.11	-0.12	0.000	DF
Oliveira	-0.05	0.04	-0.28 *	0.19 **	-0.18	0.031	DF

(Continued)

Table V.7. Continuation.

Surnames	Distance Class 1	2	3	4	5	Overall Significance	Classification
Ornelas	0.30 **	-0.46 **	-0.10	0.09	-0.10	0.001	DF
Ourique	0.32 **	-0.53 **	-0.06	0.07	-0.08	0.000	DF
Pacheco	0.71 **	-0.03	-0.56 **	-0.06	-0.34 **	0.000	IBD + D
Paim	0.22 **	-0.35 **	-0.09	0.00	-0.06	0.024	IBD + D
Paiva	0.48 **	0.05	-0.43 **	-0.06	-0.30 *	0.000	IBD + D
Pamplona	0.33 **	-0.44 **	-0.10	0.02	-0.09	0.002	IBD + D
Parreira	0.15 **	-0.40 **	-0.02	0.04	-0.05	0.001	IBD + D
Pereira	-0.05	0.04	-0.30 *	0.24 **	-0.20	0.019	I
Pimentel	0.35 **	-0.03	-0.43 **	0.02	-0.19	0.002	IBD + D
Pinheiro	-0.06	0.02	-0.33 *	0.33 **	-0.22	0.004	I
Pinto	-0.05	0.03	-0.35 *	0.31 **	-0.21	0.024	I
Pires	0.25 **	-0.32 *	-0.16	0.09	-0.14	0.019	DF
Ponte	0.48 **	-0.08	-0.48 **	-0.00	-0.20	0.000	IBD + D
Quadros	0.30 **	0.28 **	-0.47 **	-0.42 **	0.03	0.005	IBD + D
Raposo	0.20 **	-0.04	-0.33 **	0.03	-0.14	0.001	IBD + D
Rebelo	0.36 **	-0.01	-0.46 **	0.07	-0.23	0.001	DF
Rego	0.17 **	0.01	-0.34 **	0.03	-0.15	0.002	IBD + D
Resendes	0.20 *	0.32 **	-0.32 *	-0.02	-0.45 **	0.002	DF
Ricardo	0.02	0.21 **	-0.08	-0.03	-0.39 **	0.006	DF
Rocha	0.27 **	-0.41 **	-0.11	0.10	-0.13	0.004	DF
Rodrigues	0.04	0.04	-0.41 **	0.28 **	-0.22	0.011	DF
Rosa	0.28 **	-0.05	-0.20	-0.24	-0.07	0.009	IBD + D
Sampaio	0.17 *	0.03	-0.33 **	0.03	-0.17	0.046	IBD + D
Saraiva	0.10	0.10	-0.45 **	0.29 **	-0.31 *	0.002	DF
Sardinha	0.10 *	-0.06	-0.26 **	0.02	-0.09	0.022	IBD + D
Silveira	0.40 **	0.18 *	-0.41 **	-0.40 **	-0.05	0.003	IBD + D
Sousa	0.05	0.12	-0.29 *	0.18 *	-0.33 **	0.031	DF
Tavares	0.28 **	0.00	-0.44 **	0.12	-0.23	0.001	DF
Terra	0.40 **	-0.10	-0.24	-0.36 *	0.02	0.003	IBD + D
Teves	0.14 *	0.03	-0.27 **	-0.03	-0.15	0.027	IBD + D
Torres	0.56 **	-0.03	-0.53 **	-0.03	-0.24	0.000	IBD + D
Toste	0.32 **	-0.56 **	-0.04	0.07	-0.07	0.000	DF
Valadao	0.18 **	-0.46 **	-0.06	0.06 *	-0.00	0.000	IBD + D
Valerio	0.28 **	-0.11	-0.33 **	0.02	-0.14	0.012	IBD + D
Vaz	0.31 **	-0.53 **	-0.07	0.10	-0.08	0.000	DF
Ventura	0.18 *	0.04	-0.43 **	0.09	-0.16	0.017	DF
Viveiros	-0.03	-0.04	-0.19 **	0.04	-0.06	0.021	I

Distance Class (Km): 1 (0 – 49), 2 (49 – 195), 3 (195 – 269), 4 (269 – 309), 5 (309 – 605).
*=0.01< p ≤ 0.05; **=0.001< p ≤ 0.01
Classification: D (Depression); DF (Different); I (Intrusion); IBD+D (Isolation by Distance and Depression); IBD+DDP (Isolation by Distance and Double Depression); LDD (Long-Distance Differentiation).

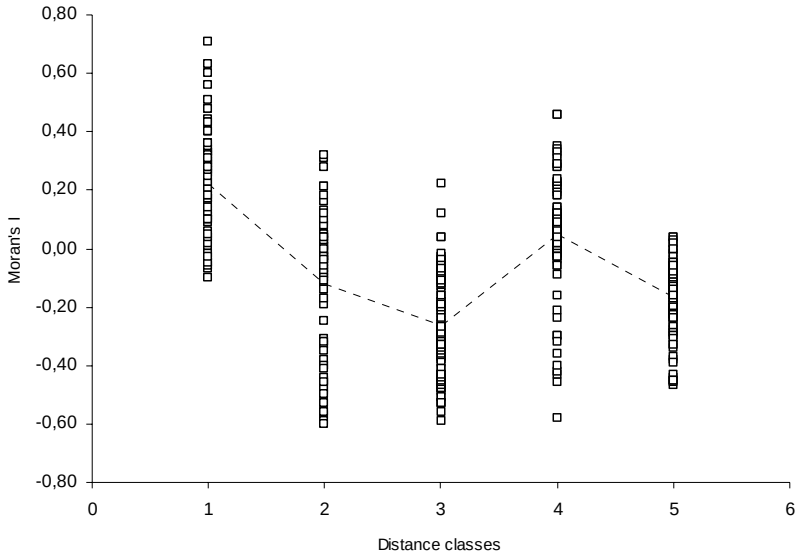


Figure V.7. Spatial correlogram of the 113 Bonferroni-significant correlograms of surname frequencies in Azores. The general trend of the Moran's *I* correlograms is shown by the dashed line connecting the mean autocorrelation coefficients for each distance class. Distance class (Km): 1 (0-49), 2 (49-195), 3 (195-269), 4 (269-309) and 5 (309-605). Note that individual variables within classes are not distinguishable.

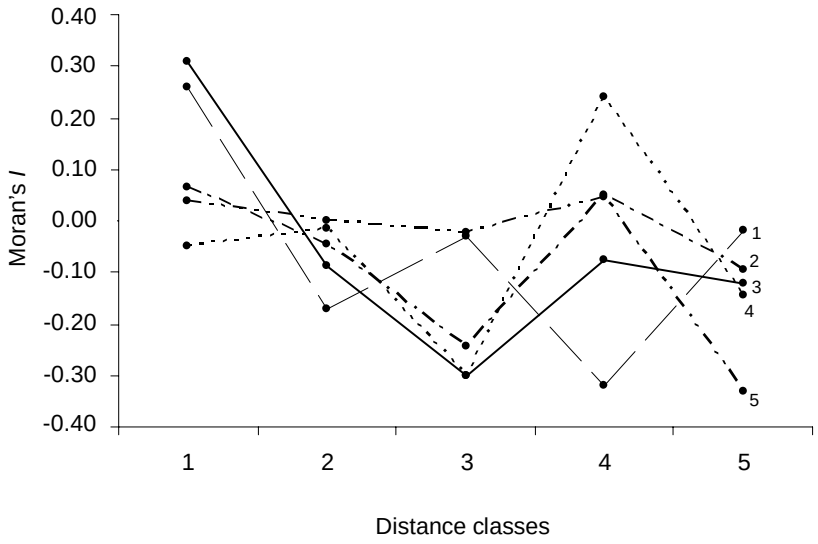


Figure V.8. Average correlograms representing the five patterns of Bonferroni significant *I* correlograms. The patterns are: 1-IBD+DDP, 2-D, 3-IBD+D, 4-I and 5-LDD.

V.3.5. Discussion

The spatial distribution model, proposed by Moran (1950), is a functional and easy method to understand the distribution of surnames. Here, we show that surname distribution can also be used to provide information on the population structure in the Azorean islands, where most of the existing surnames arrived with the first settlers. In Azores, the first 14 most frequent surnames correspond to 7% of the total population, and in Italy they correspond to 2% (Caravello *et al.* 2002). In Denmark, however, the first 14 most frequent surnames correspond to more than 50% of the total population (Caffarelli 1997).

The frequency of surnames of the 9 islands shows that São Miguel has the highest number of specific surnames. This data is compatible with the fact that São Miguel Island contains 54.4% of the Azores population. In contrast, Santa Maria, with a smaller population size than Pico and São Jorge, for example, presents 5 specific surnames. Moreover, as described in Branco and Mota-Vieira (2005), Santa Maria shows a high emigration rate. These data suggest that population size is not the major factor to be considered; instead, the dispersion patterns of individuals are important knowledge when studying the specificity of a given surname. This conclusion is corroborated by the spatial autocorrelation analysis. The major pattern obtained in the spatial analysis is IBD, which demonstrates dispersion by local movements of people over short distances (0-49 km) between close municipalities and close islands. Families with surnames Pacheco and Alvernaz correspond to the ones that moved, mainly, at short distances, because these two surnames have the highest value for Moran's *I* at distances inferior to 50 km. In addition, the spatial analysis also reflects the movement of people over great distances (269-309 km), suggesting migration to other islands (Figure V.7).

Migration flow and differential fertility may explain why some surnames have become more common and have spread over vast territories, whilst others are specific, or else, became extinct. Some examples of specific surnames in our dataset have a Spanish origin, like Escobar, Meneses, Rego and Vargas (Table V.6). This reinforces the contribution in the Azorean peopling of individuals of Spanish origin, which was

recently demonstrated by the presence of male Spanish lineages in the Azorean population (Pacheco *et al.* 2005).

Several spatial correlograms are similar and partitioned into patterns. Patterns characterized by a decline of autocorrelation in the first distance classes followed by insignificant values should be generated by the migration of people over short distances (Barbujani 1987; Sokal *et al.* 1992). This also may lead to the presence of specific surnames in the islands, as it is seen in our data. Numerous nearby inhabited centers in different municipalities (similarity in short distances – class 1) may account for the migration. The settlement proximity of the Central group with the Western group could have favored the movement of people carrying autochthonous surnames between these two groups (similarity at long distances – class 4). According to historical data (Guill 1993), Flores and Corvo were the last two islands discovered, and the first settlers arrived there were from mainland Portugal, and from the other islands of the archipelago, mainly Terceira. Moreover, the geographic and, consequently, socio-cultural features of the archipelago make easier the interaction between individuals from the Western with the Central group than with the Eastern.

A conflicting result is provided by Santos *et al.* (2003), who describe higher similarity between the Central and the Eastern groups based on mtDNA data. Recently, Montiel *et al.* (2005) reanalyzed these data in light of the Y-chromosome, and reveal that there are no differences between the three groups of islands when considered the mtDNA. However, when analyze the data concerning the Y-chromosome the author detected important differences, particularly on the Western group, which is the most differentiated in the PC analysis. These results corroborate with the results here obtained.

The results of the correlograms were interpreted considering how the actual population pairs within each distance class. The presence of the short distance positive autocorrelation may be explained by mating and migration patterns that are observed in all islands of the archipelago. In general, migration at marriage occurs between neighbouring village or country, and it is sufficiently strong to maintain family ties (Connel 1968; Pacheco *et al.* 2003). This type of migration explains the positive

autocorrelation within the first distance class observed in our dataset. Moreover, the observation that IBD is the most frequent pattern reinforces historical data, *i.e.* the peopling of Azores was a continuous process where people from the other islands contributed to the peopling of the last two islands (Flores and Corvo). On the other hand, the autocorrelation is positive in first distance class, validating the presence of specific surnames in each island. For example, São Miguel Island has the highest number of specific surnames and also has small distances between municipalities.

As surnames constitute quite a robust indicator of demographic changes, their analysis could greatly contribute to improve our knowledge of population genetic structure. Finally, the data described above show that migration and settlement history has been determinant for the spatial distribution of the present-day Azorean population.

"All men by nature desire to know".

Aristotle

CHAPTER VI

AZOREAN ANCESTRY

The Y-chromosomal heritage of the Azores Islands population

Published in Ann Hum Genet, 2005

Assessment of the Azorean ancestry by *Alu* insertion polymorphisms

Published in Am J Hum Biol, 2006

VI.1. The Y-chromosomal heritage of the Azores Islands population

VI.1.1. Summary

The Azores, a Portuguese archipelago located in the north Atlantic Ocean, had no native population when the Portuguese first arrived in the 15th century. The islands were populated mainly by Portuguese, but Jews, Moorish prisoners, African slaves, Flemish, French and Spaniards also contributed to the initial settlement. To understand the paternal origins and diversity of extant Azorean population, we typed genomic DNA samples from 172 individuals, using a combination of 10 Y-biallelic markers (YAP, SRY-1532, SRY-2627, 92R7, M9, sY81, Tat, SRY-8299, 12f2 and LLY22g) and the following Y-chromosomal STR systems: DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS385. We identified nine different haplogroups, most of which are frequent in Europe. Haplogroup J* is the second most frequent in Azores (13.4%), but it is modestly represented in mainland Portugal (6.8%). The other non-European haplogroups, N3 and E3a, which are prevalent in Asia and subSahara, respectively, have been found in Azores (0.6% and 1.2%, respectively) but not in mainland Portugal. Microsatellite data indicate that mean gene diversity (D) value for all the *loci* analysed in our sample set is 0.590, while haplotype diversity is 0.9994. Taken together, our analysis suggests that the current paternal pool of the Azorean population is, to a great extent, of Portuguese descent with significant contribution from people with other genetic backgrounds.

VI.1.2. Introduction

The Y-chromosome is a powerful tool to study human evolutionary pathways and to infer about major and local male migration movements or patterns (Jobling and Tyler-Smith 1995). The nonrecombining portion of the Y (NRY) retains a record of the mutational events that occurred along male lineages throughout evolution. Binary polymorphisms are particularly useful to identify stable paternal lineages, traced back in time over thousands of years, because of their low rate of parallel and back mutation

(Y-Chromosome Consortium 2002). The diversity within these lineages – haplogroups – can be examinated by polymorphisms that mutate more rapidly, such as, microsatellites, allowing the construction of very detailed Y-phylogenies that reveals male-specific aspects of genetic history (Qamar *et al.* 2002).

Here we report on the diversity of the Y-chromosome of Azorean individuals, using a combination of slowly evolving biallelic *loci* and rapidly evolving microsatellite *loci*. This allowed for an assessment of the relative diversity and phylogenetic context of the Azores Islands Y-chromosome pool. We aim to address the following questions: (*i*) how does the Y-chromosomal distribution in Azores fits in the context of other European populations, and (*ii*) how did geographical isolation affect Y-chromosomal distribution in Azores compared to mainland Portugal.

VI.1.3. Material and Methods

VI.1.3.1. Terminology and nomenclature

The terminology and nomenclature used here are those proposed by the Y-Chromosome Consortium (YCC NRY tree 2002). The terms "haplogroup" and "haplotype" are used according to de Knijff (2000).

VI.1.3.2. Population samples

The sample set comprised 172 unrelated healthy blood donors, from the anonymous DNA bank of São Miguel population, with signed informed consent (Mota-Vieira *et al.* 2005). The origin of the individual's father was used to sort the samples into: São Miguel (N=149), Faial (N=2), Flores (N=4), Pico (N=6), Santa Maria (N=3), São Jorge (N=2), Terceira (N=5) and Corvo (N=1), Figure VI.1. Due to disproportionate number of samples, we combined them all into a single group: Azores. Blood samples (7.5 ml) were collected by venipuncture into EDTA tubes. DNA was extracted using the PUREGENE® kit (Gentra Systems Inc.).

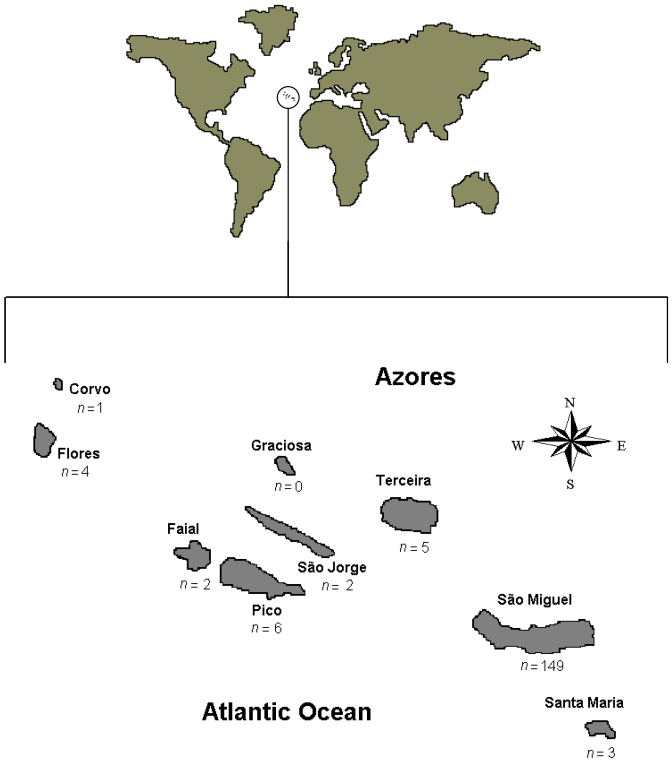


Figure VI.1. Geographic location of the Azores archipelago (*n*=number of individuals sampled). Map is not drawn to scale. The islands spread out in the area of the parallel that passes through Lisbon (39º,43'/39º,55', north latitude).

VI.1.3.3. PCR amplification of Y-SNPs and endonuclease digestion

A total of 10 Y-biallelic markers were selected based on the probability of their occurrence in the European populations (Rosser *et al.* 2000, and references therein). The base substitutions were as follows: 92R7 C→T; M9 C→G; SRY-2627 C→T; SRY-1532 A→G →A; sY81 A→G; SRY-8299 G→A; LLY22g C→A and Tat T→C. The LLY22g was typed using conditions kindly supplied by C. Tyler-Smith (personal communication). The 12f2 deletion was typed according to Rosser *et al.* (2000). Polymerase Chain Reaction (PCR) amplifications were carried out in a singleplex 20 µl reaction mixture including 1X PCR buffer, 2.5 mM $MgCl_2$, 0.1 mM dNTP mix, 1 µM of forward and reverse amplification primers, 1 U of *Taq* DNA polymerase (PROMEGA) and 40 ng of genomic DNA. PCR was carried out according to the following conditions: an initial denaturation step at 95ºC for 2 min, 30 cycles of 94ºC for 30 sec, 60ºC for 30

sec, 72ºC for 1 min, and a final extension step at 72ºC for 5 min For restriction fragment length polymorphism analysis, 1 U of the appropriate restriction enzyme in 2.5 µl of 1X digestion buffer was added directly to 25 µl of PCR reaction and incubated at the appropriate temperature for 2 hours. Digests were analysed by electrophoresis on polyacrilamide gels (12%) and visualized by ethidium bromide. Analysis of the Y-chromosomal *Alu* repeat insertion (YAP) was carried out by PCR and analysed by agarose gel electrophoresis, as described elsewhere (Hammer and Horai 1995).

VI.1.3.4. PCR amplification of Y-STRs

Seven microsatellite *loci* were typed using fluorescently labelled primers from five tetranucleotide markers (DYS389I, DYS389II, DYS390, DYS391 and DYS393), one trinucleotide repeat *locus* (DYS392), and one duplicated tetranucleotide repeat marker (DYS385). Primer sequences were obtained in the Y-STR haplotype database (www.ystr.org). The PCR protocol used is as follows: an initial denaturation at 95ºC for 15 min to activate HotStarTaq™DNA polymerase (QIAGEN); 30 cycles of 94ºC for 1 min, 51ºC for 1 min, 72ºC for 1 min, and a final 10 min extension step at 72ºC. Each 25 µl reaction contained 2 U of *Taq* DNA polymerase, 1X PCR buffer, 50 mM KCl, 4 mM $MgCl_2$, 0.25X Q Solution, 0.2 mM each of the four deoxyribonucleotide triphosphates, 0.4 µM of forward and reverse amplification primers and 50 ng of genomic DNA. An aliquot of 1 µl of each PCR product was combined with 0.5 µl CEQ™DNA size standard kit 400, 29 µl formamide deionized (Qbiogene), and run on a CEQ™8000 Genetic Analysis System (Beckman Coulter).

VI.1.3.5. Statistical analysis

Alleles are designated by the number of repeats. Since the DYS389II product contains the DYS389I, we subtracted the corresponding DYS389I repeat length from that of DYS389II, to avoid double-counting the variation at the DYS389I (Roewer *et al.* 1996). For DYS385, which is a duplicated Y-STR *locus*, the allele *locus* assignment was

performed so that for each individual, the shorter allele was assigned to one *locus* (DYS385a) and the longer to another (DYS385b).

Population differentiation between the Azores and other populations was assessed using haplogroup frequencies included in Arlequin software package (Schneider *et al.* 2000). Genetic distances, as pairwise F_{ST}, were represented in two-dimensional space using Multi Dimensional Scaling (MDS) analysis included in the SPSS software package (version 10.0).

VI.1.4. Results

VI.1.4.1. Y-chromosome biallelic polymorphisms

The biallelic *loci* used in this study divided Azorean Y-chromosomes into twelve clades, which are usually referred to as haplogroups (HGs). A Y-chromosomal HG tree with 10 biallelic markers and HG frequencies is shown in Figure VI.2. We identified 9 different HGs out of 12 possible, which indicates the degree of information of the markers selected. HG P*(xR1b8, R1a, Q3) is the most frequent, comprising 59.3% of the total sample. Interestingly, our data shows high frequency of lineage J*, the second most frequent HG in our population, comprising 13.4% of the Y-chromosomes. Lineages BR*(xB2b, CE, F1, H, JK), 11.6%, and E*(xE3), 10.5%, are both frequent in Azores. Lineage R1a has a frequency of 1.2%, four times higher than that described for the northern and southern Portuguese populations (0.3%; Rosser *et al.* 2000). In Azores, R1b8 accounts for 0.6% of the Y-chromosomes. Albeit at low frequency (1.2%), we have also detected the subSaharan HG E3a (Figure VI.2). In addition, lineage N3, which is primarily found in Asians, is present in Azores at a frequency of 0.6%.

In order to test the hypothesis of a random distribution of HGs among population groups, we computed F_{ST} values using HG frequencies as implemented by the Arlequin. HG frequency data for northern and southern Portuguese, Spanish, Basque, east Anglian, Belgian, French, Dutch, Bavarian, German, Sardinian, Italian, Turkish, Greek, Algerian, Canarians, Caboverdean and northern African were retrieved from Rosser *et*

al. (2000), Flores *et al.* (2003) and Gonçalves *et al.* (2003). Population differentiation between the Azores and those listed above was calculated. No significant difference was observed between the Azoreans and the northern and southern Portuguese, Belgian, French or Italian samples (p=0.05), suggesting no population differentiation. In contrast, comparison with the remaining populations reveals a significant difference (p<0.05). These data corroborates with the analysis of pairwise genetic distances in the two-dimensional space analysis (Figure VI.3). Noteworthy, MDS revealed that genetic relationship among populations corresponds tightly to their relative geographical distances.

VI.1.4.2. Y-chromosome STR polymorphisms

A Y-chromosomal haplotype was constructed for each individual, using seven *loci* (see Material and Methods). Overall, 118 different haplotypes were observed in the 172 sample set (68.6% discriminatory capacity). Haplotype diversity is high (0.9994), due to high variability of Y-STRs. Allele frequencies and gene diversity values are listed in Table VI.1. The mean gene diversity (D) value for the *loci* is 0.590 (values range from 0.4592 to 0.8212, Table VI.1).

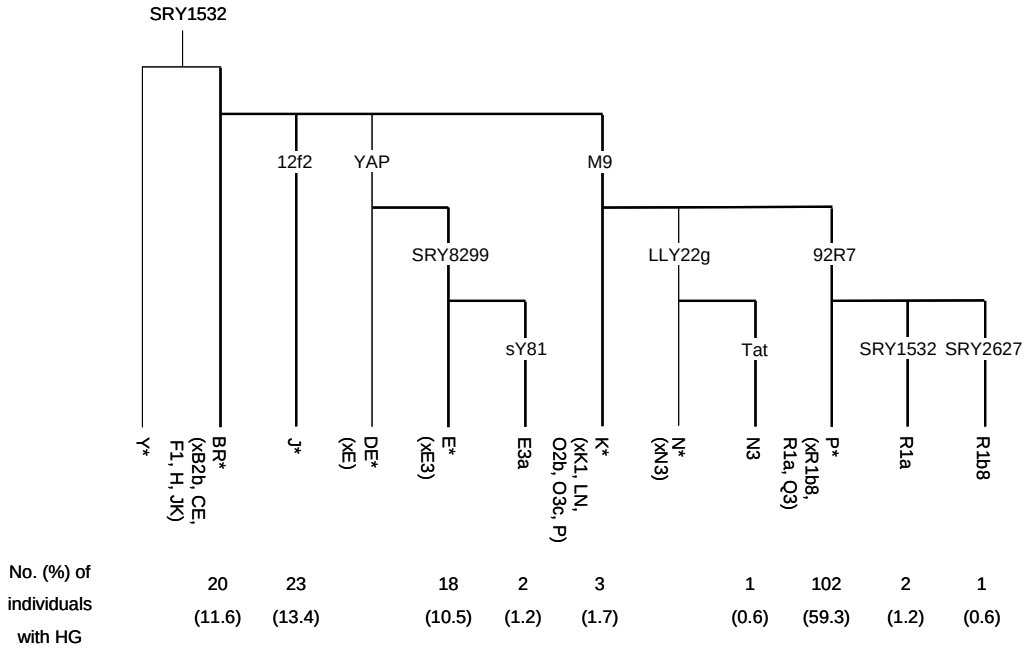


Figure VI.2. Phylogenetic tree of the Y-chromosome haplogroups and their percent frequencies in the Azorean sample. Bold lines indicates HG present in the Azorean population.

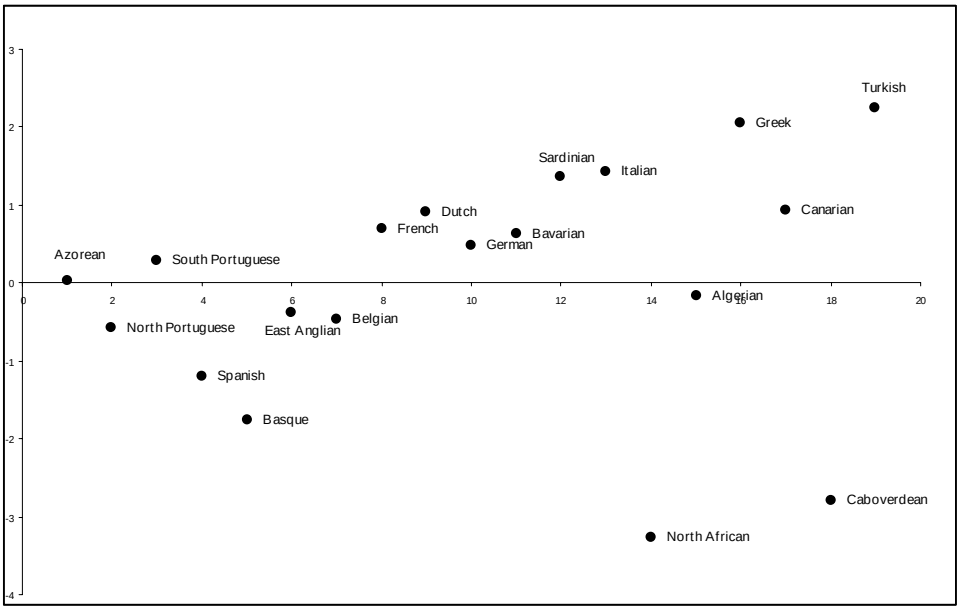


Figure VI.3. Multidimensional scaling of genetic relationships between populations based on Y-STRs. Note the position of the African samples that reflects the major division between the populations.

Table VI.1. Allele frequencies and gene diversity value at 7 Y-chromosome STR *loci* in Azorean population (*h*=Gene diversity, D=mean gene diversity).

Allele	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	Haplotype	DYS385
9				0.0640			9-14	0.0058
10				0.4419		0.0116	9-15	0.0058
11				0.4535	0.3605		10-14	0.0116
12	0.1395			0.0407	0.0058	0.1744	11-11	0.0116
13	0.6395				0.5581	0.7093	11-12	0.0058
14	0.2093	0.0058			0.0756	0.0988	11-13	0.0407
15	0.0116	0.0523				0.0058	11-14	0.4012
16		0.6453					11-15	0.0872
17		0.2326					12-12	0.0233
18		0.0523					12-13	0.0116
19		0.0116					12-14	0.0291
20							12-15	0.0349
21			0.0291				12-16	0.0058
22			0.0756				12-17	0.0058
23			0.3081				12-19	0.0058
24			0.5058				13-13	0.0291
25			0.0640				13-14	0.0349
26			0.0116				13-15	0.0174
27			0.0058				13-16	0.0465
							13-17	0.0291
							13-18	0.0058
							14-14	0.0349
							14-15	0.0174
							15-15	0.0058
							16-16	0.0523
							16-17	0.0058
							16-19	0.0058
							17-17	0.0116
							17-18	0.0174
h	0.5307	0.5269	0.6421	0.5968	0.5560	0.4592		0.8212
D=0.590								

To investigate the separation of recently diverged populations, we performed a *locus* by *locus* analysis between the Azorean population and those we assumed to be the closest, e.g. the Madeirans (Fernandes *et al.* 2001), central Portuguese (Carvalho *et al.* 2000)

and northern mainland Portuguese (Gonzalez-Neira *et al.* 2000), using microsatellite analysis. Pairwise F_{ST} showed no statistical differences ($p<0.05$) to DYS389II, DYS391 and DYS393 *loci*. However, excepting DYS389II *locus*, the other *loci* show statistical difference ($p<0.05$) between the Azoreans and the central mainland Portuguese. The comparison of Azoreans and northern Portuguese show that the difference is found only at the DYS390 *locus*. Taken together, the data suggests no genetic differentiation between northern Portuguese, Madeirans and Azoreans.

VI.1.4.3. Y-chromosome STR polymorphism within haplogroups

When combining the SNPs with the STRs the number of haplotypes increased from 118 (STRs alone) to 123 (SNPs and STRs) and the discriminatory capacity raised from 68.6% to 71.5% (Table VI.2). The most common haplotypes were found on a P*(xR1b8, R1a, Q3) background. Haplotype H7 (13-16-24-10-13-13-11/14) occurred 10 times (5.8%), H6 (13-16-24-11-13-13-11/14) accounted for 9 individuals (5.2%) and the third most frequent haplotype, H15 (13-16-23-11-13-13-11/14), was found 6 times (3.5%). Of the 172 males there were 98 unique haplotypes (56.9%).

The two most common haplotypes in Azores, H7 (5.8%) and H6 (5.2%), are represented in the YHRD – Y-Chromosome Haplotype Reference Database at 1.49% and 3.42%, respectively. As of July 2004, this database contains 15,545 haplotypes from 114 different European regions. Haplotype 13-16-24-11-13-13-11/14 is recorded at 3.42% in the European database, but at only 0.58% (H79) in Azores. In addition, our data show low frequency (17.4%) of population-specific haplotypes. Of the remaining 82.6% nonunique haplotypes, the majority are shared with the mainland Portuguese and Madeirans (51.2%), Germans (64.5%), Spanish (56.3%) and Italians (50%). High numbers of nonunique haplotypes and consequent haplotype sharing indicate a close relationship between populations (Kayser *et al.* 2001). Two haplotypes were shared by two different HG backgrounds (Table VI.2), one between P*(xR1b8, R1a, Q3) and J*, and another between BR*(xB2b, CE, F1, H, JK) and E*(xE3). The presence of identical

Y-chromosome STR haplotypes found on different SNP HGs is evidence of recurrent mutations, likely to occur at STR *loci*.

VI.1.5. Discussion

VI.1.5.1. Prevalent Y-chromosome lineages in Azores Islands

The non-random distribution of distinctive stable HGs provides patterns of genetic affinity and clues concerning past human movements. Here we investigated the genetic background of the male Azorean population, and discussed the results under the light of existing historical records.

HG J*, defined by the 12f2 deletion, is largely confined to Caucasoid populations, with its highest frequencies being found in Middle eastern populations. It is thought to have originated in the Middle east where its frequency exceeds one-third of the Y-chromosomes of Jewish, Turkish and Arab populations (Bosh *et al.* 2001; Nebel *et al.* 2001). Our data shows that in Azores this haplogroup is the second most common, with a frequency of 13.4%, twice as high as in mainland Portugal (6.8%; Rosser *et al.* 2000). Using a sampling strategy based on the three geographical groups of the Azores Islands, Montiel and colleagues (2005) found lineage J at a lower frequency (8.6%) for the whole archipelago, although their study revealed similar frequency (14.5%) for the islands of the Central group. The high frequency of lineage J raises the question of whether Jewish early settlers left a significant imprint in the genetic pool of the Azorean male population. The overall northwest (NW) African contribution to the Iberian Y-chromosome pool has been calculated as 7%, with the highest level of contribution (14%) being found in Andalusians, southern Iberia (Bosch *et al.* 2001), a result that is consistent with the population movement associated with Islamic rule in Iberia (Pereira *et al.* 2000). The frequency of the NW African lineage E*(xE3) in mainland Portugal and Azores (11.7% and 10.5%, respectively) is similar.

Table VI.2. Frequencies of Y-chromosome haplotypes by haplogroup in the Azorean population.

Haplogroup	H	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS385	Frequency
P*(xR1b8,R1a,Q3)	1	12	16	24	10	13	13	11-15	1
N=102	2	12	16	24	11	13	13	12-14	1
GD=0.9986	3	13	16	23	11	13	13	12-13	1
	4	13	16	23	10	13	13	12-14	2
	5	13	16	24	11	13	14	11-13	2
	6	13	16	24	11	13	13	11-14	9
	7	13	16	24	10	13	13	11-14	10
	8	13	16	24	10	14	13	11-14	1
	9	13	16	24	11	13	13	12-15	1
	10	13	16	24	10	14	12	11-14	1
	11	13	17	23	11	13	13	12-14	1
	12	14	16	25	11	13	13	11-15	1
	13	13	16	23	11	13	12	13-16	1
	14	13	16	23	12	13	13	11-14	1
	15	13	16	23	11	13	13	11-14	6
	16	13	16	24	11	13	13	9-14	1
	17	13	17	23	11	13	13	11-13	1
	18	13	17	23	10	13	13	11-14	1
	19	13	16	24	11	13	13	9-15	1
	20	13	16	22	11	13	13	11-14	1
	21	14	16	23	11	13	13	11-14	3
	22	13	16	24	11	13	12	11-15	1
	23	12	14	24	12	14	13	11-14	1
	24	13	16	24	11	13	12	11-14	2
	25	14	16	24	11	14	13	11-15	2
	26	12	17	24	11	14	13	11-14	1
	27	13	17	24	11	13	14	11-15	1
	28	13	17	24	10	13	14	11-15	1
	29	13	16	24	11	13	13	11-15	2
	30	14	15	24	10	13	12	11-11	1
	31	13	16	25	10	13	13	13-13	1
	32	13	17	24	11	13	14	11-14	1
	33	13	18	23	11	13	13	11-14	1
	34	13	15	24	11	13	13	11-14	1
	35	13	16	23	11	14	13	11-14	2
	36	14	16	24	11	13	12	11-14	1
	37	14	15	24	10	13	13	11-14	3
	38	12	17	24	12	13	13	11-14	1
	39	13	17	24	11	13	13	11-15	1
	40	13	16	24	11	13	14	11-14	3
	41	13	16	23	10	14	13	11-15	1
	42	13	17	24	11	13	13	11-12	1
	43	13	17	23	11	13	14	11-15	1
	44	14	16	24	11	14	13	11-14	1
	45	12	16	24	11	13	13	11-14	2
	46	12	17	24	10	13	13	11-13	1
	47	13	16	24	10	13	13	11-11	1
	48	13	16	24	10	13	14	11-14	1
	49	14	16	25	11	13	13	11-14	1
	50	13	16	23	11	13	13	12-14	1
	51	14	16	24	11	13	13	11-14	3
	52	14	17	23	11	13	13	11-14	1
	53	14	16	24	11	13	13	10-14	2
	54	14	16	24	11	13	13	11-15	1
	55	13	17	24	12	13	13	11-15	1
	56	13	16	25	11	13	13	11-14	1
	57	14	15	24	11	14	12	13-14	1
	58	14	15	25	10	13	13	11-14	1
	59	15	16	25	10	13	13	11-15	1
	60	13	17	25	11	11	13	11-14	1
	61	13	16	23	12	13	12	12-15	1
	62	13	16	23	11	13	12	11-14	1
	63	13	16	24	12	13	13	11-14	1
	64	13	17	24	10	13	13	11-14	1

Table VI.2. Continuation.

Haplogroup	H	DYS389I	DYS389II	DYS390	DYS391	DYS392	DYS393	DYS385	Frequency
J*	65	13	16	26	10	11	12	13-13	2
N=23	66	13	17	23	10	11	12	13-18	1
GD=0.9872	67	13	15	23	10	11	13	12-12	1
	68	13	16	22	10	11	13	12-19	1
	69	12	16	24	10	11	12	12-17	1
	70	13	16	27	10	11	12	13-13	1
	71	13	16	25	9	11	12	13-17	1
	72	13	16	22	9	11	12	13-17	1
	73	13	16	23	10	11	12	13-16	2
	74	13	18	25	10	11	12	14-14	1
	75	13	16	24	10	13	14	11-14	1
	76	13	18	23	11	11	12	13-16	1
	77	14	16	23	10	11	12	14-14	1
	78	13	16	23	9	11	12	13-16	2
	79	13	16	24	11	13	13	11-14	1
	80	14	17	23	10	11	12	13-15	1
	81	13	16	25	11	11	13	12-16	1
	82	13	16	23	9	11	12	14-15	1
	83	13	17	23	10	11	12	13-17	1
	84	13	17	24	10	11	12	13-17	1
BR*(xB2b,CE,F1,H,JK)	85	12	17	22	10	11	14	14-15	1
N=20	86	12	16	23	10	11	13	12-13	1
GD=0.9842	87	15	15	23	10	11	13	12-12	1
	88	12	16	22	10	11	13	13-15	1
	89	12	16	22	10	11	13	13-14	2
	90	12	16	22	10	11	12	13-14	1
	91	13	16	23	10	13	14	14-15	1
	92	12	16	22	10	11	13	12-15	2
	93	14	18	22	10	11	14	13-13	1
	94	12	16	21	11	11	14	11-13	1
	95	12	16	23	10	11	13	13-15	1
	96	12	17	23	10	11	13	14-14	1
	97	12	17	21	10	11	15	13-17	1
	98	12	18	24	10	11	13	16-16	1
	99	14	16	23	10	11	13	12-12	2
	100	12	17	22	12	11	13	13-14	1
	101	13	16	23	11	12	14	15-15	1
E*(xE3)	102	14	17	21	9	11	13	12-15	1
N=18	103	13	17	22	9	11	13	12-15	1
GD=0.9815	104	13	17	24	10	11	13	17-18	3
	105	13	17	23	10	11	13	16-16	1
	106	13	19	24	10	11	13	16-16	2
	107	14	16	24	9	11	13	13-14	1
	108	13	16	23	9	11	13	14-14	1
	109	14	16	24	9	11	13	14-14	2
	110	13	18	24	10	11	13	16-17	1
	111	13	18	23	10	11	14	17-17	1
	112	14	17	24	11	11	13	16-16	1
	113	14	17	21	10	11	13	16-16	1
	114	13	17	23	11	11	13	16-19	1
	115	12	18	24	10	11	13	16-16	1
K*(xK1,LN,O2b,O3c,P)	116	13	17	23	10	13	10	12-16	2
N=3	117	14	18	23	10	14	12	17-17	1
GD=0.7278									
R1a	118	13	17	24	11	11	13	11-14	1
N=2	119	13	17	25	11	11	13	11-14	1
GD=0.5000									
E3a	120	14	17	21	11	11	13	16-16	1
N=2	121	13	17	24	10	11	13	16-16	1
GD=0.5000									
N3	122	14	16	23	11	14	13	11-13	1
R1b8	123	13	17	24	10	13	13	11-14	1

GD=genotype diversity,
H=Haplotype

Montiel and colleagues (2005) also found comparable values (13.0%) for the archipelago. The results obtained by us and the other group suggest several hypotheses for the presence of this lineage in the present-day population of Azores: a direct input of Moorish prisoners, the influence of early Portuguese settlers, or a contribution of both Moorish prisoners and Portuguese.

Lineage E3a, defined by mutation sY81, shows a subSaharan distribution pattern. This HG is the most frequent in west African populations, and their presence can be interpreted as resulting from subSaharan gene flow. The occurrence of lineage E3a in Azores is the result of African influence, since it has been detected neither in Europe, nor in Iberian samples (Semino *et al.* 2000; Bosh *et al.* 2001; Rosser *et al.* 2000). The presence of subSaharan African slaves in the archipelago since the beginning of the settlement is well documented (Matos 1989). Therefore, we conclude that the 1.2% Y-chromosomes with the E3a background represents the male descendants of black slaves from Guinea, Cape Verde and São Tomé.

Lineage N3, defined by Tat biallelic polymorphism, is specific to Asians and northern Europeans and has not been found in Iberian Peninsula or in other European countries (Rosser *et al.* 2000; Helgason *et al.* 2000). This mutation probably arose in the Mongolia/ China area, and the present distribution stretches from Japan to Norway (Zerjal *et al.* 1997). The presence of this lineage in Azores (0.6%) is intriguing. Historical records of the presence of Asians or Mongolians in the archipelago are not known, but Bruges-Armas and colleagues (1999) have recently described the presence of Mongolian HLA genes at a high frequency in Terceira Island population (Azores). Thus, it is possible that the presence of Lineage N3 may have been introduced at the expansion of the trade navigation between Europe, America and Asia, during the 16th and 17th centuries, when the Azores had a strategic role due to its geographic position (Russel-Wood 1998).

Lineage R1b8, defined by a C→T base substitution at the SRY-2627, arose recently in Iberia. This lineage has its highest frequency in Basques (11%) and Catalans (22%), whereas in other regions these chromosomes are rare or absent (Hurles *et al.* 1999). In Azores, its frequency is marginal (0.6%), probably reflecting the descendants of the

Spaniards, who came to the islands during the reign of Spain over Portugal, from 1580 to 1640 (Matos 1989).

Lineage R1a is most frequent in central eastern Europe, comprising approximately half of the chromosomes in the Russian, Polish and Slovakian samples. In contrast, frequencies in the southeast and southwest Europe are low. In our sample set, R1a is four times higher then in mainland Portugal (Rosser *et al.* 2000), which may be explained by the following reasons: (*i*) this chromosome arrived with Portuguese settlers only, and subsequently increased in frequency, (*ii*) some chromosomes came in with Portuguese settlers, while others came in directly from central eastern Europeans, and (*iii*) they are an exclusive contribution from central eastern Europe. Historical records and papers exploring historical settlement show that some Europeans (e.g. Flemish) contributed to the peopling of the Azores, so we believe that all the hypotheses above are possible.

VI.1.5.2. Variability of Y-chromosome STRs in Azores Islands

Comparisons of allelic frequencies between our sample set and those obtained in central mainland Portugal (Carvalho *et al.* 2000) show differences. Indeed, historical records demonstrate that the first Portuguese settlers were mainly from the north and south Portugal. The mean gene diversity value across *loci* in the Azorean sample (D=0.590) is higher than the value reported for northern Portugal (D=0.517), from which Azoreans are believed to be partially derived (Guill 1993). It is also higher than that observed for the Europeans (D=0.503). Likewise, haplotype diversity value in Azores (0.9994) is higher than in northern Portugal (0.980) and Europe (0.985). Unexpectedly, the Azoreans share more haplotypes with the Germans than with the Portuguese, but due to a relative high mutation frequency of Y-STRs, Y-haplotypes can be shared identical by state that are not identical by descent (de Knijff 2000). However, we conclude that the diversity found in Azorean Y-chromosome is derived from the admixture of Portuguese with other populations.

One advantage of Y-chromosome markers, compared with mtDNA, is that Y-chromosome polymorphisms seem to show higher degree of population specificity (Seielstad *et al.* 1998), making them more informative for tracing population relationships. The comparison between the paternal Y-chromosome (present study and Montiel *et al.* 2005) and the maternal mtDNA (Santos *et al.* 2003) shows some evidences of differential sex-specific influences. Here, the paternal Middle east influence was estimated at 13.4%, which is higher than the 7.5% obtained by Santos and colleagues (2003). Another difference was the smaller contribution from Africans. We estimated a clear African Y-chromosome contribution of 1.2%, whereas they identify an 11.3% contribution of African mtDNA. The Y-chromosome and mtDNA results are, in general, concordant; they both indicate the same history for the peopling of the Azores and suggest that there was some gender differentiation in the population pathways.

VI.1.6. Concluding remarks

The presence of HGs of widespread distribution in Europe, in combination with others of clear subSaharan, Asian and Middle east origin reflects the diverse patterns defining the extant Azorean Y-chromosome pool. We conclude that the current paternal Y-chromosome pool in the Azores is of Portuguese descent, with a considerable contribution of individuals from multiple origins.

VI.2. Assessment of the Azorean ancestry by *Alu* insertion polymorphisms

VI.2.1. Summary

The knowledge of the population ancestry from genetic markers is essential, for example, to understand the history of human migration and to carry out admixture and association studies. Here we assess the genome ancestry of the Azorean population through the analysis of six *Alu* polymorphic sites (TPA-25, ACE, APO, B65, PV92 and D1) in 65 Azoreans and 30 mainland Portuguese unrelated blood donors and compare the data with those obtained by Y-chomosome and mtDNA. Allele frequencies were calculated by direct counting. Statistical analysis was performed using Arlequin 2.0. Nei's genetic distance was calculated with DISPAN software, and trees were constructed by Neighbor-Joining (NJ) using PHYLIP 3.63. The results show that all *Alu* insertions were polymorphic. APO is the closest to fixation. The less frequent insertions are PV92 and D1 in Azores and mainland Portugal, respectively. ACE and TPA-25 show the highest values of heterozygosity in both populations. Allele frequencies are very similar to those obtained in European populations. These results are validated by the Y-chromosome and mtDNA data, where the European represent the majority of the maternal and paternal lineages. Overall, these data are reflected in the phylogenetic tree, in which Azores and Portugal branch with Catalans, Andalusians, Morrocans and Algerians. We conclude that the Azores shows no significant genetic differences from mainland Portugal and is an outbred population. Moreover, the data validate the use of *Alu* insertion polymorphisms to assess the origin and history of human populations.

VI.2.2. Introduction

Y-chromosome and mtDNA have been extensively used to characterize populations in terms of diversity and origin. However, the full picture of the histories of populations requires studies of markers in the recombining parts of the nuclear DNA, namely the autosomes (Kidd *et al.* 2000). Polymorphic *Alu* insertions represent an important source of nuclear genetic variability and their use is advantageous, once, they are: identical by

descent, widely dispersed throughout the human genome, subject to very limited amounts of gene conversion, rapidly and easily genotyped, and selectively neutral when located in noncoding regions (Batzer *et al.* 1996; Comas *et al.* 2000).

Recently, studies on Y-chromosome lineages (Pacheco *et al.* 2005; Montiel *et al.* 2005) and mtDNA (Santos *et al.* 2003) in the Azores population demonstrated that the current paternal pool of the Azoreans is of Portuguese descent with significant contribution from people with other genetic background. Our main purpose is to compare the results from Y-chromosome and mtDNA with those obtained here through the study of the genetic diversity and ancestry of the Azores population using six *Alu* insertions. Moreover, we intend to assess the genetic differentiation between Azores and mainland Portugal.

VI.2.3. Material and Methods

VI.2.3.1. Population samples

The sample set, comprising 65 Azoreans and 30 mainland Portuguese unrelated blood donors, were obtained from a biobank constructed according to International ethical guidelines (Mota-Vieira *et al.* 2005).

VI.2.3.2. Alu genotyping

Six human-specific *Alu* insertion polymorphisms (B65, ACE, D1, APO, PV92 and TPA25) were studied, using sets of oligonucleotide primer-pairs described previously (Batzer *et al.* 1996). Polymerase Chain Reaction (PCR) amplifications were carried out in a singleplex 15 µl reaction mixture including 1X PCR buffer, 2.5 mM $MgCl_2$, 0.8 mM dNTP mix (0.2 mM each), 10 µM of forward and reverse primers, 2U of *HotStarTaq* DNA polymerase (QIAGEN) and 50 ng of genomic DNA. PCR conditions for B65, D1, PV92 and APO were as follows: (1X) 95ºC/ 15 min; (30X) 94ºC/ 1 min, optimal annealing temperature for 2 min, 72ºC/ 1 min; with a final extension step at

72ºC/ 10 min. Annealing time for TPA-25 and ACE markers was only 1 min. PCR products were visualized by electrophoresis in 4% agarose gel stained with Syber Green (Molecular Probes).

VI.2.3.3. Statistical analysis

We selected previously published data (Romualdi *et al.* 2002; Comas *et al.* 2000) on 17 populations, namely: African American, Armenian, Bantu Speakers, Bretons, Darginian, European-American, French, German, Greek, Hungarian, Swiss, Syrians, Turks, Catalans, Andalusians, Moroccans and Algerians to perform population comparisons. The selection was based on the historical data for the Azorean peopling and the geographical location of the populations.

Allele frequencies were calculated by direct counting and Hardy-Weinberg equilibrium was assessed by an exact test provided by the Arlequin program 2.0 (Schneider *et al.* 1996). The inbreeding coefficient, F_{IS}, was calculated by Genetic Data Analysis (GDA) software package (Lewis and Zaykin 2000). Statistical significance of genic and genetic differentiation between *loci* and populations was estimated by the GENEPOP[32] web version program (Raymond and Rousset 1995).

F_{ST} genetic distances were computed between pairs of populations by means of the DISPAN software (Ota 1993) and the distance matrix was used to construct a Neighbor-Joining (NJ) tree with PHYLIP 3.63 (Felsenstein 1993). The NJ tree was rooted by setting the frequency of each insertion to zero (ancestral), as previously described (Batzer *et al.* 1996). We used TreeView 1.6.6 (Page 1996) to display tree phylogenies obtained by Neighbor-Joining.

[32] GENEPOP web version, http://genepop.curtin.edu.au.

VI.2.4. Results and Discussion

The frequency distribution of six *Alu* polymorphisms was determined in a sample set comprising 95 individuals from Azores and mainland Portugal (Table VI.3). All *Alu* insertions were polymorphic in both populations, being APO the closest to fixation. The less frequent insertion is PV92 and D1 in Azores and Portugal, respectively. ACE and TPA-25 show the highest values of heterozygosity in both populations[33]. The data also show that all markers were in Hardy-Weinberg equilibrium.

We observe a wide range of *Alu* insertion frequencies, from 0.208 (PV92) to 0.946 (APO; Table VI.3). Nevertheless, when we focus on genetic differentiation between populations, which gives us the differences in the genotypic distribution *locus* by *locus*, the estimation using GENEPOP program shows no significant differences between the Azores and mainland Portugal.

The inbreeding coefficient, F_{IS}, represents a measure of reduction in the genetic variability of a population. We observe that Azores shows a higher value when compared to Portugal. However, the difference is not statistically significant (p=0.069).

Table VI.3. *Alu* insertion frequencies, heterozygosity and gene diversity for Azores and mainland Portugal.

		Alu insertion polymorphism					
Population	N	TPA-25	ACE	APO	PV92	D1	B65
Azores	65						
Frequency		0.592	0.385	0.946	0.208	0.254	0.585
Heterozygosity		0.424	0.485	0.106	0.257	0.348	0.409
HW (*p value*)		0.323	1.000	1.000	0.033	0.524	0.211
Locus diversity		0.493	0.481	0.117	0.343	0.392	0.493
Av. gene diversity		0.383 +/- 0.233					
F_{IS}		0.117					
Portugal	30						
Frequency		0.600	0.367	0.917	0.283	0.233	0.500
Heterozygosity		0.517	0.483	0.034	0.345	0.275	0.483
HW (*p value*)		0.665	1.000	0.073	1.000	0.453	1.000
Locus diversity		0.496	0.480	0.128	0.404	0.370	0.517
Av. gene diversity		0.392 +/- 0.240					
F_{IS}		0.094					

[33] To have a greater dispersion of these results, the *Alu* frequencies were registered in the ALFRED database (Rajeevan *et al.* 2003, http://alfred.med.yale.edu/alfred/index.asp).

Moreover, there are no significant differences in gene diversity and heterozygosity between Azores and Portugal. This indicates that both populations are outbred and no deviation from equilibrium is present.

In order to assess the relationship between the two populations analysed in the present study, and compare them with other worldwide populations previously reported (Romualdi *et al.* 2002; Comas *et al.* 2000), F_{ST} genetic distances were calculated and depicted in a NJ tree (Figure VI.4). The tree topology clearly sets Azores and Portugal far from the hypothetical ancestral population, which is closer to African-Americans and Bantu speakers. In addition, we observe the proximity of the Azores and Portugal to other European and north African populations. These results are confirmed by Y-chromosome and mtDNA studies (Pacheco *et al.* 2005; Santos *et al.* 2003), where a mixed composition of European and African haplogroups is evidenciated. For example, we identified 59.3% of European and 10.5% of northwest African paternal lineages in the genetic pool of Azores. However, since a NJ tree imposes a bifurcating model onto a distance matrix, which may be inadequate for closely related populations, such as, Azores and Portugal, we also performed a PC analysis based on the *Alu* frequencies (Figure VI.4). The first and second PC accounts for 88.8% and 5.9% of the genetic variance observed, respectively, and their plot shows a similar pattern to that shown in the NJ tree. As all populations display close proximity in the PC analysis, we performed an AMOVA analysis. As expected, only 0.04% accounts for variation among groups.

Overall, the genetic relationships by means of NJ tree and PC analysis reveal that Azores is closely related to mainland Portugal. Both maternal (Santos *et al.* 2003) and paternal (Pacheco *et al.* 2005) studies demonstrate that mainland Portuguese were the main contributors to the peopling of Azores. The data presented here support this conclusion. However, the comparisons between Azorean Y-chromosome and mtDNA show some evidence of differential sex-specific influences (Montiel *et al.* 2005), which was not detected by our data based on autosomal *Alu* polymorphisms.

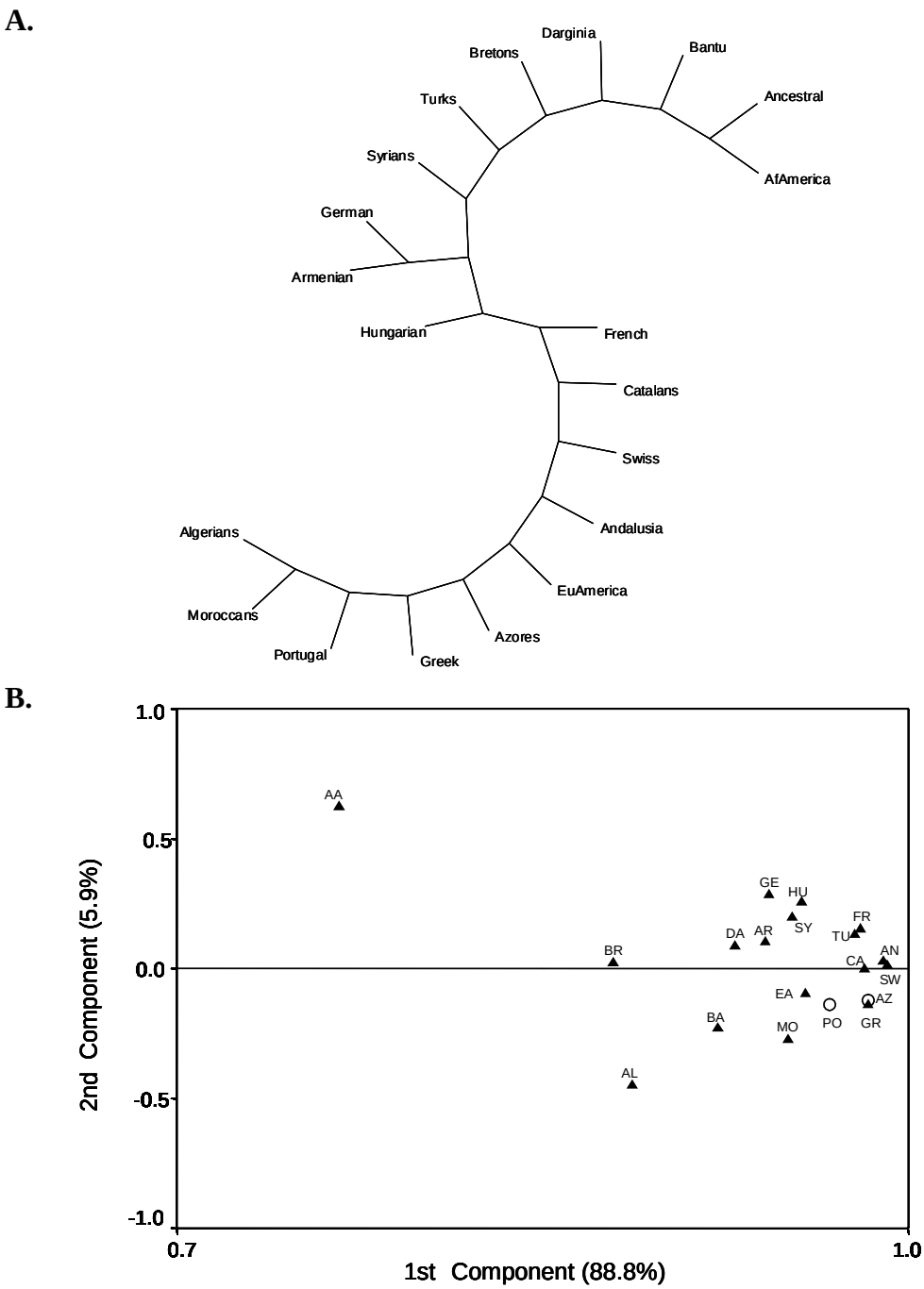


Figure VI.4. Population relationships based on six *Alu* markers. **A.** Neighbor-Joining tree using F_{ST} genetic distances. The following populations, AfAmerica, EuAmerica and Darginia represent African Americans, European Americans and Darginians, respectively. **B.** Principal component analysis based on allele frequencies. AZ, Azores; PO, Portugal; AA, African American; AR, Armenian; BA, Bantu Speakers; BR, Bretons; DA, Darginian; EA, European American; FR, French; GE, German; GR, Greek Cypriot; HU, Hungarian; SW, Swiss; SY, Syrians; TU, Turk Cypriot; CA, Catalans; AN, Andalusians; MO, Moroccans; AL, Algerians.

VI.2.5. Concluding remarks

Alu insertions are widely distributed throughout the human genome, constituting convenient markers to assess genetic diversity between human populations. Here we show that *Alu* frequencies in Azores and mainland Portugal are very similar to other European regions. Despite being geographically isolated, the Azores show no genetic differentiation when compared to mainland Portugal, which may only be explained by its recent historic settlement (~500 years). Moreover, the results here presented reveal that Azores is an outbred population with high genetic diversity. In summary, our data also support the use of *Alu* insertion polymorphisms to assess the origin and history of populations.

"A journey of a thousand miles begins with a single step."

Confucius

CHAPTER VII

AZOREAN GENETIC DIVERSITY AND STRUCTURE

Genetic signature of the São Miguel Island population (Azores) assessed by 21 microsatellite *loci*

Published in Am J Hum Biol, 2007

Azores islands: genetic origin, gene flow and diversity patterns

2007 submitted

Evaluation of linkage disequilibrium on the Xq13.3 region: comparison between the Azores Islands and mainland Portugal

Published in Am J Hum Biol, 2007

Linkage disequilibrium on Xq13.3, NRY and HLA regions in São Miguel Island (Azores) population

2007 submitted

VII.1. Genetic signature of the São Miguel Island population (Azores) assessed by 21 microsatellite *loci*

VII.1.1. Summary

To study the genetic diversity of São Miguel's population we compared 21 microsatellite *loci* in 204 individuals from São Miguel Island and 103 individuals from mainland Portugal. The results show that São Miguel and mainland Portugal populations have an average gene diversity of 0.767 and 0.765, respectively. Allele frequencies of all markers are comparable to other European populations. This result is corroborated by the genetic relationships analysis based on the NJ tree and principal component, where São Miguel, and probably, Azores is closely related to mainland Portugal. Overall, the data suggests that São Miguel population does not show population structure and is behaving as an outbred population with high genetic diversity.

VII.1.2. Introduction

The genetic variation of modern human populations, including disease-causing variation, results of many evolutionary processes, most of which are still unknown (Tishkoff and Varrelli 2003). Understanding these processes will shed light on how past demography shaped variation in the human genome. In this study, we characterize the overall diversity of São Miguel's population, based on the analysis of 21 autosomal STRs. Our main purpose is to estimate the genetic heterogeneity of the island population and infer its genetic structure in order to understand its past history and genetic evolution.

VII.1.3. Material and Methods

VII.1.3.1. Population samples

The sample population, composed of 204 healthy unrelated individuals, was obtained from the anonymous Azorean DNA bank located at the main Hospital in São Miguel

Island (Mota-Vieira *et al.* 2005). In addition, 103 mainland Portuguese individuals were analysed. The collection the samples followed the international ethical guidelines for sample collection, processing and storage.

VII.1.3.2. STR typing

Twenty-one microsatellite *loci* (TPOX, D3S1358, FGA, CSF1PO, D5S818, D6S265, TNFα, D7S820, D8S1179, D10S525, TH01, vWA, D13S317, D14S306, FES/FPS, D16S539, D17S976, D18S51, D19S433, D20S161 and D22S417) were typed using fluorescently labelled primers described previously in Human Databases (STRBase[34] and Human Genome Database[35]). PCR amplifications were carried out and run on a CEQ™8000 Genetic Analysis System (Beckman Coulter).

VII.1.3.3. Statistical analysis

Allele frequencies were calculated by direct counting; Hardy-Weinberg equilibrium, gene diversity and inbreeding coefficient were assessed by the GENEPOP web version software. F_{ST} related genetic distances were computed between pairs of populations by means of DISPAN and the distance matrix was used to construct a Neighbor-Joining (NJ) tree using PHYLIP 3.63. We used TreeView 1.6.6 to display tree phylogenies obtained from NJ. The F_{ST} values were calculated using data of allele frequencies, deposited in the ALFRED database, for 11 STRs (TPOX, D3S1358, FGA, CSF1PO, D5S818, D7S820, D8S1179, TH01, vWA, D13S317 and D18S51), since the information for the remaining microsatellites was not available. The following populations were selected from the same database: north and center Portugal, north Spain, Madeira, Cape Verde, Andalusia, Belgium, Italy, Morocco, Fang, Arabs, Indian and Turks.

[34] STRBase, http://www.cstl.nist.gov/biotec/strbase.
[35] Human Genome Database - GDB, http://www.gdb.org.

VII.1.4. Results

The genetic diversity was determined in 204 São Miguel individuals and 103 mainland Portuguese and based on the allele distribution for the 21 *loci*. Allele frequencies are supplied in Appendix IX.1. In Table VII.1 we describe the Hardy-Weinberg equilibrium, the gene diversity and the inbreeding coefficient for both populations. All markers are in Hardy-Weinberg equilibrium, considering $p<0.01$ (99% confidence). Microssatellite data reveals that in São Miguel, the gene diversity values range from 0.623 for TPOX to 0.904 for D17S976. The same markers show similar values in the mainland Portuguese sample. Overall, the average gene diversity is 0.767 for São Miguel Island, which is a similar value to that found in mainland Portugal (0.765, Table VII.1).

Table VII.1. Hardy-Weinberg equilibrium (HWE), gene diversity (GD) and inbreeding coefficient (F_{IS}) for São Miguel and mainland Portugal based on 21 STRs.

Microsatellite *locus*	**Chromosome location**	**São Miguel**			**mainland Portugal**		
		HWE	GD	F_{IS}	HWE	GD	F_{IS}
TPOX	2p23	0.2793	0.623	-0.0307	0.9459	0.630	-0.0776
D3S1358	3p21	0.1456	0.793	0.0121	0.0189	0.803	0.0215
FGA	4q28	0.3806	0.857	0.0333	0.1315	0.870	0.0295
CSF1PO	5q33.3	0.1716	0.719	-0.0634	0.4771	0.711	-0.0368
D5S818	5q21	0.7140	0.694	0.0532	0.0582	0.705	-0.0874
D6S265	6p21	0.0158	0.754	0.0181	0.0278	0.771	0.1061
TNFα	6p21	0.2539	0.868	0.0177	0.0226	0.874	0.1004
D7S820	7q	0.0239	0.810	0.0799	0.0101	0.812	0.0075
D8S1179	8q24.1	0.1571	0.818	0.0113	0.6259	0.803	0.0335
D10S525	10p11	0.0153	0.666	-0.0086	0.0639	0.712	0.0593
TH01	11p15	0.6432	0.801	-0.0463	0.6272	0.779	0.1152
vWA	12p12	0.1335	0.795	-0.0116	0.5869	0.826	-0.0463
D13S317	13q22	0.1293	0.796	0.0453	0.0182	0.824	0.1049
D14S306	14q	0.2617	0.784	0.0316	0.6014	0.817	0.0619
FES-FPS	15q25	0.4007	0.697	0.0579	0.3233	0.703	0.1028
D16S539	16q22	0.3124	0.767	0.0347	0.6174	0.794	-0.0143
D17S976	17p11	0.2288	0.904	-0.0251	0.6041	0.925	0.0132
D18S51	18q21.3	0.0850	0.884	-0.0038	0.3238	0.885	0.0242
D19S433	19q12	0.0304	0.788	0.0360	0.0289	0.818	0.1223
D20S161	20p	0.6970	0.638	-0.0212	0.6131	0.691	0.0169
D22S417	22q13	0.4706	0.851	-0.0026	0.8077	0.838	-0.0431
Av. gene diversity			0.7670			0.7650	
Total F_{IS}			0.0111			0.0326	

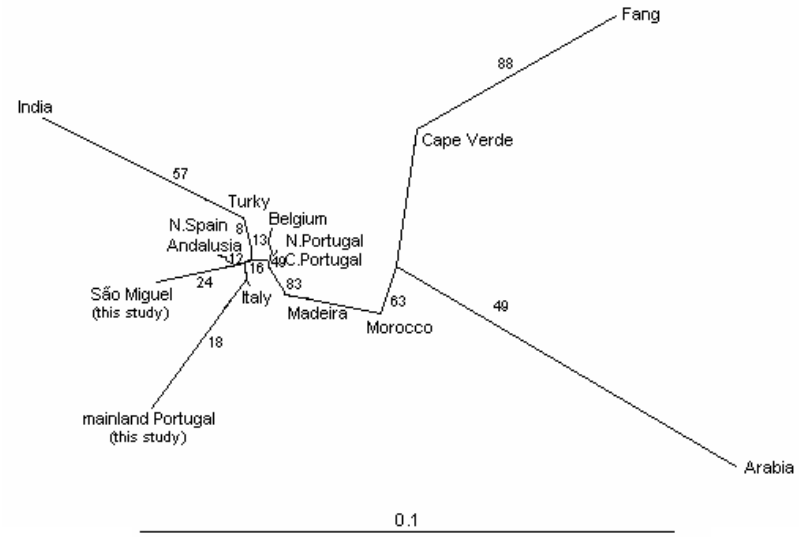
The assessment of the inbreeding coefficient was performed by the calculation of F_{IS}. The level of inbreeding for each marker, for the São Miguel sample, ranges from –0.0634 to 0.0799 for CSF1PO and D7S820, respectively. In general, the total value of F_{IS} is 0.0111 for São Miguel and 0.0326 for mainland Portugal (Table VII.1).

To investigate the relationships between São Miguel, mainland Portuguese and other European and African populations, we used F_{ST} genetic distances depicted in a NJ tree (Figure VII.1). The data shows a close proximity between all European populations, where São Miguel clusters. Noteworthy, Fang and Arabia are the most divergent and show genetic proximity with the Morocco and Cape Verde populations. However, since a NJ tree imposes a bifurcating model onto a distance matrix, which may be inadequate for closely related populations, such as, São Miguel and Portugal, we also performed a PC analysis (Figure VII.1). The first and second PC accounts for 73.9% and 11.9% of the genetic variance observed, respectively, and their plot shows a similar pattern to that shown in the NJ tree. Overall, the genetic relationships reveal that São Miguel is closely related to mainland Portugal.

VII.1.5. Discussion

In order to assess the patterns of genetic diversity in São Miguel and in mainland Portugal, we genotyped 21 microsatellite *loci* in 204 islanders and 103 mainland Portuguese subjects. In general, the data suggest high gene diversity for both populations, with no significant difference between them. The comparison of F_{IS} values for the mainland (0.0326) and the São Miguel (0.0111) samples suggests higher inbreeding in the mainland. Although there is a significant difference (χ^2, $p<0.001$) in F_{IS} values, the observed trend is not in agreement with results obtained in a comparative study of consanguineous marriages (first cousins, uncle-niece and aunt-nephew) registered by the National Institute of Statistics for Azores, Madeira and mainland Portugal from 1931 to 2000 (Pacheco *et al.* 2003). The small values for this parameter in both populations suggest that mainland and São Miguel do not show genetic structure and are behaving as expanded populations with high genetic diversity.

A.



B.

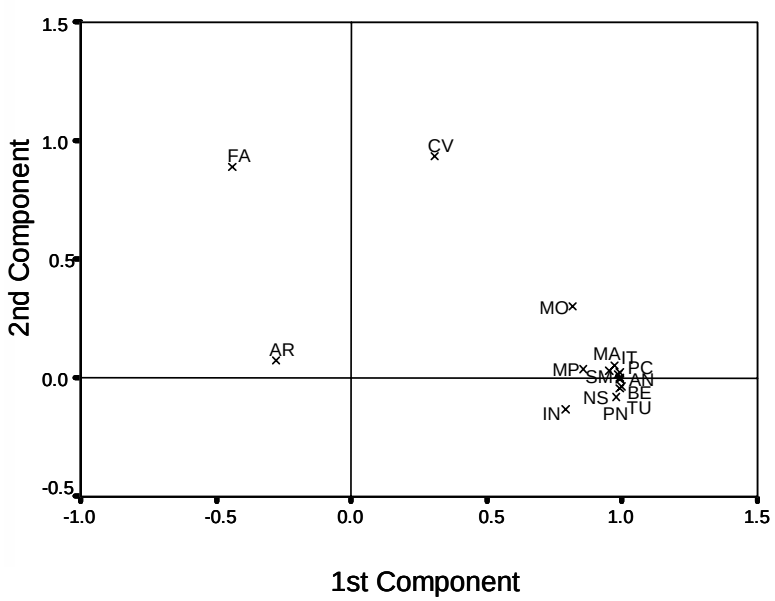


Figure VII.1. Population relationships based on 11 STRs **A.** Neighbor-joining tree using Nei's genetic distances. The numbers in the NJ tree represent the bootstrap values (%) obtained with 10,000 iteractions. **B.** Principal component analysis based on allele frequencies. The populations are represented as follows: SM, São Miguel; PN, north Portugal; PC, center Portugal; MA, Madeira; MP, mainland Portugal; NS, north Spain; AN, Andalusia; BE, Belgium; IT, Italy; MO, Morocco and CV, Cape Verde.

Differences in STR allele frequencies among populations can correctly reveal their genetic relationships. This study shows that São Miguel population exhibits an average gene diversity value (0.767) similar to other European populations (0.773; Tishkoff and Varrelli 2003), a higher value when compared to south American populations (0.697, Mesa *et al.* 2000) and a lower value than the African populations (0.792; Tishkoff and

Varrelli 2003). Furthermore, allele frequencies of all markers are comparable to other European populations. The NJ tree shows São Miguel clustering with the mainland Portuguese sample. This last sample does not cluster with north and center Portugal, probably because it is composed mainly by individuals from the south region. This observation agrees with other genetic studies (Pacheco *et al.* 2005, Montiel *et al.* 2005, Gonçalves *et al.* 2005), where the mainland south region is genetically different when compared with the other two regions, north and center. In addition, we observe a clustering of these populations with north Spain, Italy, Belgium, Madeira and Andalusia. In general, the results agree with a previous genetic study of the Azorean and the mainland Portugal populations, based on *Alu* insertion polymorphisms (Branco *et al.* 2006).

The genetic reconstruction of human origins and history requires evidence from different parts of the genome. Previous studies have reported a high genetic variability and heterogeneity of the Azorean population based on the maternal (Santos *et al.* 2003) and paternal (Pacheco *et al.* 2005) lineages. The results, based on microsatellites, support these observations and corroborate historical evidence of the settlement of São Miguel Island and, consequently, of Azores archipelago. Thus, the data suggests that São Miguel and probably the Azorean genetic signature results from the major contribution of Portuguese. Understanding the background of neutral human genetic variation provides insights about the allelic structure of health-related genetic variation (Bamshad *et al.* 2004). Therefore, the knowledge here obtained will be crucial to predict and explain the genotypes implicated in genetic diseases in the Azorean population.

VII.2. Azores islands: genetic origin, gene flow and diversity pattern

VII.2.1. Summary

The Azores are an archipelago located in the north Atlantic Ocean (parallel 38) composed of nine islands, dispersed over three geographical groups: The Eastern Group (São Miguel and Santa Maria), the Central (Terceira, Graciosa, Pico, São Jorge and Faial) and the Western (Flores and Corvo). Taking into consideration the geographical and settlement history differences of the archipelago, we assessed the genetic diversity pattern and the internal migration of the Azorean population, based on the analysis of 15 STR *loci* in 592 unrelated individuals. The results of this evaluation reveal that Terceira displays the highest value of gene diversity (0.7979) and Corvo the lowest (0.7717). Gene flow analysis indicates that Corvo has the lowest values of migration, 23.35, whereas São Miguel and Terceira present the highest values of emigration, 108.14 and 87.66, respectively. Taken together, the data demonstrate that, despite settlement diversity, no genetic difference between the islands population is observable today. This may be explained by the internal migration. Overall, the Azorean population can be analysed as a homogeneous genetic group presenting, possibly, the same drug-reaction profile. In terms of genomic medicine, these results will have a significant impact in the design of future genetic and pharmacogenomic studies in the Azorean population.

VII.2.2. Introduction

Population-specific genetic variation has been reported to be crucial for the genetic understanding of human demography and history. Moreover, several studies have emphasised its use in many fields of biomedical research, such as, the variation of disease prevalence in different regions and in pharmacogenetics (Cavalli-Sforza and Feldman 2003; The International HapMap Consortium 2005; Tishkoff and Kidd 2004; Foster and Sharp 2004; Suarez-Kurtz and Pena 2006). Therefore, a clear knowledge of the genetic variation of a population is of great interest. Our main objective is to answer questions such as: What is the genetic relationship between the different islands? Given the recent origin of the Azorean population, is the historic differential settlement revealed by the autosomal markers? What are the patterns of gene flow between the

islands' populations? In addition, we intend to assess the overall genetic heterogeneity of the Azorean population and compare it with other well described populations.

VII.2.3. Material and Methods

VII.2.3.1. Population samples

The study of the genetic diversity was based on a sample composed of 592 healthy Azoreans, obtained from the anonymous DNA bank located at the Hospital of Divino Espirito Santo (Ponta Delgada, São Miguel Island), the central hospital of the Azores. This bank was built according to the international ethical guidelines for sample collection, processing and storage (Mota-Vieira *et al.* 2005). The sample distribution *per* geographic group and island was the following: Eastern group, 166 (São Miguel, 114; Santa Maria, 52); Central group, 320 (Terceira, 103; Pico, 66; São Jorge, 51; Faial, 53; Graciosa, 47) and the Western group, 106 (Flores 76; Corvo, 30).

VII.2.3.2. STR genotyping

The PCR co-amplification of the fifteen STR *loci* (Penta-E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Penta-D, CSF1PO, D16S539, D7S820, D13S317 and D5S818) and Amelogenin was performed using the multiplex STR system PowerPlex® 16 (Promega), according to the manufacturer's instructions. Amplification was carried out on a DNA thermocycler GeneAmp® PCR System 2700 (Applied Biosystems) in a 10 µl PCR reaction with 2.5 ng of template DNA. PCR products were mixed with deionised formamide and internal lane standard ILS-600 (Applied Biosystems), and separated on an ABI 3130 Genetic Analyser. The sizing and genotyping were analyzed using GeneMapper® ID 3.2 software, and allele designations were made by comparison with the allelic ladders provided in the kit.

VII.2.3.3. Statistical analysis

Allele frequencies were calculated by direct counting; the Hardy-Weinberg equilibrium and gene diversity were assessed by the Arlequin software (Schneider *et al.* 2000). Slatkins FST genetic distance matrix was computed between pairs of populations by Arlequin and used to perform Principal components analysis. The F_{ST} values were calculated using data of allele frequencies, deposited in the ALFRED database (Rajeevan *et al.* 2003), for 13 STRs (D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, CSF1PO, D16S539, D7S820, D13S317 and D5S818). The following populations were chosen from the same database taking into consideration their possible relation to the Azorean Islands: Fang, Guinea, Mozambique, African American, Andalusia, Belgium, Italy, Spain, Basque, Ashkenazi Jew, Portugal, Brazil, Morocco, Han and Arab. Penta-E and Penta-D markers were not considered, since they were not described for all of these populations.

In order to estimate rates of migration among the islands' populations, we used the method implemented in the Migrate 2.1.2 software (Beerli and Felsenstein 1999). This method uses a maximum likelihood framework based on coalescence theory and support the one-step mutation model for microsatellites. Moreover, Migrate software provides by default estimates of M=4Nem, where *Ne* is the effective population size and *m* the actual migration rate. In order to avoid influence of the differences of Ne between all islands we selected randomly from each sample 30 individuals, which corresponds to the smaller sample size (Corvo Island).

VII.2.4. Results

The assessment of genetic diversity of all the Azorean Islands' populations was based on the allele distribution of 15 STR markers. Table VII.2 shows the Hardy-Weinberg equilibrium (HWE) *p* values and the gene diversity (GD) for each marker. All markers are in HWE, considering a 99% confidence ($p<0.01$), and are relatively polymorphic. The average number of alleles *per locus* is 11, ranging from 6 (TH01) to 20 (FGA). Comparing the allele composition between our sample and published data (ALFRED

Table VII.2. Hardy-Weinberg equilibrium (HWE) and gene diversity (GD) for 15 STR markers in the Azorean islands.

Microsatellite markers	**São Miguel** (N=114)		**Santa Maria** (N=52)		**Terceira** (N=103)		**Faial** (N=53)		**Pico** (N=66)		**São Jorge** (N=51)		**Graciosa** (N=114)		**Flores** (N=76)		**Corvo** (N=30)	
	HWE	GD	HWE	GD	HWE	GD	HWE	GD	HWE	GD	HWE	GD	HWE	GD	HWE	GD	HWE	GD
TPOX	0.0443	0.6213	0.2777	0.6103	0.3931	0.6577	0.5979	0.6470	0.7000	0.5752	0.8630	0.6184	0.8374	0.6792	0.7022	0.6101	0.0530	0.6046
D3S1358	0.3741	0.8029	0.7107	0.7804	0.6002	0.7742	0.1870	0.7761	0.1628	0.8014	0.3884	0.7976	0.5974	0.7796	0.0947	0.7892	0.1787	0.7483
FGA	0.1641	0.8591	0.1175	0.8546	0.1369	0.8690	0.1189	0.8714	0.1555	0.8688	0.0741	0.8376	0.5132	0.8663	0.0209	0.8525	0.8118	0.8672
CSF1PO	0.5604	0.7226	0.0903	0.7106	0.4015	0.7362	0.2633	0.7290	0.2020	0.7152	0.1591	0.7280	0.6688	0.6996	0.0356	0.7169	0.0147	0.7649
D5S818	0.0411	0.7276	0.4063	0.6750	0.6926	0.7105	0.9864	0.6840	0.8901	0.6931	0.9978	0.6922	0.6670	0.6533	0.3150	0.73	0.5144	0.7328
D7S820	0.3032	0.8067	0.1083	0.8090	0.9165	0.7909	0.1717	0.7776	0.3021	0.8111	0.8015	0.7924	0.2022	0.8154	0.9813	0.793	0.2132	0.7862
D8S1179	0.4164	0.8237	0.1507	0.7956	0.5760	0.8338	0.8131	0.8126	0.8742	0.8232	0.0863	0.8261	0.2541	0.8182	0.1015	0.7976	0.0678	0.7891
TH01	0.5384	0.782	0.1373	0.7732	0.3427	0.7998	0.6453	0.7739	0.2983	0.7881	0.6658	0.7916	0.0544	0.7847	0.2781	0.7961	0.6408	0.6902
vWA	0.1661	0.8074	0.9177	0.7860	0.3108	0.8103	0.4599	0.8179	0.5584	0.8232	0.6428	0.7978	0.3595	0.8305	0.7890	0.8252	0.0406	0.8052
D13S317	0.9575	0.7659	0.6319	0.8122	0.7652	0.7556	0.2536	0.8050	0.6019	0.7910	0.1454	0.7998	0.3079	0.7791	0.0119	0.7484	0.4769	0.7316
Penta-E	0.3713	0.8773	0.4503	0.9040	0.5376	0.8866	0.2869	0.8862	0.0730	0.8723	0.2348	0.8482	0.0733	0.8575	0.2549	0.8796	0.2160	0.8144
D16S539	0.0152	0.7594	0.9042	0.7837	0.8439	0.8008	0.3637	0.7322	0.3216	0.7476	0.9811	0.7622	0.9029	0.7539	0.1124	0.7878	0.8864	0.7764
D18S51	0.2827	0.8829	0.1554	0.8742	0.4849	0.8742	0.1799	0.8687	0.7086	0.8740	0.7743	0.8775	0.4097	0.8661	0.9942	0.8675	0.7968	0.8201
D21S11	0.1704	0.8359	0.8799	0.8213	0.2836	0.8295	0.6705	0.8111	0.8461	0.8365	0.8246	0.8145	0.9792	0.8198	0.1441	0.8259	0.1513	0.7954
Penta-D	0.4765	0.8191	0.2852	0.8177	0.0696	0.8393	0.0509	0.8282	0.0821	0.8261	0.1697	0.8349	0.6744	0.7946	0.3290	0.8423	0.2460	0.8500
Average GD		0.7929		0.7872		**0.7979**		0.7880		0.7897		0.7879		0.7865		0.7908		**0.7717**

Database; Rajeevan *et al.* 2003), we observe rare alleles in the Azores, namely D18S51*24 and FGA*25.2. The most interesting is FGA*25.2, which was found in São Miguel and Faial Islands, and is particularly frequent in India. In general, Penta-E and D18S51 show the highest values of gene diversity (around 0.87). The marker with the lowest gene diversity is TPOX with values varying from 0.575 to 0.679 for Pico and Graciosa, respectively. The gene diversity values reveal that Terceira shows the highest value (0.7979) and Corvo presents the lowest (0.7717). However, all values are similar between islands and do not show a statistically significant difference (χ^2, p=0.999). The average gene diversity for the whole Azorean population is 0.788.

To understand the gene flow patterns between islands, we calculated the migration (emigration and immigration) rates through the Migrate software (Table VII.3). The data suggest that Corvo is the island with the lowest values of migration. São Miguel and Terceira islands present the highest values of emigration, 108.14 and 87.66, respectively. On the other hand, Santa Maria shows the highest value (79.11) of immigration followed by Graciosa with 69.04. The Azorean population has an average migration value of 51.57.

The relationship between all islands was assessed by F_{ST} genetic distances and displayed by Principal Component (PC). The PC results (Figure VII.2) demonstrates that Corvo is the most genetically different island when compared with the other islands. Moreover, the data show a closer proximity between the Azorean Central (Terceira, Pico, Faial, São Jorge and Graciosa) and Eastern (São Miguel and Santa Maria) groups. These results are supported by the AMOVA analysis, where the Western group is the most different when compared with the other two groups. Nevertheless, these differences are not significant, only 1% of variance is observed between all groups of islands. The first and second PC accounts for 82.6% and 9.6% of the genetic variance, respectively. Moreover, to assess if genetic distances were correlated with geographic distances, we performed a Mantel test. The results show a relative correlation (r=0.457) between both distances with about 21% of the genetic variance explained by the geographic distance.

Table VII.3. Migration rates among all Azorean islands.

	São Miguel	**Santa Maria**	**Terceira**	**Faial**	**Pico**	**São Jorge**	**Graciosa**	**Flores**	**Corvo**	**Av. Immig.**
São Miguel	-	22.43	61.21	24.05	29.68	22.74	20.51	34.23	12.18	**28.38**
Santa Maria	171.57	-	115.14	51.51	64.77	49.24	45.83	85.98	48.86	**79.11**
Terceira	101.45	35.85	-	38.19	43.90	33.64	30.27	57.93	17.28	**44.81**
Faial	118.35	50.00	107.70	-	53.85	35.51	35.80	60.36	25.74	**60.91**
Pico	126.67	45.76	103.66	42.98	-	47.03	35.65	67.25	22.50	**61.44**
São Jorge	107.24	43.48	92.17	42.61	58.26	-	35.07	60.00	17.97	**57.10**
Graciosa	128.93	53.02	115.58	52.64	62.17	49.97	-	65.23	24.79	**69.04**
Flores	71.25	28.81	72.67	28.67	40.03	30.23	21.15	-	17.46	**38.78**
Corvo	39.63	24.70	33.12	23.93	18.19	12.25	18.19	26.42	-	**24.55**
Av. Emig.	**108.14**	**38.01**	**87.66**	**38.07**	**46.36**	**35.08**	**30.31**	**57.17**	**23.35**	**51.57**

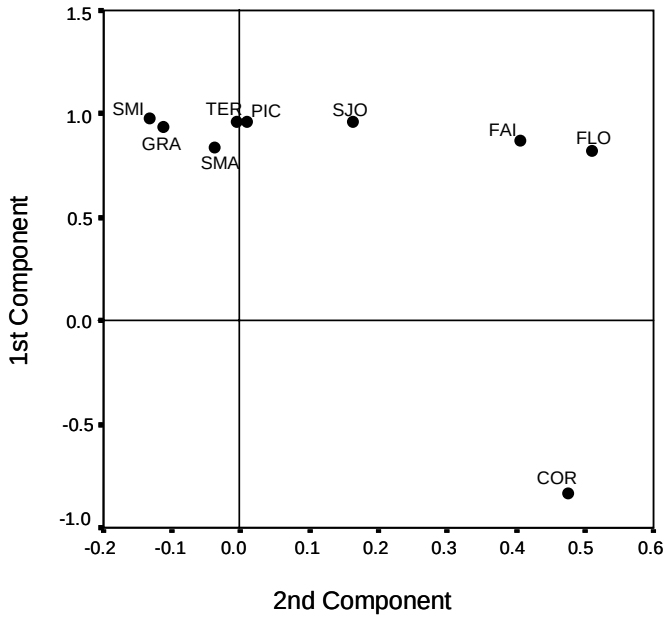


Figure VII.2. Principal component analysis based on allele frequencies in Azores.

To compare the Azorean with other European and African populations, we used data deposited in the ALFRED database (Rajeevan *et al.* 2003). In order to enhance the differences in allele distribution, we realized a joint analysis dividing the total Azorean population in the three corresponding geographical groups, namely Eastern, Central and Western. The results of the F_{ST} genetic distances are depicted in a PC plot (Figure VII.3). The data show a close proximity between all European populations, where the three Azorean groups cluster. The data also demonstrate a close proximity between all African populations. However, the Morocco population is more related to the European populations, as expected. Brazil and Arabs are located in an intermediate position between the Europeans and Africans. Overall, the genetic relationships by means of PC analysis reveal that the Azorean population is closely related to that from mainland Portugal.

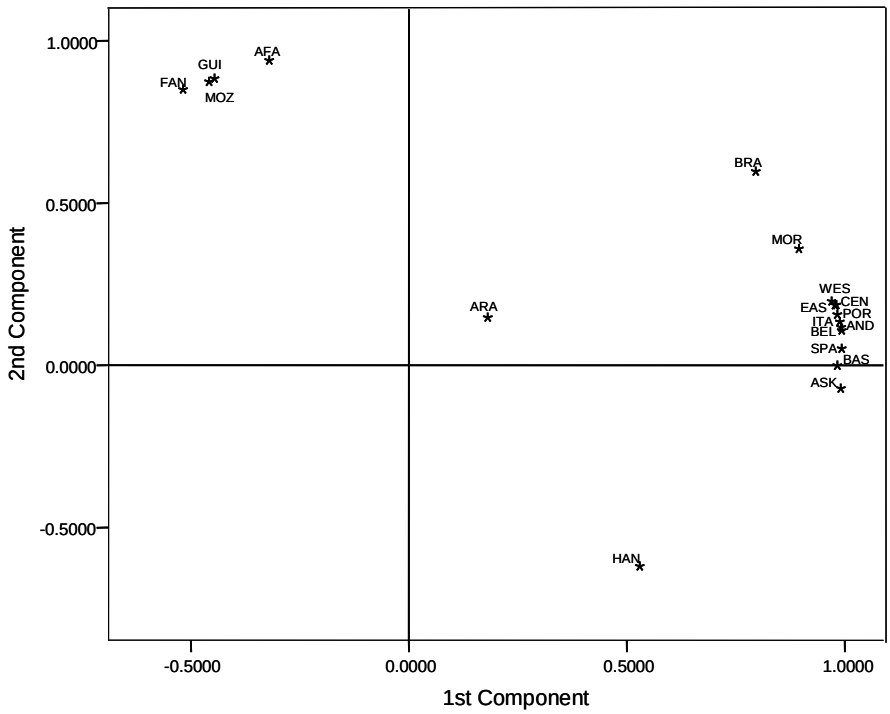


Figure VII.3. Principal component analysis based on Slatkins F_{ST} genetic distance using 13 STRs. The populations are represented as follows: WES, Azorean Western group; CEN, Azorean Central group, EAS, Azorean Eastern group; POR, Portugal; AND, Andalusia; SPA, Spain; BEL, Belgium; ITA, Italy; MOR, Morocco; ASK, Ashkenazi Jews; BAS, Basques, BRA, Brasil; FAN, Fang; GUI, Guinea, MOZ, Mozambique, AFA, African American, HAN, Chinese Han and ARA, Arabs.

VII.2.5. Discussion

Population genetic variation studies have demonstrated that there is an overall low level of differentiation in human populations (Excoffier 2003); however, local factors, such as, geography and differential settlement, can greatly enhance genetic discontinuity. To assess these factors in the current Azorean population, we describe the genetic diversity patterns through the genotyping of 15 microsatellite *loci* in a total of 592 individuals. In general, the data demonstrate no significant difference in gene diversity between all islands. The results demonstrate that Corvo presents the lowest value of gene diversity (0.7717) when compared with the other islands. Similar data were obtained in a previous surname study (Branco and Mota-Vieira 2005). Overall, the results from this work show that the Azorean population exhibits an average gene diversity value (0.788), which is similar to other European populations (0.773; Tishkoff and Varrelli

2003), higher than the South American populations (0.697; Mesa *et al.* 2003), and lower than the African populations (0.792; Tishkoff and Varrelli 2003). Altogether, the data suggest that the Azorean people present high genetic diversity as a result of the archipelago's settlement history. On the other hand, it may be argued that, STRs are highly variable, because they have high mutation rates and, therefore, after a population bottleneck, these markers would recover their diversity faster than other markers. Nevertheless, autosomal markers, such as, *Alu* insertion polymorphisms, studied in the Azorean population (Branco *et al.* 2006), show the same pattern of genetic diversity.

Interestingly, we identified in two Azorean islands the presence of a rare allele – FGA*25.2 – not found in mainland Portugal, but prevalent in Indian populations. During the 16th century, the commercial trade between Portugal and India (at the time under Portuguese rule) was very important (Correia 1948). The Azores, because of its geographic location in the north Atlantic Ocean, played a strategic role during that period. Thus, these data may suggest the presence of individuals of Indian origin in the archipelago. Alternatively, it may indicate the possibility of mutation in the FGA allele.

Migration is one of the main forces shaping genetic diversity of human populations. It can affect genomic variation within a population, for example, by the redistribution of genes geographically. Thus, understanding the causes, patterns and effects of migrations is fundamental for interpreting the evolutionary history of a population (Cavalli-Sforza and Feldman 2003). The data presented here show that Corvo stands out with the lowest values of migration, suggesting that people have become sedentary. Nevertheless, Corvo has the lowest population size (Ne). This last parameter affects the population's genetic diversity apportionment, as observed in the present study, where Corvo shows the lowest values of diversity. Therefore, the low values of migration obtained in this island can be a direct influence of Ne. São Miguel and Terceira, with the highest values of emigration, have also the highest levels of gene diversity indicating that people of these islands were the main contributors in the settlement of the other islands. Nevertheless, the majority of the islands' populations show low migrant proportions, but we observe a high genetic similarity between them. Overall, the results indicate that the islands' populations did not evolve independently, but rather maintained connections through the exchange of migrants. Other archipelagos, such as, Cape Verde, still maintain genetic differences between groups of islands, namely Cape Verde

North and Cape Verde South, as a result of their settlement history (Fernandes *et al.* 2003). Nevertheless, probably the same result would be obtained if (*i*) the islands had been peopled from the same population without a strong bottleneck, and (*ii*) the original populations had been different but the differences between islands had been smoothed out by migration. On the other hand, migration among islands can elucidate why geography explains only 21% of the genetic variance, indicating that it contributed to a mixture of the Azorean population as a whole.

In general, scientists agree that the characterization of regional diversity patterns has several implications in biomedical research, with a strong input in the local health care. The PC analysis (Figure VII.2) demonstrate a strong genetic similarity between all the islands' populations. These data are corroborated by the genetic diversity values, where no significant differences (χ^2, p=0.999) between islands were obtained. Nonetheless, geography should be considered, since it is possible to observe a higher proximity among islands of the same Azorean group. This observation is supported by the Mantel test were ther is a correlation of 0.457 between genetic and geographic distances. The comparison of the Azorean groups with other European and African populations demonstrates a strong input of Europeans, the majority of which from mainland Portugal in the origin of the archipelago's population. Allelic frequencies change in populations owing to two factors – natural selection and genetic drift –, both can ultimately lead to the elimination or fixation of a particular gene (Cavalli-Sforza and Feldman 2003). Considering the geography of the Azores archipelago, which could potentiate the action of several genetic processes, like genetic drift, the overall results do not suggest the influence of genetic drift nor natural selection.

Nuclear genetic variation allows the characterization of the overall genetic similarities of populations that are the result of all historical phenomena (Kidd *et al.* 2000). Our results based on microsatellite data demonstrate that, despite reports of differential settlement for each island, there is no genetic difference between the islands' population today. Genetically structured populations may be composed of two or more subpopulations with distinct drug-reaction profiles and thus in some contexts it would be better to consider them separately (Wilson *et al.* 2001; Schaak *et al.* 2007; Suarez-Kurtz and Pena 2006). The data described here show that the Azorean population is an outbred population with no genetic structure. This suggests that, despite

living in different islands, the Azorean population can be treated as a homogeneous genetic group, which consequently, would present, possibly, the same drug-response pattern. Sistonen *et al.* (2007) studying CYP2D6 worldwide genetic variation observed that patterns of variation, within and among populations, are similar to those observed for other autosomal markers (e.g. microsatellites and protein polymorphisms), suggesting that the diversity observed at the *CYP2D6 locus* reflects the same factors affecting variation at random genome markers. In terms of genomic medicine, the results obtained in the present work play an important role in the design of future genetic and pharmacogenomic studies in the Azorean population.

VII.3. Evaluation of linkage disequilibrium on the Xq13.3 region: comparison between the Azores Islands and mainland Portugal

VII.3.1. Summary

The design of genetic studies of complex diseases is dependent on the extent and distribution of linkage disequilibrium (LD) across the genome in different populations. Here, we characterize the extent of LD in the Azores (Western, Central and Eastern islands groups) and mainland Portugal populations. LD was evaluated in the Xq13.3 region by genotyping eight STR markers spanning 20.9 Mb. Standardized multiallelic disequilibrium coefficient (D') analysis indicates that the Western group presents higher values when compared with the Central and Eastern groups. However, all islands groups show values of D' lower than 0.5 and 0.33, suggesting no extensive LD in these populations. Taken together, the data show that the Azorean population presents a lower D' (0.142) than mainland Portugal (0.226). Although, both populations do not show extensive LD, the easy reconstruction of large pedigrees in the Azorean population is a valuable resource for the fine mapping of disease genes.

VII.3.2. Introduction

Linkage disequilibrium (LD) is defined as a non-random association of alleles at different *loci* on the same chromosome. Studying the extent of LD and population structure is a good starting point for the investigation of complex traits (Angius *et al.* 2002). The Azores is a Portuguese archipelago composed of nine islands, located in north Atlantic Ocean. Its settlement began in 1439 with Portuguese individuals, but a significant contribution from people with other genetic backgrounds, including Flemish, Spanish, French, Italian, German, Scotish, Jewish, and also from Moorish prisoners and black slaves from Guinea, Cape Verde and São Tomé also occurred. Nowadays, the Azorean population is composed of 241,763 inhabitants (National Institute of Statistics – Portugal, 2001 Census). Recently, the genetic background of the Azorean population has been thoroughly analysed using autosomal (Branco *et al.* 2006, 2007; Spinola *et al.* 2005), mitochondrial (Santos *et al.* 2003) and Y-chromosome (Pacheco *et al.* 2005a; Montiel *et al.* 2005) markers. These studies report a high genetic variability and

heterogeneity of the Azorean population, which can be explained by the settling history of the islands. Here, we characterize LD at Xq13.3 in the Azorean and mainland Portugal populations. It was also our purpose to assess the pattern of LD in the different groups of islands of the archipelago, and compare them with the mainland population and other well described populations.

VII.3.3. Material and Methods

VII.3.3.1. Population samples

The study of the X-chromosome LD extent was based on a sample composed of 432 healthy Azoreans (408 males and 24 females) and 97 individuals from mainland Portugal, obtained from the anonymous Azorean DNA bank (Mota-Vieira *et al.* 2005). The sample distribution *per* group and island was the following: Eastern group, 207 (São Miguel, 185; Santa Maria, 22); Central group, 150 (Terceira, 54; Pico, 29; São Jorge, 23; Faial, 25; Graciosa, 19) and the Western group, 75 (Flores 59; Corvo, 16). The origin of all females was from Flores Island.

VII.3.3.2. STRs typing

Linkage disequilibrium was evaluated in Xq13.3 This region was analyzed by genotyping eight microsatellite markers – DXS983, DXS1066, DXS986, DXS8092, DXS8082, DXS1225, DXS8037 and DXS995 – spanning approximately 6.9 centiMorgans (cM) or 20.9 megabases (Mb). The exact location, in base pairs (bp), on the Human Genome Map of these microsatellites was reported by Kaessmann *et al.* (2002). The markers were genotyped using fluorescently labelled primers described previously in the Human Genome Database (GDB, www.gdb.org).

Polymerase Chain Reaction (PCR) amplification was carried out in a singleplex 15 µl reaction mixture. An aliquot of 1 µl of each PCR product was combined with 0.5 µl CEQ™DNA size standard kit 400, 29 µl formamide deionized (Qbiogene), and run on a CEQ™8000 Genetic Analysis System (Beckman Coulter).

VII.3.3.3. Statistical analysis

Allele frequencies were calculated by direct counting. Average gene diversity estimation, based on X-markers, was performed using the Arlequin software. Estimation of the X-haplotypes was obtained through the expectation maximum (EM) algorithm, an iterative procedure from multilocus genotype data with unknown gamete phase implemented in Arlequin. To increase the power in LD calculations we included 24 females in the Flores Island sample. Therefore, the number of haplotypes in the Western group population increased from 75 to 93. Estimation of standardized multiallelic disequilibrium coefficient, D', was performed using the Haploxt application from the GOLD software. This program calculates disequilibrium statistics from haplotype data. Disequilibrium across each *locus* was plotted using the same software.

VII.3.4. Results

Understanding the background genetic variation of a population is essential in the characterization of LD. Table VII.4 describes the number of haplotypes, gene diversity and standardized multiallelic disequilibrium coefficient (D') based on X-linked markers for all populations. The Azorean Western group shows a higher genetic diversity (0.718) when compared with the other two groups. Overall, the Azorean population, as

Table VII.4. Haplotype number (HN), gene diversity (GD) and standardized multiallelic disequilibrium coefficient (D') for Azorean and mainland Portugal populations.

Populations	**HN**	**GD**	**D'**
Azores			
Western group	93	0.718	0.328
Central group	150	0.690	0.189
Eastern group	207	0.686	0.176
Total	450	0.695	0.142
mainland Portugal	97	0.683	0.226

a whole, shows higher genetic diversity (0.695) when compared to mainland Portugal (0.683). On the other hand, there is no statistically significant difference of gene diversity values for all populations (χ^2, p=0.236).

We observe that the Azorean Western group presents higher values of average D' when compared with the Central and Eastern groups (Table VII.4). However, we selected randomly 75 individuals from the Azorean Central and Eastern samples and calculated average D'. The values obtained were not statistically different from those for Western group (data not shown). This result confirms that the difference in D' values in populations is not statistically significant.

To compare the extent of LD over physical distance, we plotted the average standardized multiallelic disequilibrium coefficient (D') with stratified physical distances (Figure VII.4). All groups show values of D' lower than 0.5 with higher values for shorter distances. Mainland Portugal presents a higher value of average D' when compared with the whole Azorean sample.

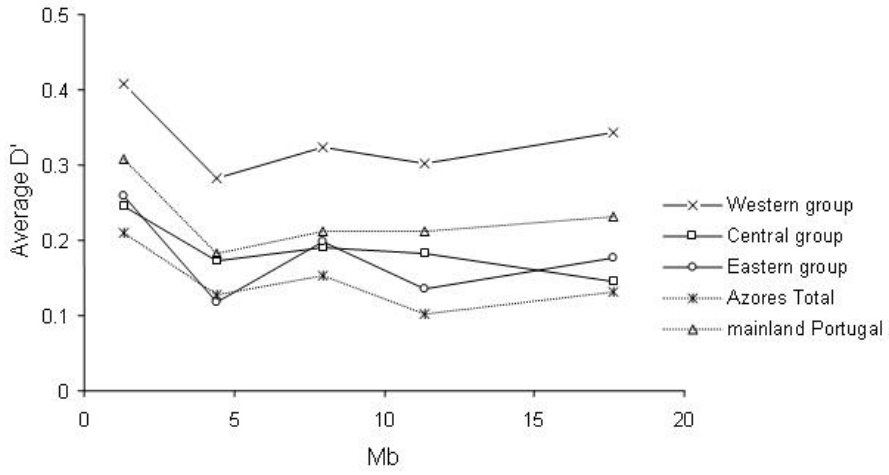


Figure VII.4. Comparison of the LD extent in Azores and mainland Portugal, evaluated as average multiallelic D' values *versus* physical distances.

VII.3.5. Discussion

The knowledge of genetic diversity in a population is crucial for a better understanding of the genomic patterns relevant for mapping disease genes, such as the distribution and

extent of LD. Our results demonstrate that the Azorean population presents a high genetic diversity comparable to the mainland Portuguese population.

There is some controversy related to the amount of useful LD for mapping studies. According to Abecasis *et al.* (2001), the value of D'=0.33, which corresponds to a 10-fold increase in the required sample size, is commonly taken as the minimum usable amount of LD. On the other hand, Reich *et al.* (2001) considers that D'>0.5 is useful. None of the samples analysed in the present study show values higher than 0.5 or 0.33, suggesting no LD for all populations. In general, the pattern of LD observed is different when compared to the populations of Niolo, Corte and Bozio in Corsica (Latini *et al.* 2004), indicating a smaller extent of LD in the Azorean and mainland Portugal populations. Although, there are limitations concerning the sample size and marker density of the present study, the results are corroborated by those obtained by Service *et al.* (2006), where the Azoreans presented the lowest values of LD when compared with populations considered genetic isolates.

The existence of high LD over large chromosomal regions is characteristic of populations with reduced haplotype and allelic diversity (Varilo *et al.* 2000). Our results show that both Azores and mainland Portugal present characteristics of expanded populations. The extent of LD is influenced, among other factors, by genetic drift, admixture and inbreeding. The LD distribution here described is a consequence of a high genetic diversity determined by the Azorean settlement history and demography. Therefore, the data show that admixture is the contributing factor to the present LD pattern in the Azorean population. The fact that the majority of Azoreans lives in small rural localities with large families (more than 3 children *per* generation) and the easy access to church and city hall records, facilitates the reconstruction of extended family pedigrees. In addition, according to a comparative study of consanguineous marriages (first cousins, uncle-niece and aunt-nephew) registered by the National Institute of Statistics for Azores, Madeira and mainland Portugal from 1931 to 2000, the Azores present the highest values of consanguinity (Pacheco *et al.* 2003). These features associated with the geographical, the demographic and the environmental characteristics suggest that the Azorean population may be a valuable resource for fine mapping of disease genes.

VII.4. Linkage disequilibrium on Xq13.3, NRY and HLA regions in São Miguel Island (Azores) population

VII.4.1. Summary

The design of genetic studies of complex diseases is dependent on the extent and distribution of linkage disequilibrium (LD) across the genome in different populations. Here, we characterize the extent of LD in the São Miguel Island population. Genetic diversity and LD were evaluated in Xq13.3, nonrecombining portion of the Y-chromosome (NRY) and HLA (6q21) regions in healthy blood donors of São Miguel Island population.

Haplotype analysis revealed 100% discriminatory power for the X- and Y-STRs, and 94.3% for the HLA *loci*, demonstrating that the São Miguel population is very genetically diverse. Standardized multiallelic LD, D', in the three genomic regions show values lower than 0.33, suggesting no extensive LD in this population. As expected, the highest D' values are found for shorter distances. The D' results also indicate that there is a higher LD for the NRY region when compared to HLA and Xq13.3. Taken together, the data demonstrate that the São Miguel Island population presents a low D' (0.241). The results suggest that the identification of identical by descent (IBD) regions surrounding disease susceptibility gene or other complex trait *loci* in this population, as well as in the Azoreans, would require a very high density of markers.

VII.4.2. Introduction

It is well known that LD varies across genomic regions; therefore, for association studies to be feasible, with an optimal distribution of markers, the level of LD should be estimated for each region. Here, we examine the extent of LD in three genomic regions – Xq13.3, nonrecombining portion of the Y-chromosome (NRY) and HLA (6q21) – in the São Miguel Island population, in order to evaluate the use of LD for future studies of mapping disease susceptibility genes.

VII.4.3. Material and Methods

VII.4.3.1. Population samples and genotyping

Linkage disequilibrium was evaluated in Xq13.3, NRY and HLA (6q21). The sample set was composed of healthy blood donors living in São Miguel Island obtained from the anonymous DNA bank located at the Hospital of Divino Espirito Santo of Ponta Delgada, EPE (Mota Vieira *et al.* 2005). LD for X- and Y-chromosomes was assessed only in males (189 and 149, respectively), whereas the analysis of the HLA region consisted of 106 individuals of both sexes (8 females and 98 males).

The Xq13.3 region was analyzed by genotyping eight microsatellite markers – DXS983, DXS1066, DXS986, DXS8092, DXS8082, DXS1225, DXS8037 and DXS995 – spanning approximately 6.9 centiMorgans (cM) or 20.9 megabases (Mb). The exact location, in base pairs (bp), on the Human Genome Map of these microsatellites was reported by Kaessmann *et al.* (2002). The markers were genotyped using fluorescently labelled primers described previously in the Human Genome Database (GDB, www.gdb.org). PCR conditions were described in Branco *et al.* (2007b).

Genotyping of Y STRs and HLA class I (A, B and Cw) and class II (DRB1, DQB1, DPA1 and DPB1) are described in Pacheco *et al.* (2005a,b). We also typed two dinucleotide STRs located in the HLA region, D6S265 and TNFα (Branco *et al.* 2007a).

VII.4.3.2. Statistical analysis

Allele frequencies were calculated by direct counting. Average gene diversity estimation was performed using the Arlequin software. Estimation of the HLA haplotypes was obtained through the expectation maximum (EM) algorithm, an iterative procedure from multilocus genotype data with unknown gamete phase implemented in Arlequin. A total of 200 haplotypes were obtained. Estimation of standardized multiallelic disequilibrium coefficient, D', was performed using the Haploxt application from the GOLD software. This program calculates disequilibrium statistics from haplotype data.

VII.4.4. Results and Discussion

Understanding the background genetic variation of a population is essential in the characterization of LD. We investigated the gene diversity in Xq13.3, NRY and HLA regions in the São Miguel Island population. The results demonstrate that this population is very diverse (Table VII.5). Haplotype analysis reveals 100% discriminatory power for the X- and Y-markers, because each individual presents a different haplotype, and 94.3% for the HLA markers. In general, the data agree on previous works (Branco *et al.* 2007a, Pacheco *et al.* 2005a), where Azoreans and São Miguel islanders show higher values of genetic diversity than mainland Portugal and other European populations. This may be a direct consequence of the Azorean settlement, where a major contribution of mainland Portuguese males and, to a lesser extent, Flemish, Spanish, French, Italians, Germans, Scottish, Jews, Moorish and blacks from Guinea, Cabo Verde and São Tomé is observed.

Table VII.5. Haplotype number (HN), gene diversity (GD) and standardized multiallelic disequilibrium coefficient (D') for the three genomic regions in the São Miguel Island population.

Genomic Region	HN	GD	D'
Xq13.3	189	0.691	0.172
NRY	149	0.574	0.282
HLA	200	0.843	0.275
Average	**179**	**0.703**	**0.243**

Considering only the HLA markers, the haplotype analysis reveals interesting features. For instance, while the A*01 B*08 DRB1*03 haplotype, known to be of Indo European Celtic origin, is present in centre and north Portugal at relatively low frequencies of 3% and 2.2%, respectively (Arnaiz Villena *et al.* 1997), it is the most frequent in São Miguel (8%, data not shown). According to Spínola *et al.* (2005), the presence of this haplotype results from a colonizing event from people originating from the centre of Portugal. However, we can also hypothesise a direct influence of Celts or Barbarian in the Azorean population, since the frequency of this haplotype in São Miguel is more than twice the frequency in mainland Portugal. Another hypothesis is the occurrence of

genetic drift, however, other studies of genetic diversity do not corroborate this theory (Branco *et al.* 2006, 2007a,b; Pacheco *et al.* 2005a,b).

Since LD varies among genomic regions within the same population, we investigated in the São Miguel population the extent of this parameter in Xq13.3, NRY and HLA regions. Figure VII.5 shows the plot of average D' over the physical distance. We observe a decrease of LD values for shorter distances (<5 Mb) for all regions. As expected, the highest value (>0.5) obtained in the X-chromosome corresponds to the association of DXS1225 DXS8082, which is the smallest physical distance between all markers.

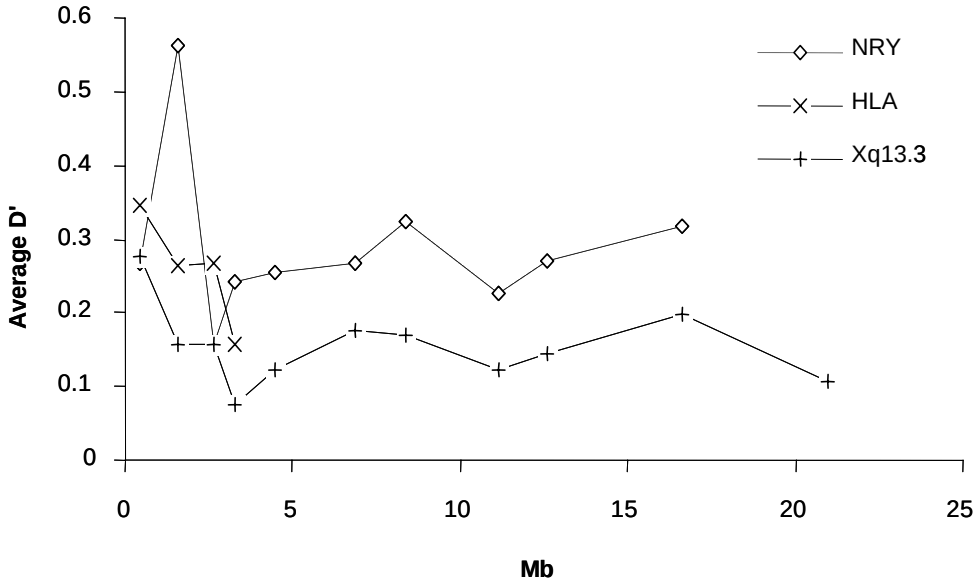


Figure VII.5. Comparison of the LD extent in Xq13.3, NRY and HLA regions, evaluated as average multiallelic D' values *versus* physical distances for the São Miguel Island population.

Because LD is generated by evolutionary processes, which are not regular in statistical terms, it is important to assess the patterns of LD both in sex and autosomal chromosomes. The comparison of D' on Xq13.3, NRY and HLA regions shows a smaller LD on the Xq13.3 (Table VII.5). The data indicate a higher LD for the NRY, followed by the HLA region. The HLA results are in agreement with those of Meyer et al. (2006), where a significant LD between all HLA *loci* is reported in the studied populations. The distribution of LD between Y-linked alleles is expected to be

substantially larger than for the X-linked markers, because Y-alleles have only one forth the effective population size. The data here obtained confirm this expectation. Nevertheless, the highest peak observed in Figure VII.5 corresponds to the association between DYS392-DYS385. This was not expected, since this region does not present recombination; however, it may reflect the influence of stochastic processes, such as random sampling.

There is some controversy related to the amount of useful LD for mapping studies. According to Abecasis *et al.* (2001), the value of D'=0.33, which corresponds to a 10 fold increase in the required sample size, is commonly taken as the minimum usable amount of LD. On the other hand, Reich *et al.* (2001) considers that D'>0.5 is useful. None of the samples analysed in the present study show values higher than 0.5 or 0.33, indicating no LD for all São Miguel population. These results are corroborated by those obtained by Service *et al.* (2006) and Branco *et al.* (2007b), where the Azoreans presented the lowest values of LD when compared with isolated and outbred populations. Taken together, the data suggest that the identification of identical by descent (IBD) regions surrounding disease susceptibility gene or other complex trait *loci* in the São Miguel population, as well as in the Azoreans, would require a very high density of markers.

"The important thing is not to stop questioning. Curiosity has its own reason for existing. One cannot help but be in awe when he contemplates the mysteries of eternity, of life, of the marvelous structure of reality. It is enough if one tries merely to comprehend a little of this mystery every day."

Albert Einstein

CHAPTER VIII

GENERAL DISCUSSION

VIII. General Discussion[36]

The study of genetic variation and heritance leads to the comprehension of genetics in general, with a practical value for human welfare. The knowledge of the contribution that genes make to the development of diseases – for example, cancer, heart disease and diabetes –, played an important role in the perception that such studies can potentially improve human health. Moreover, the characterization of genetic diversity provides a powerful tool for understanding and describing human evolution. Here, we show a broader view of the genetic structure of the Azorean population.

The Azores, the biggest Portuguese archipelago, is located in the north Atlantic Ocean. It is composed of nine volcanic islands unevenly distributed by three geographic groups: the Eastern group with two islands – São Miguel and Santa Maria –, the Central which includes five islands – Terceira, Pico, Faial, São Jorge and Graciosa –, and the Western group with Flores and Corvo. Although, Azoreans constitute a young population (<27 generations), there has been reports of increased frequencies of diseases, among others, congenital heart diseases (Cymbron *et al.* 2006), schizophrenia and psychosis (Pato *et al.* 2005; Sklar *et al.* 2004), autism (Oliveira *et al.* 2007), as well as Machado-Joseph disease (Lima *et al.* 2001). To understand this genetic panorama in the Azorean population, it revealed necessary and imperative to study its genetic background. The present thesis aims to contribute to this objective and two main approaches were followed: the surnames and the molecular markers analysis. Both approaches have advantages and criticisms. Surnames constitute a good tool when studying recent movements of individuals between subpopulations. However, surnames do not take in consideration the possibility that they may be polyphyletic, this is the same surname presents different origin and, consequently, different ancestors. Situations such as (*i*) a surname acquired because it was beneficial, for instance, in commercial trades; (*ii*) slaves from rich and important families usually acquired the surname of his owner and; (*iii*) cases of non-paternity, constitute good examples of polyphyletism. The overall results in this thesis, in addition to the inherent evolution of surnames, corroborate

[36] In this section some unpublished data are included, since they contribute to improve the discussion. They also increase and validate the analysis performed during the present thesis.

the polyphyiletic nature of surnames. Considering molecular markers, they also present discrepancies; for example, molecular markers are subject to evolutionary forces, which are not accounted in most of the simple methodologies to study populations, and their diversity is influenced by random fluctutions in sampling.

VIII.1. Genetic origin of the Azorean population

In the present PhD thesis, the understanding of the genetic origins of the Azorean population was a main concern. To achieve this goal two main studies were performed, the Y-chromosome lineages (Pacheco *et al.* 2005) and the *Alu* insertion (Branco *et al.* 2006). The nonrecombining portion of the Y-chromosome retains a record of the mutational events that occurred along male lineages throughout evolution (Y-Chromosome Consortium 2002). Overall, the results obtained revealed nine different haplogroups, most of which are frequent in Europe. Haplogroup J* is the second most frequent in Azores (13.4%), but it is modestly represented in mainland Portugal (6.8%). The other non-European haplogroups – N3 and E3a –, which are prevalent in Asia and subSahara, respectively, have been found in Azores (0.6% and 1.2%, respectively) but not in mainland Portugal. Two other studies, Gonçalves *et al.* (2005) and Montiel *et al.* (2005), also studied the Y-chromosome lineages of the Azorean population. In general, all studies evidence the four major haplogroups: P*(xR1b8,R1a,Q3), J*, BR*(xB2b,CE,F1,H,JK) and E*(xE3) that account for the majority of the male lineages in the Azores. Nevertheless, slight differences in frequency of these haplogroups are observed. All studies report that the main contributors to the genetic origin of the Azores are, as expected, the mainland Portuguese. Moreover, all studies agree that an important contribution of Middle eastern (HG J*) and north African (HG E*(xE3)) populations is observed. Without any doubt, Y-chromosome and mtDNA studies are crucial to address the origin of the population; however, a population loses mtDNA when a woman has only sons and Y-chromosome DNA when a man has only daughters. Consequently, these genetic markers may give less correct information on broad ancestry of most genes in a population. A full picture of the histories of populations requires studies of markers in the recombining parts of the nuclear DNA, namely the autosomes. Albeith several types of markers can be used to achieve this, *Alu* insertion

polymorphisms present some interesting advantages. These markers arose within the human population as a unique event in human evolutionary history, making *Alu* repeats identical by descent from a common ancestor (Batzer and Deininger 2002). Moreover, the ancestral state, which is absence of the *Alu* insertion, is always known. The allele frequencies for each *Alu* polymorphism in Azoreans are very similar to those obtained in European populations. Although, Comas *et al.* (2000) revealed a clear differentiation between north African and Iberian populations, our results show a strong proximity between mainland Portuguese and Moroccans. Historical data may support this proximity. Historians mention that the conquest of Ceuta in 1415 by the Portuguese was the first step in the "Portuguese expansion". Ceuta was considered a strategic market and a start point for the exploration of the African littoral (Serrão 1978). On the other hand, we also see a close relation with Spanish populations, namely, Catalans and Andalusians. This is reflected in the phylogenetic tree where Azores and mainland Portugal branch with Catalans, Andalusians, Moroccans and Algerians (Figure VI.4). Overall, the data are in concordance with the ones obtained by Y-chromosome studies (Pacheco *et al.* 2005; Montiel *et al.* 2005; Gonçalves *et al.* 2005) and the historical facts, reinforcing the contribution of Spanish individuals in the Azorean peopling. Furthermore, the *Alu* analysis also suggests the existence of a different demographic history and patterns of population evolution between European and African populations; for example, the African groups, with the exception of Algerians and Moroccans, are closer to the ancestral population in contrast to European populations (Figure VI.4). mtDNA studies in the Azorean population also corroborate the major presence of mainland female Portuguese settlers (Santos *et al.* 2003). However, these authors also report around 35% of unique female lineages. In general, the *Alu* markers and Y-chromnosome studies do not corroborate this observation.

Spinola *et al.* (2005) questions the identification of the lineage N3, specific to Asians and northern Europeans (Rosser *et al.* 2000; Helgason *et al.* 2000), since they did not found any results supporting this observation based on HLA *loci*. Historical records of the presence of Asians or Mongolians in the archipelago are not known. On the other hand, the HLA data showed the presence of haplotype A*02-B*44-DRB1*04 at a frequency of 1.42%. This haplotype, possibly oriental in its origin, has previously been described in the Azores (Bruges-Armas *et al.* 1999). The

introduction of this genetic contribution occurred probably during the expansion of the trade navigation between Europe, America and Asia, in the 16th and 17th centuries, when the Azores had a strategic role due to its geographic position (Russel-Wood 1998).

Genetic distance methods describe allele frequency similarities between populations or groups, indicating the degree of proximity between them. The works based on 21 STRs in São Miguel's population (Branco *et al.* 2007, in press) and on 15 STRs in all Azorean islands (Branco *et al.* 2007, submitted) indicate a very close proximity with mainland Portugal and other European and African populations. These results are also corroborated by studies performed on HLA *loci* (Spinola *et al.* 2005; Pacheco *et al.* personal communication). In conclusion, all studies point to the main importance of mainland Portuguese in the genetic origin of the Azorean population. Moreover, the presence of African and other European populations is not negligible. All data confirm and complement the gaps in the settlement history of the Azores archipelago.

VIII.2. Genetic diversity, relationship and linkage disequilibrium in the Azorean islanders

The evolution of populations is dependent on several mechanisms such as, migration, genetic drift, selection and mutation, all affecting the patterns of diversity of neutral and disease variants. Consequently, the measure of diversity of neutral markers allows the inference of how these processes are shaping the overall signature of a population and has further implications in the general diseases apportionment. In the present thesis, the diversity of the Azorean population was addressed considering different STR markers, located in different chromosomes (autosomal, Y and X), *Alu* insertion polymorphisms and surnames. The average diversity obtained in the different studies show that, in general, the Azorean population is very diverse, presenting values higher than those found in mainland Portugal. Only the *Alu* insertion polymorphisms are the exception, with mainland presenting a higher diversity. Nonetheless, all differences between values are not statistically significant. Considering the results obtained for the Y- and X-chromosomes and *Alu* insertions in

the Azorean population, we observe that the diversity value is higher in the X-chromosome (0.695), followed by the Y- (0.590), and last the *Alu* insertions (0.383). These results are explained by the fact that both the X- and Y-chromosomes have lower effective sizes (3/4 and 1/4, respectively), when compared with autosomal chromosomes, and also present lower rates of recombination (Schaffner 2004). The *Alu* insertions are biallelic markers and, consequently, show a smaller level of diversity regarding microsatellite data (Venter *et al.* 2001). Variability based on the STR markers in the autosomal chromosomes indicate as well, that the Azores is a very diverse population. Similar values of diversity are obtained when comparing the Azores (0.788) with mainland Portugal (0.782; Bosch *et al.* 2000; Perez-Lezaun *et al.* 2000), Madeira (0.773; Fernandes *et al.* 2001) and Cape Verde (0.791; Fernandes *et al.* 2003). The results from the genetic characterization of São Miguel Island's population reveal a smaller value of diversity (0.767) considering 21 STRs (Branco *et al.* 2007, in press) compared to the higher value (0.792) analysing 15 STRs (Branco *et al.* 2007, submitted). The same trend occurs in the mainland Portugal population where a value of (0.765) is observed. Therefore, the accurate value of global variability is dependent on the number of markers used.

Interestingly, the study of abundance of surnames and microsatellite in Azores revealed that the most diverse islands are Terceira and São Miguel. However, a slight discrepancy is present. In the surname study, the islands with less diversity are Graciosa and Santa Maria, in contrast to the STR data (Branco *et al.* 2007, submitted) where Corvo is the less diverse island followed by Graciosa. Nevertheless, both studies agree that the smallest islands – Corvo, Graciosa and Santa Maria –, present, as expected, the lowest values of variability. Curiously, in both STRs and surnames analysis, Faial and São Jorge show no difference of diversity and abundance of surnames, this is, both islands are very similar genetically. These results validate the use of surnames as a tool to understand genetic diversity patterns of a population.

Studies of HLA markers in mainland Portugal (Spinola *et al.* 2005a), based on 3 *loci* (A, B and DRB1), and in Azores (Spinola *et al.* 2005b), based in 6 *loci* (A, B, Cw, DRB1, DQA1 and DQB1), demonstrate values of average diversity of 0.92. The results obtained in the present thesis, based in 7 *loci* (A, B, Cw, DRB1, DQA1,

DQB1 and DPA1) presented a smaller value (0.83). Nevertheless, this may be explained by the difference in number of analysed *loci* and by the fact that Spinola *et al.* (2005) used a high-resolution methodology[37] to genotype HLA.

The analysis of relationship between islands was assessed using surnames (Branco and Mota-Vieira 2005) and 15 STR markers (Branco *et al.* 2007, submitted). Two different images appear: surnames show a closer proximity between the Central and Western groups, and the molecular markers give the Central closer to the Eastern group. One hypothesis to explain this discrepancy is that surnames are, probably, revealing more recent movement of individuals. Actually, it is common knowledge that people in the Western group travel more easily to the Central islands than to the Eastern group. On the other hand, the microsatellite data is probably demonstrating a deeper relationship that dates from the time of the settlement. This is corroborated by the software Migrate, which has a methodology based on the coalescent theory. Corvo and Flores were the last islands to be settled. Another observation supporting this information is the fact that in the surname analysis, Faial and Pico, the closest islands, cluster together. Nowadays, there are daily boat connections between these islands. However, this clustering does not happen in the microsatellite data, where São Jorge and Faial are genetically more similar. Historical records mention the presence of Flemish individuals more intensively in these islands. Therefore, we conclude that surnames are evidencing a more recent image showing the socio-economic features of the islands, while the microsatellite data is revealing the evolution based on the settlement characteristics of the archipelago. Both approaches complement each other.

The patterns of genetic diversity of a population have a direct influence in the linkage disequilibrium extent. With the development of technology, analysis of LD has been found to improve the knowledge of human evolution and origin. Moreover, it has also been used to identify genes causing disease. The overall results demonstrate that both the Azoreans and mainland Portugal do not show extensive LD. This may be a direct consequence of the large genetic diversity of these populations. Several studies have demonstrated the use of isolated populations in the characterization of complex diseases (Angius *et al.* 2001; Varillo *et al.* 2000). The

[37] This methodology, which enables an HLA genotyping with a resolution of ≤6 digits (ex. HLA-B510101) is mostly used in transplant medicine.

geography of the archipelago jointly with the cultural background of the Azoreans and the surname analysis seemed to indicate, *à priori*, that the Azoreans were an isolated population. The misleading conclusion from surnames can be explained by the fact that surnames represent only one *locus*. It is common knowledge that for a full characterization of a population it is necessary several *loci*. Moreover, the surnames comparisons were based on countries, some of which with millions of telephone users (Barrai *et al.* 2000, 1999; Mourrieras *et al.* 1995). The overall values obtained from surname data were smaller in the Azores and this induced the conclusion of low diversity and isolation of this population (Branco and Mota-Vieira 2005). Nevertheless, the analysis of surnames in mainland Portugal would be considerably informative in terms of comparison. Despite, the Azores are not an isolated population and show LD only for short physical distances, there are some characteristic that make it a possible resource for future genetic studies, namely, the same environmental conditions and the possibility to construct large pedigrees through church and other civil records. The same environment allows a better control on external factors that may be influencing the development of a complex disease. The large pedigrees permit to develop reliable linkage studies with statistical significance. In summary, the overall data suggest that the identification of identical by descent (IBD) regions surrounding disease susceptibility genes or other complex trait *loci* in the São Miguel, as well as in the Azoreans, will require a very high density of markers. On the other hand, in a near future, the HapMap project will produce data that will considerably increase the power of IBD mapping.

VIII.3. Inbreeding and population structure

The assessment of inbreeding in human populations plays a fundamental role in the identification of population subdivision, which has significant consequences in the design of association mapping and pharmacogenomic studies. Moreover, it is well known that genetic variation is higher within individuals in a population (Tishkoff and Varrelli 2003); therefore, the spectrum of genetic diseases may be influenced by the level of molecular similarity of individuals.

The inbreeding coefficient calculated using surnames for the São Miguel Island (0.0016) is almost seven times smaller than those obtained through 21 STR markers (Branco *et al.* 2007, in press). The STR values of inbreeding could be inflated by the fact that its calculation is based on allele identity, this is, microsatellites that are identical by state may not be from the same ancestor (Rousset 2002). In surname analysis each surname is considered separately and, therefore, this problem is not apparent. Nevertheless, both analysis show that the São Miguel population is outbred. Another study using surnames by Santos *et al.* (2005) shows a value of F_{ST} of 0.00709 for the Flores Island. This value is higher than that obtained in this thesis (0.0038). However, in both studies, the total surnames found are similar, 291 for electoral records (Santos *et al.* 2005) and 223 for telephone users (Branco *et al.* 2005). Probably these differences are explained by the different methods to calculate the same parameter. Both samples do not show microdifferentiation.

The values of inbreeding (F_{IS}) obtained in the *Alu* study report a much higher value for the whole Azorean population (0.117). *Alu* polymorphisms have only two alleles (presence or absence of the insertion); consequently, this reduces the power to detect efficiently the inbreeding. The estimated value for the Azorean sample, based in the analysis of 15 STR markers (Branco *et al.* 2007, submitted), show a similar value (0.0196) to that found in the São Miguel population using 21 STRs (Branco *et al.* 2007, in press), and an higher value when compared with surname analysis (0.0039). Regarding the results of the 15 STRs and surnames for each island, there are some inconsistencies in both approaches (Table VIII.1). The STR markers show that Graciosa is the less inbred followed by Pico. Conversely, Corvo is the more inbred population followed by Flores. The surname analysis shows Graciosa and Santa Maria as being the most inbred islands and São Miguel and Terceira the less inbred. These differences are explained by the nature of the two systems. Once more, surnames simulte one *locus* with several alleles. As mentioned above, the inbreeding estimated through STRs is based on allele identity, and identity by state does not necessarily imply the same ancestor. Therefore, both estimates have problems and no accurate value is retrieved; nonetheless, all analyses demonstrate that the Azorean population is an open population. Additionally, and according to Wright (1984), values smaller than 0.05, such those obtained for both the Azorean and mainland Portugal populations, represent little genetic differentiation. On the other hand, to

assess if there where differences in the allelic composition between islands, which could reveal the presence of population stratification as result of the geography of the archipelago, we performed the analysis of genetic differentiation. The various analyses demonstrate no genetic differentiation between islands, as well as, between the whole Azorean and mainland populations (Table VIII.2).

Furthermore, to detect population structure we used the STRUCTURE software. The analysis was performed varying K, which corresponds to the different source populations, from 2 to 7. The assignment of individuals to K distinct source populations was based on the 21 autosomal STRs (Branco *et al.* 2007, in press; Chapter VII). The results indicate the absence of structure in both São Miguel and mainland Portugal populations (Figure VIII.1). Moreover, we were unable to see a clear clustering of individuals by location, suggesting a high genetic similarity of both populations. These observations are in agreement with other studies

Table VIII.1. Inbreeding coefficient based on surnames and allele frequencies of 15 STR *loci* in all Azorean islands.

Islands	**F_{IS}**	
	Surnames*	**STRs**
São Miguel	0.0033	0.0066
Santa Maria	0.0064	0.0098
Terceira	0.0027	0.0111
Faial	0.0056	0.0200
Pico	0.0048	0.0062
São Jorge	0.0056	0.0328
Graciosa	0.0158	-0.0099
Flores	0.0038	0.0383
Corvo	0.0062	0.0613
Azores (whole)	**0.0039**	**0.0196**

* Surnames results are described in Section V.2 of the present thesis.

(Rosenberg *et al.* 2002; Perez-Lezaun *et al.* 1997), where a high similarity between European populations is reported. The analysis using the STRUCTURE software may not perform well considering a small number of markers, such as, this study (21 STRs); nevertheless, as all data indicate that the Azorean population does not show structure.

Table VIII.2. Genetic differentiation between populations considering 11 autosomal STR markers[38] and Azores as a whole.

Population group	G_{ST}	Min.	Max.
Azores Islands	0.0128	0.0066 (D7S820)	0.0203 (TPOX)
São Miguel			
Santa Maria			
Terceira			
Graciosa			
Faial			
Pico			
São Jorge			
Flores			
Corvo			
Portuguese	0.0079	0.0011 (CSF1PO)	0.0154 (D3S1358)
Azores			
North Portuguese			
Center Portuguese			
Madeirans			
Portuguese (this study)			
Europeans (with Azores)	0.0055	0.0014 (TPOX)	0.0137 (D3S1358)
Azores			
North Spanish			
Andalusians			
Belgian			
Italians			
Europeans (without Azores)	0.0066	0.0020 (TPOX)	0.0136 (D13S317)
Portuguese (this study)			
North Spanish			
Andalusians			
Belgian			
Italians			
Africans (with Azores)	0.0131	0.0026 (vWA)	0.0195 (D3S1358)
Azores			
Moroccans			
Cape Verdeans			
Africans (without Azores)	0.0140	0.0064 (FGA)	0.0249 (D13S317)
Portuguese (this study)			
Moroccans			
Cape Verdeans			
Overall[39]	0.0102	0.0062 (D7S820)	0.0157 (TH01)

[38] Genetic differentiation calculation was based only on 11 STRs (TPOX, D3S1358, FGA, CSF1PO, D5S818, D7S820, D8S1179, TH01, vWA, D13S317 and D18S51), since the information for the remaining microsatellites was not available in ALFRED and other databases as well as in the literature.

[39] The overall group includes the following populations: São Miguel, Santa Maria, Terceira, Graciosa, Faial, Pico, São Jorge, Flores, Corvo, Portuguese, north Portuguese, center Portuguese, Madeirans, Portuguese (this study), north Spanish, Andalusians, Belgian, Italians, Africans, Moroccans and Cape Verdeans.

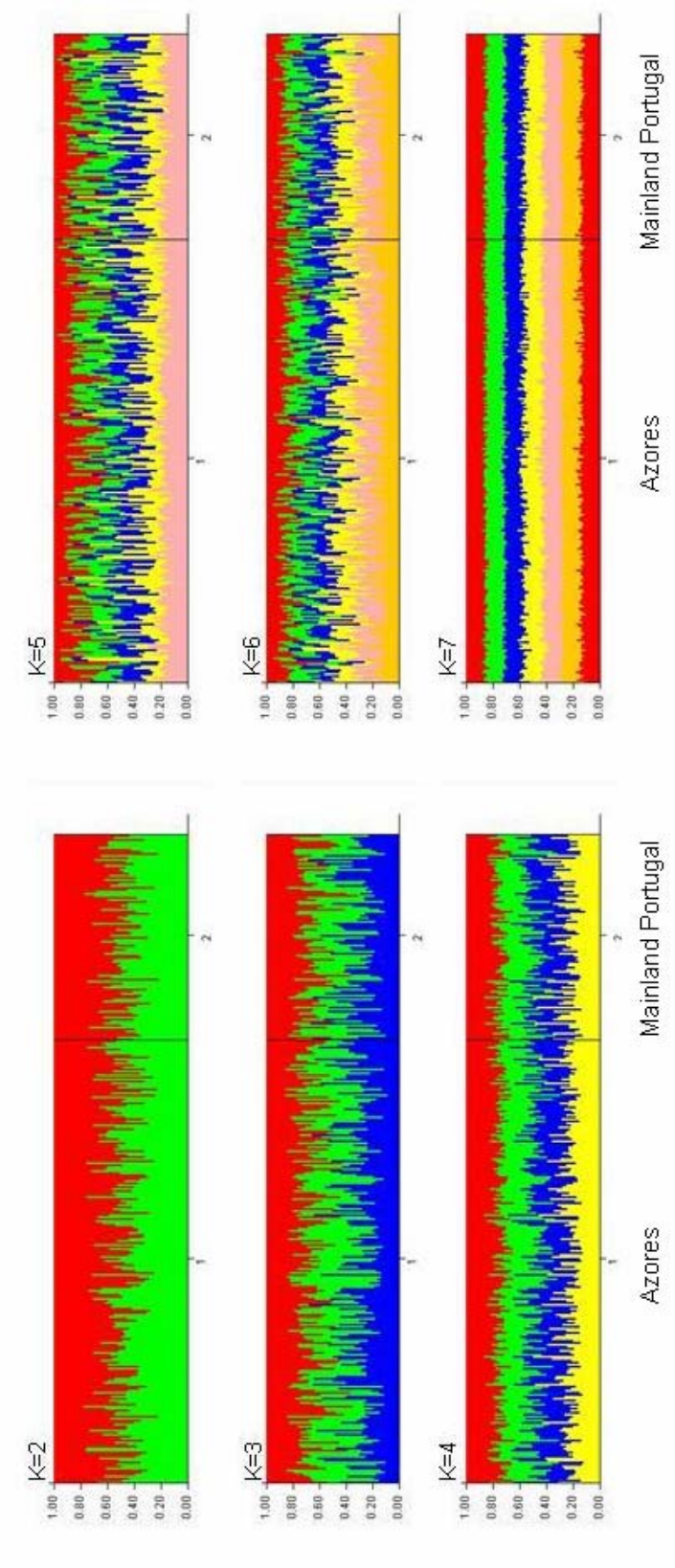


Figure VIII.1. Population structure for the Azorean and mainland Portugal populations based on 21 STR markers. K represents the number of clusters. In all runs, each separate cluster is represented by a colour. The individuals are represented by lines, each partitioned into coloured segments according to the individual's estimated membership fractions.

VIII.4. Gene flow patterns

Migration or gene flow constitutes one important phenomenon that influences the diversity patterns and, consequently, the evolution of populations. The understanding of how individuals disperse within small groups of the same population has significant impact in the establishment of a reference population and, therefore, in medical healthcare, as well as, in the design of genetic studies. Migration rates were estimated initially by surnames and then by microsatellite data. These estimates in both studies evidence the movement of people towards the biggest islands, namely São Miguel and Terceira. However, while surnames point Corvo as the island with the largest migration rate, the microsatellite data show that people in that island have become sedentary. As stressed before, surnames correspond to one *locus*. On the other hand, migration is largely dependent on the abundance of surnames. This parameter is also directly obtained from the isonymy values; therefore, populations with smaller number of diversity of surnames would present higher migration rates. Nevertheless, in general, there is relative gene flow among islanders and this has contributed to the overall genetic background of the Azorean population.

Another study to characterize the patterns of gene flow was the spatial analysis based on surnames. Five different patterns were obtained, of which the most relevant is isolation by distance and depression (41.6%). However, 43.4% of surnames had no defined pattern. This analysis reports a majority of positive values of Moran's *I* for distances lower than 49 km and between 269 and 309 km, indicating high similarity between closer municipalities and between distant municipalities whose populations show historic and socio-cultural affinities, which agrees with the historical demography of the Azorean population (Cabral *et al.* 2005).

To test for the effects of gene flow and genetic drift on population relationships, we performed a centroid analysis, as described by Harpending and Ward (1982), based on *Alu* insertion polymorphisms. Briefly, this model assumes a simple linear relationship between the heterozygosity of a population and the genetic distance of the population from the centroid (ri). The centroid is defined as the mean allelic frequency of the populations. Surprisingly, Moroccans, Catalans, Andalusians, French, Azoreans and mainland Portuguese are located above the theoretical prediction, indicating that these populations have experienced more gene flow than

the average (Figure VIII.2). Low gene flow is indicative of a certain degree of genetic isolation (de Pancorbo *et al.* 2001). According to Batzer *et al.* (1996), European populations are located below the theoretical prediction. In contrast, Azores and mainland Portugal, despite being European populations, are experiencing high gene flow, making them open populations. Moreover, this result also demonstrates that Azores does not show characteristics of an isolated population, albeit it is a mid-Atlantic archipelago. The data also confirm the high variability observed in these two populations. Nonetheless, populations that fall below the theoretical regression line experience significantly more drift. Contrary to what have been suggested by Santos *et al.* (2003), gene flow results show that the Azoreans may not be experiencing genetic drift.

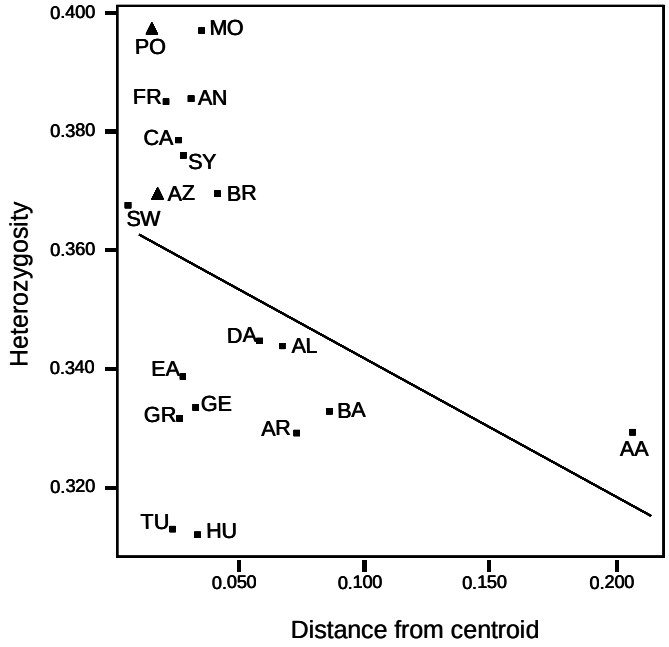


Figure VIII.2. Centroid analysis based on *Alu* frequencies. AZ, Azores; PO, Portugal. AA, African American; AR, Armenian; BA, Bantu Speakers; BR, Bretons; DA, Darginian; EA, European American; FR, French; GE, German; GR, Greek Cypriot; HU, Hungarian; SW, Swiss; SY, Syrians; TU, Turk Cypriot; CA, Catalans; AN, Andalusians; MO, Moroccans; AL, Algerians.

Considering that migration and admixture are intimaly related concepts, we tried to calculate the admixture proportions in the Azorean population. However, the type of markers used to analyse this population are not the best choice considering the available softwares. STRs are highly polymorphic and can not be assigned to specific populations. Because the Azoreans are of European descent, it is very difficult to

define the admixed proportions. Probably, the data produced by the HapMap project will help to make this characterization, once a map of SNPs characteristic to each population will be produced.

VIII.5. Concluding remarks and future perspectives

The main objective of the present thesis was to characterize the genetic background of the Azorean population, through the study of molecular and non-molecular markers. Both markers have advantages and criticisms, but their analysis are complementary. In general, the results obtained along this thesis improved the knowledge of the genetic signature of the Azorean population: the Azoreans are a young outbred population with high genetic diversity, relative gene flow among its individuals, and without extensive LD. Moreover, the overall patterns of diversity are a direct consequence of the archipelago settlement history. In conclusion, the results here reported complement the past, by connecting genetics and history; improve the knowledge of the present, since the genetic background is responsible for the current disease carriage; and will contribute to predict the future in terms of disease distribution and frequency.

The advance in knowledge and technology lead to pose new scientific thoughts and questions and, therefore, the present thesis cannot be considered as the final line in the understanding of the genetic features of the Azorean population. It constitutes a starting point. Knowing that different peoples contributed to the genetic background of this population, questions such as, what are the admixture proportions of each contributor? in which way these proportions are contributing to the neutral genetic variation, as well as, to the disease carriage? what implications these admixture proportions play in farmacogenetic drug-response?, can be addressed. In addition, in a near future, the HapMap project intends to produce a haplotype map showing which haplotypes are characteristic of each population. This will allow the development of admixture mapping marker panels that, applied to the Azorean population, could help to clarify the above questions. Recently, Tang *et al.* (2007), by examining the genome-wide distribution of ancestry in Puerto Ricans, report a strong statistical evidence of recent selection in three chromosomal regions (6p, 8q

and 11q). These authors suggest that admixed populations may constitute powerfull tools in the study of natural selection. This evolutionary force can be responsible by geographical differences in diversity and disease carriage. Moreover, according to Guthery e*t al.* (2007), even if the bulk of alleles underlying complex health-related traits are common SNPs, geographic ancestry might be an important predictor of whether a person carries a risk allele. Therefore, a correct assignment of admixture in the Azoreans may help in the understanding of the patterns of selection and in mapping disease causing genes in this population.

Genetic association studies offer a powerful approach to identify the multiple variants of small effect that modulate susceptibility to complex diseases. However, the lack of data replication indicates that there are many factors influencing gene mapping, namely, natural selection, population admixture, recombination and consanguinity. Pacheco *et al.* (2003) based on marriage records for the period 1931 to 2000 (National Institute of Statistics) demonstrated that Azores presents higher consanguinity than mainland Portugal and Madeira Islands. Because consanguinity increases homozygosity, the assessment of the extent of homozygosity tracts in proximate regions of highly informative markers, such as STRs, could contribute to understand the role of consanguinity in this population. For example, it may be involved in the increase of complex disease frequencies, such as congenital heart diseases (Cabral *et al.* 2007) and autism (Oliveira *et al.* 2007). Therefore, a full characterization of the forces acting in the genetic background of Azoreans will probably play a relevant role in the understanding of the genomic basis of diseases in this population.

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APPENDIXES

Appendix IX.1 Allele frequencies for 21 STR *loci* in São Miguel and mainland Portugal populations.

		Frequency				Frequency	
Locus	**Allele**	**São Miguel**	**m. Portugal**	***Locus***	**Allele**	**São Miguel**	**m. Portugal**
TPOX				**D7S820**			
	7	0.0030	-		7	0.0303	0.0310
	8	0.4880	0.4450		8	0.1250	0.1300
	9	0.1110	0.1030		9	0.1250	0.1530
	10	0.0660	0.0710		10	0.2600	0.2820
	11	0.2840	0.3260		11	0.2330	0.1980
	12	0.0480	0.0550		12	0.1910	0.1600
D3S1358					13	0.0330	0.0380
	13	0.0080	-		14	0.0027	0.0080
	14	0.1150	0.1020	**D8S1179**			
	15	0.2490	0.2520		8	0.0109	0.0080
	16	0.2290	0.2520		9	0.0272	0.0230
	17	0.2380	0.2280		10	0.0842	0.0920
	18	0.1420	0.1340		11	0.1060	0.0840
	19	0.0190	0.0320		12	0.1277	0.0990
D19S433					13	0.2636	0.2970
	11	0.0028	-		14	0.2174	0.2210
	12	0.0028	-		15	0.1549	0.1070
	12.2	0.0028	-		16	0.0054	0.0460
	13	0.0223	0.0080		17	0.0027	0.0230
	13.2	0.1260	-	**D17S976**			
	14	0.2150	0.1310		19.3	0.1873	0.1430
	15	0.0084	0.2460		20	-	0.0070
	15.2	0.3240	0.0230		21	0.0379	0.0430
	16	0.0140	0.3380		21.3	0.0076	-
	16.2	0.1731	0.0150		22	0.1114	0.0570
	17	0.0220	0.1310		23	0.1089	0.1210
	17.2	0.0614	0.0540		24	0.0810	0.0930
	18	0.0170	0.0460		25	0.0456	0.0570
	19	0.0084	0.0080		26	0.0304	0.0640
D18S51					27	0.0456	0.0790
	11	0.0103	0.0350		27.3	0.0709	0.0710
	12	0.0026	-		28.3	0.0557	0.0930
	13	0.1211	0.1280		29.3	0.0911	0.0500
	14	0.1366	0.1210		30	-	0.0220
	15	0.1366	0.1420		30.3	0.0532	0.0140
	16	0.1366	0.0920		31.3	0.0405	0.0430
	17	0.1134	0.1350		32.3	0.0152	0.0220
	18	0.1392	0.1700		34	0.0101	0.0140
	19	0.1082	0.0710		35	0.0076	0.0070
	20	0.0412	0.0430				
	21	0.0412	0.0280				
	22	0.0052	0.0140				
	23	0.0052	0.0140				
	24	0.0026	0.0070				

Appendix IX.1 *cont.*

		Frequency				Frequency	
Locus	**Allele**	**São Miguel**	**m. Portugal**	***Locus***	**Allele**	**São Miguel**	**m. Portugal**
CSF1PO				**D13S317**			
	7	0.0055	0.0080		8	0.1142	-
	8	0.0055	0.0080		9	0.0668	0.0980
	9	0.0222	0.0160		10	0.0529	0.0900
	10	0.2740	0.2890		11	0.2896	0.0680
	11	0.3380	0.3280		12	0.2396	0.2410
	12	0.2990	0.3280		13	0.1616	0.2930
	13	0.0503	0.0230		14	0.0641	0.1800
	14	0.0055	-		15	0.0056	0.0300
D5S818					16	0.0056	-
	8	0.0100	-	**D14S306**			
	9	0.0440	0.0300		1	0.0028	0.0150
	10	0.1320	0.0680		2	0.0055	0.0380
	11	0.4730	0.3080		3	0.0305	0.1150
	12	0.3070	0.3160		4	0.1470	0.2670
	13	0.0340	0.2630		5	0.2880	0.1220
	14	-	0.0150		6	0.1523	0.2750
FGA					7	0.2576	0.1450
	17	0.0027	-		8	0.1025	0.0150
	18	0.0054	-		9	0.0083	0.0080
	19	0.0458	0.0900		10	0.0055	-
	20	0.1833	0.1430	**vWA**			
	20.2	0.0108	-		14	0.1084	0.0070
	21	0.1430	0.1430		15	0.1599	0.1590
	21.2	0.0054	0.0150		16	0.2358	0.1300
	22	0.1560	0.1500		17	0.2791	0.2320
	22.2	0.008	0.0220		18	0.1626	0.1740
	23	0.1810	0.1810		19	0.0434	0.1960
	23.2	0.0027	-		20	0.0108	0.1020
	24	0.1510	0.1650	**TNFα**			
	25	0.0752	0.0530		1	0.0260	0.0220
	25.2	0.0027	-		2	0.2120	0.1870
	26	0.0135	0.0220		3	0.0110	0.0310
	27	0.0108	0.0080		4	0.1130	0.0750
	28	0.0027	0.0080		5	0.0590	0.1120
D22S417					6	0.1160	0.1640
	3	-	0.0220		7	0.1140	0.0890
	4	0.2059	0.2520		8	0.0130	0.0070
	5	0.1711	0.2300		9	0.0230	0.0370
	6	0.0989	0.0890		10	0.1710	0.1420
	7	0.2086	0.1330		11	0.1110	0.0820
	8	0.1096	0.0520		12	-	0.0070
	9	0.0348	0.0520		13	0.0230	0.0450
	10	0.0856	0.1410		14	0.0080	-
	11	0.0561	0.0220				
	12	0.0187	-				
	13	0.0107	0.0070				

Appendix IX.1 *cont.*

		Frequency				Frequency	
Locus	**Allele**	**São Miguel**	**m. Portugal**	***Locus***	**Allele**	**São Miguel**	**m. Portugal**
D6S265				**D20S161**			
	2	0.1460	0.1340		14	0.0063	0.0080
	3	0.0110	-		17	0.2453	0.2600
	4	0.3230	0.3230		18	0.4591	0.3950
	5	0.2220	0.1340		19	0.2358	0.2440
	6	0.2470	0.1570		20	0.0283	0.0760
	7	0.0080	0.1260		21	0.0252	0.0170
	10	-	-		11	0.1110	0.0820
	11	0.0060	-		12	-	0.0070
	12	0.0370	0.1260		13	0.0230	0.0450
					14	0.0080	-
FES/FPS				**D10S525**			
	8	0.0094	0.0080		3	0.2240	0.1490
	9	0.0032	0.0240		4	0.3170	0.3640
	10	0.3070	0.3310		5	0.4210	0.4210
	11	0.3861	0.3390		6	0.0380	0.0580
	12	0.2342	0.2660		7	-	0.0080
	13	0.0569	0.0240	**D16S539**			
	14	0.0032	0.0080		8	0.0310	0.0220
TH01					9	0.1140	0.0880
	6	0.2270	0.1780		10	0.0510	0.0880
	7	0.1970	0.0890		11	0.3160	0.2430
	8	0.1330	0.1850		12	0.2650	0.2870
	9	0.2240	0.2340		13	0.2010	0.2210
	9.3	0.2080	0.3060		14	0.0190	0.0510
					15	0.0030	-

Appendix IX.2. Allele frequencies for 15 STR *loci* in all Azorean islands.

Markers	**Islands**								
	São Miguel	**Santa Maria**	**Terceira**	**Faial**	**Pico**	**São Jorge**	**Graciosa**	**Flores**	**Corvo**
D3S1358	**N=207**	**N=93**	**N=187**	**N=99**	**N=123**	**N=85**	**N=85**	**N=130**	**N=54**
13	0.0048	-	-	0.0101	-	-	-	-	-
14	0.1256	0.1630	0.1016	0.0909	0.0984	0.1294	0.1548	0.1000	0.0556
15	0.2319	0.3043	0.3155	0.2222	0.2049	0.2589	0.2500	0.2692	0.2222
16	0.2173	0.2501	0.1818	0.3233	0.1803	0.2353	0.3095	0.2154	0.3889
17	0.2319	0.1630	0.2460	0.2121	0.2787	0.2235	0.2024	0.2385	0.2037
18	0.1546	0.1087	0.1444	0.1414	0.2049	0.1294	0.0833	0.1615	0.1296
19	0.0242	0.0109	0.0107	-	0.0328	0.0235	-	0.0077	-
20	0.0097	-	-	-	-	-	-	0.0077	-
TPOX	**N=192**	**N=81**	**N=169**	**N=89**	**N=102**	**N=85**	**N=83**	**N=127**	**N=44**
6	0.0052	0.0123	0.0059	-	-	-	-	0.0236	-
7	-	-	0.0059	-	-	-	-	-	-
8	0.4896	0.5062	0.4438	0.4831	0.5098	0.5059	0.3976	0.5039	0.5000
9	0.0781	0.1728	0.1479	0.1685	0.0392	0.1412	0.0964	0.0394	0.1591
10	0.0521	0.0247	0.0533	0.0787	0.0882	0.0353	0.0964	0.0945	0.2500
11	0.3281	0.2717	0.2840	0.2472	0.3334	0.2705	0.3614	0.2835	0.0909
12	0.0417	0.0123	0.0592	0.0225	0.0294	0.0353	0.0482	0.0551	-
13	0.0052	-	-	-	-	0.0118	-	-	-
D21S11	**N=205**	**N=98**	**N=183**	**N=94**	**N=126**	**N=95**	**N=89**	**N=135**	**N=51**
24.2	0.0049	-	-	-	-	0.0105	-	0.0296	-
27	0.0341	0.0102	-	0.0532	0.0238	0.0421	-	0.0074	-
28	0.1561	0.2041	0.1366	0.2021	0.1508	0.1579	0.0899	0.2074	0.2353
29	0.2341	0.1327	0.1913	0.2766	0.2461	0.3263	0.2921	0.2742	0.2942
30	0.2341	0.2857	0.2624	0.1703	0.2064	0.1368	0.2022	0.1407	0.1569
30.2	0.0488	0.0204	0.0546	0.0319	0.0079	0.0316	0.0112	0.0889	0.0784
31	0.0634	0.0612	0.0874	0.0319	0.0476	0.0737	0.1461	0.0222	-
31.2	0.0976	0.1531	0.1257	0.1596	0.1270	0.0737	0.1124	0.1333	0.0392
32	0.0098	0.0204	0.0109	-	0.0079	0.0211	-	0.0074	-
32.2	0.0878	0.0714	0.1038	0.0638	0.1429	0.0947	0.1124	0.0593	0.1176
33.2	0.0293	0.0408	0.0273	0.0106	0.0317	0.0105	0.0337	0.0296	0.0784
34.2	-	-	-	-	0.0079	0.0211	-	-	-
TH01	**N=206**	**N=97**	**N=186**	**N=95**	**N=115**	**N=92**	**N=90**	**N=135**	**N=50**
6	0.2039	0.1443	0.1882	0.2105	0.2609	0.2609	0.2778	0.1926	0.1000
7	0.2039	0.2371	0.2043	0.1368	0.1652	0.1087	0.1778	0.1407	0.2200
8	0.1019	0.0722	0.1505	0.1263	0.1478	0.1413	0.0889	0.1704	0.2200
9	0.2087	0.2165	0.1828	0.1895	0.1565	0.2065	0.2333	0.2370	0.0400
9.3	0.2816	0.3196	0.2634	0.3369	0.2696	0.2717	0.2222	0.2593	0.4200
10	-	0.0103	0.0108	-	-	0.0109	-	-	-

Appendix IX.2. *cont.*

Markers	Islands								
	São Miguel	**Santa Maria**	**Terceira**	**Faial**	**Pico**	**São Jorge**	**Graciosa**	**Flores**	**Corvo**
D5S818	**N=196**	**N=84**	**N=182**	**N=91**	**N=118**	**N=87**	**N=74**	**N=133**	**N=50**
7	0.0204	-	0.0055	0.0110	0.0169	-	-	-	-
8	0.0102	-	0.0110	-	-	0.0115	-	0.0075	-
9	0.0255	0.0357	0.0604	0.0110	0.0339	0.0230	0.0270	0.0526	0.0200
10	0.1020	0.0476	0.0659	0.0330	0.0339	0.0690	0.0270	0.0677	0.2000
11	0.3215	0.2976	0.4012	0.3516	0.3644	0.2414	0.2703	0.3534	0.3600
12	0.3520	0.4286	0.2967	0.3846	0.3644	0.4482	0.4595	0.2932	0.2600
13	0.1480	0.1786	0.1538	0.1978	0.1780	0.1379	0.2027	0.2030	0.1600
14	0.0153	0.0119	0.0055	0.0110	0.0085	0.0690	0.0135	0.0226	-
15	0.0051	-	-	-	-	-	-	-	-
D13S317	**N=202**	**N=98**	**N=180**	**N=92**	**N=118**	**N=87**	**N=83**	**N=132**	**N=49**
8	0.0990	0.1327	0.1333	0.1522	0.0847	0.1149	0.1325	0.1061	0.0816
9	0.0495	0.1020	0.0444	0.0761	0.1017	0.0575	0.0843	0.0758	0.0816
10	0.0396	0.0510	0.0389	0.0870	0.0678	0.0920	0.0120	0.0682	0.2245
11	0.3316	0.2449	0.3500	0.2935	0.2458	0.2184	0.3013	0.2802	0.1633
12	0.2624	0.2858	0.2779	0.2608	0.3136	0.3103	0.3013	0.3560	0.3878
13	0.1485	0.0918	0.1111	0.0978	0.1525	0.0575	0.1325	0.0758	0.0408
14	0.0644	0.0918	0.0444	0.0326	0.0339	0.1034	0.0241	0.0379	0.0204
15	0.0050	-	-	-	-	0.0460	0.0120	-	-
D16S539	**N=196**	**N=94**	**N=183**	**N=88**	**N=113**	**N=89**	**N=83**	**N=128**	**N=54**
8	0.0255	0.0426	0.0164	0.0341	0.0531	0.0674	0.0120	0.0313	-
9	0.1276	0.1383	0.1530	0.0795	0.0885	0.0899	0.1687	0.1094	0.1296
10	0.0357	0.0426	0.1148	0.0455	0.0531	0.1124	0.0723	0.0781	0.0185
11	0.3214	0.2765	0.2732	0.3750	0.2212	0.3708	0.1446	0.2812	0.2407
12	0.2602	0.2872	0.2459	0.2727	0.3894	0.2247	0.3735	0.2187	0.2778
13	0.1990	0.1809	0.1475	0.1818	0.1593	0.0899	0.1928	0.2422	0.2778
14	0.0306	0.0213	0.0492	0.0114	0.0354	0.0449	0.0361	0.0391	0.0556
15	-	0.0106	-	-	-	-	-	-	-
D8S1179	**N=208**	**N=89**	**N=192**	**N=95**	**N=124**	**N=93**	**N=91**	**N=140**	**N=52**
8	0.0242	0.0112	0.0052	0.0211	-	-	-	-	0.0192
9	0.0097	-	0.0052	0.0105	-	0.0108	0.0330	0.0143	0.0192
10	0.0773	0.0674	0.1094	0.0947	0.1290	0.1290	0.0659	0.1286	0.1154
11	0.1256	0.1573	0.1458	0.0632	0.1048	0.1075	0.0440	0.0357	0.0577
12	0.0966	0.1124	0.1406	0.1368	0.1532	0.1183	0.2198	0.1571	0.1346
13	0.2464	0.2922	0.2552	0.2947	0.2662	0.2796	0.2636	0.2929	0.3463
14	0.2222	0.1685	0.1615	0.2000	0.1935	0.1720	0.2198	0.2429	0.1538
15	0.1787	0.1910	0.1458	0.1579	0.1452	0.1613	0.1209	0.1214	0.1538
16	0.0145	-	0.0313	0.0211	0.0081	0.0215	0.0330	0.0071	-
17	0.0048	-	-	-	-	-	-	-	-

Appendix IX.2. *cont.*

Markers	**Islands**								
	São Miguel	**Santa Maria**	**Terceira**	**Faial**	**Pico**	**São Jorge**	**Graciosa**	**Flores**	**Corvo**
D18S51	**N=208**	**N=93**	**N=195**	**N=100**	**N=122**	**N=95**	**N=86**	**N=145**	**N=56**
9	-	-	-	-	-	-	-	0.0069	0.0179
10	0.0144	0.0430	0.0102	0.0100	0.0164	-	0.0465	0.0138	-
11	-	0.0108	0.0154	-	-	0.0105	-	-	-
12	0.1154	0.1183	0.1487	0.2000	0.1639	0.1579	0.1744	0.1655	0.1428
13	0.1442	0.1398	0.1590	0.1300	0.1311	0.1579	0.1744	0.1586	0.0357
14	0.1442	0.1934	0.1795	0.1300	0.1639	0.1158	0.0349	0.1862	0.0893
15	0.1298	0.0968	0.1026	0.1600	0.1721	0.1158	0.1628	0.1034	0.1071
16	0.1394	0.1290	0.1333	0.1300	0.1066	0.1684	0.1512	0.1655	0.0893
17	0.1250	0.1505	0.1026	0.1000	0.1066	0.1158	0.1396	0.0414	0.3036
18	0.1058	0.0538	0.0821	0.0500	0.0574	0.1053	0.0581	0.0414	-
19	0.0385	0.0323	0.0462	0.0800	0.0492	0.0421	0.0349	0.0690	0.1964
20	0.0385	0.0215	0.0102	-	0.0164	-	0.0116	0.0345	0.0179
21	0.0048	0.0108	0.0102	-	-	0.0105	0.0116	0.0069	-
22	-	-	-	0.0100	0.0082	-	-	0.0069	-
24	-	-	-	-	0.0082	-	-	-	-
CSF1PO	**N=195**	**N=85**	**N=169**	**N=84**	**N=106**	**N=83**	**N=75**	**N=121**	**N=46**
7	0.0103	0.0118	0.0118	-	-	-	0.0267	-	-
8	0.0051	-	0.0178	-	0.0094	0.0120	0.0133	-	-
9	0.0205	0.0353	0.0178	0.0595	-	0.0241	0.0133	0.0083	-
10	0.2717	0.2000	0.2663	0.2976	0.3208	0.3013	0.2933	0.3058	0.3261
11	0.3385	0.2470	0.3077	0.3452	0.3208	0.3012	0.3734	0.3718	0.2826
12	0.2821	0.4117	0.2958	0.2144	0.2641	0.3013	0.2667	0.2149	0.1739
13	0.0718	0.0471	0.0414	0.0595	0.0377	0.0361	0.0133	0.0579	0.1739
14	-	0.0353	0.0296	0.0238	0.0472	0.0120	-	0.0413	0.0435
15	-	0.0118	0.0118	-	-	0.0120	-	-	-
D7S820	**N=210**	**N=98**	**N=186**	**N=97**	**N=116**	**N=92**	**N=83**	**N=136**	**N=53**
7	0.0238	0.0408	0.0108	0.0103	0.0345	-	0.0120	0.0074	0.0566
8	0.1619	0.1531	0.1398	0.0928	0.1638	0.1087	0.1808	0.2206	0.1321
9	0.1095	0.0918	0.1022	0.0928	0.1034	0.1413	0.1325	0.1029	0.1509
10	0.2715	0.2245	0.2687	0.2990	0.2759	0.2934	0.2530	0.2794	0.3019
11	0.2095	0.2143	0.2742	0.2887	0.1983	0.2283	0.2048	0.1985	0.2453
12	0.1857	0.2551	0.1559	0.1649	0.1810	0.1848	0.1808	0.1544	0.0943
13	0.0333	0.0204	0.0484	0.0515	0.0431	0.0326	0.0241	0.0368	-
14	0.0048	-	-	-	-	0.0109	0.0120	-	0.0189

Appendix IX.2. *cont.*

Markers	**Islands**								
	São Miguel	**Santa Maria**	**Terceira**	**Faial**	**Pico**	**São Jorge**	**Graciosa**	**Flores**	**Corvo**
VWA	**N=206**	**N=92**	**N=187**	**N=99**	**N=126**	**N=92**	**N=84**	**N=135**	**N=52**
12	0.0049	-	-	-	-	-	-	-	-
13	0.0049	-	-	-	-	-	-	-	-
14	0.1068	0.1087	0.1123	0.1515	0.1349	0.1196	0.1190	0.1407	0.0385
15	0.1408	0.1304	0.1337	0.1111	0.1190	0.1196	0.1429	0.1630	0.1346
16	0.2572	0.3152	0.2353	0.2021	0.2143	0.1413	0.1667	0.2148	0.2692
17	0.2330	0.1848	0.2620	0.2525	0.2540	0.2608	0.2380	0.2296	0.3078
18	0.1795	0.1848	0.1551	0.1919	0.1667	0.2935	0.1905	0.1556	0.1538
19	0.0534	0.0435	0.0856	0.0808	0.0952	0.0543	0.1429	0.0815	0.0769
20	0.0146	0.0326	0.0160	0.0101	0.0159	0.0109	-	0.0148	0.0192
21	0.0049	-	-	-	-	-	-	-	-
FGA	**N=213**	**N=93**	**N=191**	**N=98**	**N=124**	**N=95**	**N=90**	**N=137**	**N=57**
16	0.0047	-	-	-	-	-	-	-	-
17	-	-	0.0052	-	0.0080	0.0105	-	-	-
18	0.0094	-	0.0105	0.0102	0.0080	0.0211	-	-	-
19	0.0423	0.0430	0.0524	0.0714	0.0565	0.0105	0.1667	0.0219	0.0877
19.2	-	-	-	-	0.0081	-	-	-	-
20	0.1690	0.1290	0.1780	0.1735	0.1855	0.1895	0.2000	0.1825	0.2105
21	0.1548	0.1936	0.1624	0.1327	0.1532	0.1158	0.1333	0.1897	0.1930
21.2	-	0.0108	0.0052	-	0.0161	-	-	-	-
22	0.1690	0.1720	0.1518	0.1837	0.1694	0.2631	0.1333	0.2044	0.1404
22.2	0.0141	-	0.0157	-	-	-	-	0.0073	-
23	0.2113	0.2043	0.1204	0.1735	0.1371	0.1053	0.1333	0.0730	0.1053
23.2	-	0.0215	-	0.0102	0.0081	0.0105	0.0111	0.0438	0.0175
24	0.1174	0.0860	0.1675	0.1020	0.1532	0.1158	0.1223	0.1533	0.0877
24.2	-	0.0108	-	0.0204	-	-	-	0.0146	-
25	0.0798	0.1075	0.0838	0.0714	0.0565	0.1053	0.0556	0.0876	0.1228
25.2	0.0047	-	-	0.0204	-	-	-	-	-
26	0.0141	0.0215	0.0419	0.0306	0.0242	0.0421	0.0444	0.0146	0.0351
27	0.0094	-	0.0052	-	0.0161	-	-	-	-
28	-	-	-	-	-	0.0105	-	-	-
30	-	-	-	-	-	-	-	0.0073	-

Appendix IX.2. *cont.*

Markers	**Islands**								
	São Miguel	**Santa Maria**	**Terceira**	**Faial**	**Pico**	**São Jorge**	**Graciosa**	**Flores**	**Corvo**
Penta-E	**N=208**	**N=98**	**N=192**	**N=100**	**N=120**	**N=89**	**N=87**	**N=137**	**N=54**
5	0.0433	0.0714	0.1198	0.0900	0.0917	0.1011	0.0805	0.0730	0.0185
6	-	-	0.0104	0.0100	-	-	0.0115	0.0146	-
7	0.1731	0.0918	0.1406	0.1700	0.1417	0.2135	0.1954	0.0876	0.0370
8	0.0240	0.0408	0.0104	0.0100	0.0083	0.0112	-	0.0073	-
9	0.0192	-	0.0156	0.0200	0.0417	0.0112	0.0115	0.0438	-
10	0.1010	0.0816	0.0885	0.1000	0.0500	0.1124	0.0805	0.0730	0.1297
11	0.1490	0.1225	0.1303	0.1500	0.1417	0.0787	0.1608	0.1387	0.0741
12	0.1732	0.1838	0.1979	0.1900	0.2000	0.2472	0.1954	0.2335	0.3519
13	0.1394	0.0816	0.0573	0.1100	0.1750	0.0899	0.1724	0.0949	0.1112
14	0.0288	0.1123	0.0625	0.0300	0.0333	0.0225	-	0.0803	0.1296
15	0.0529	0.0816	0.0313	0.0100	0.0083	0.0337	-	0.0365	0.0370
16	0.0240	0.0306	0.0365	0.0300	0.0583	0.0562	0.0230	0.0511	0.0370
17	0.0337	0.0408	0.0625	0.0200	0.0417	0.0112	0.0345	0.0657	0.0370
18	0.0096	0.0204	0.0052	0.0200	-	-	-	-	0.0185
19	0.0192	0.0306	0.0260	0.0400	0.0083	-	0.0345	-	0.0185
20	-	0.0102	-	-	-	0.0112	-	-	-
21	0.0048	-	0.0052	-	-	-	-	-	-
22	0.0048	-	-	-	-	-	-	-	-
Penta-D	**N=207**	**N=97**	**N=182**	**N=88**	**N=114**	**N=90**	**N=84**	**N=134**	**N=56**
2.2	0.0097	-	0.0055	-	-	-	0.0119	-	0.0357
5	0.0097	-	0.0055	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	0.0357
8	0.0145	0.0206	0.0220	0.0682	0.0175	0.0111	0.0119	0.0224	0.0357
9	0.2222	0.2062	0.1648	0.1819	0.2455	0.1444	0.2738	0.1493	0.1964
10	0.1014	0.1031	0.1319	0.1136	0.1667	0.1222	0.0595	0.1940	0.0714
11	0.1159	0.1031	0.1538	0.2045	0.1404	0.1222	0.1310	0.1716	0.1964
12	0.2029	0.2990	0.1978	0.1591	0.1316	0.2000	0.2500	0.1791	0.2322
13	0.2464	0.1649	0.2033	0.2386	0.2281	0.2667	0.1905	0.1791	0.1607
14	0.0676	0.0825	0.0989	0.0227	0.0614	0.0667	0.0714	0.0746	0.0179
15	0.0097	0.0103	0.0055	0.0114	0.0088	0.0667	-	0.0299	0.0179
16	-	0.0103	0.0110	-	-	-	-	-	-

Appendix IX.3 Allele frequencies for 8 STR *loci* located on the X-chromosome in all Azorean islands and mainland Portugal.

Markers	**Populations**									
	São Miguel N=185	**Santa Maria** N=22	**Terceira** N=54	**Faial** N=25	**Pico** N=29	**São Jorge** N=23	**Graciosa** N=19	**Flores** N=35	**Corvo** N=16	**m.Portugal** N=97
DXS986										
1	-	-	-	-	-	0.0526	-	-	-	-
3	-	-	-	-	-	0.0526	-	-	-	0.0103
4	-	-	0.0435	0.0400	0.0345	-	-	-	-	-
5	0.0703	0.0703	0.1304	0.0800	0.0690	-	0.1429	-	0.0909	0.0722
6	0.0541	0.0541	-	0.0400	-	0.1053	0.1714	-	0.0909	0.0619
7	0.4216	0.4216	0.4782	0.4000	0.5171	0.3158	0.3429	0.1875	0.7273	0.4948
8	0.2108	0.2108	0.2174	0.2400	0.2414	0.4211	0.1714	0.3125	0.0909	0.2474
9	0.0595	0.0595	-	0.0400	0.0345	-	0.0286	0.1250	-	-
10	0.0162	0.0162	-	-	-	-	-	-	-	-
11	0.0378	0.0378	0.0435	0.0400	-	-	-	0.0625	-	-
12	0.0865	0.0865	0.0435	-	0.0690	-	0.0571	0.0625	-	0.0619
13	0.0270	0.0270	0.0435	0.0400	0.0345	-	0.0857	0.2500	-	0.0103
14	0.0054	0.0054	-	0.0800	-	-	-	-	-	0.0309
15	0.0108	0.0108	-	-	-	0.0526	-	-	-	0.0103
DXS1225										
1	0.0162	-	-	-	0.0345	-	-	0.0625	-	0.0103
2	0.2109	0.2407	0.1304	0.1600	0.1379	0.1579	0.1713	0.0625	0.0909	0.2372
3	0.0054	-	-	-	-	-	-	-	-	-
4	0.0162	-	-	-	-	-	-	-	0.0455	-
5	0.1839	0.1852	0.1304	0.2000	0.0690	0.1579	0.1429	0.0625	0.1817	0.1340
6	0.0054	0.0185	-	-	0.0345	-	-	-	-	0.0103
7	0.0270	0.0741	0.1304	0.0800	0.0690	0.1053	0.1429	-	-	0.0515
8	0.0162	-	-	0.0400	-	-	-	0.2500	-	0.0103
9	0.0054	0.0185	-	0.1200	-	-	0.0571	0.0625	-	0.0206
10	0.0216	0.0185	0.0870	0.0400	0.0345	0.1053	0.0286	-	0.0455	0.0103
11	0.4054	0.3890	0.3913	0.2800	0.4482	0.4210	0.4000	0.3750	0.5000	0.4640
12	0.0162	0.0185	0.0435	0.0800	0.0345	0.0526	-	-	0.0455	0.0103
13	0.0108	0.0185	-	-	0.0345	-	0.0286	0.1250	0.0909	0.0103
14	0.0486	0.0185	0.0870	-	0.1034	-	0.0286	-	-	0.0309
15	0.0108	-	-	-	-	-	-	-	-	-

Appendix IX.3 *cont.*

Markers	Populations									
	São Miguel N=185	**Santa Maria** N=22	**Terceira** N=54	**Faial** N=25	**Pico** N=29	**São Jorge** N=23	**Graciosa** N=19	**Flores** N=35	**Corvo** N=16	**m.Portugal** N=97
DXS8082										
1	0.0054	-	-	-	-	-	-	0.0625	-	-
2	-	-	-	-	-	-	-	-	-	0.0103
3	0.0486	0.0926	0.0870	0.0800	0.0690	0.1053	0.1714	0.2500	-	0.0515
4	-	-	-	-	-	-	-	-	-	0.0103
5	0.0108	0.0185	0.0870	-	0.0345	-	-	-	-	0.0103
6	0.0054	-	-	0.1200	0.0345	-	-	0.0625	-	0.0103
7	0.4162	0.4260	0.4346	0.4000	0.5171	0.4736	0.5144	0.5625	0.6363	0.4641
8	0.1892	0.1111	0.1739	0.0400	0.1379	0.1053	0.0571	-	0.0909	0.1237
9	0.0216	-	-	0.0400	0.0345	-	-	-	-	0.0309
10	0.0270	-	-	0.0400	-	-	0.0286	-	0.1364	0.0103
11	0.0595	0.1481	0.0870	0.1200	0.0690	0.1579	0.0857	0.0625	-	0.0309
12	0.1514	0.1296	0.0870	0.1200	0.0690	0.1053	0.0857	-	0.0455	0.1959
13	0.0649	0.0556	0.0435	0.0400	0.0345	0.0526	0.0571	-	0.0909	0.0309
14	-	0.0185	-	-	-	-	-	-	-	0.0206
DXS8092										
1	0.0054	-	-	-	-	-	-	-	-	0.0103
2	-	-	-	-	-	-	-	-	-	0.0103
3	0.0324	0.0185	-	-	0.0345	0.0526	-	-	0.0909	0.0515
4	0.0541	0.1111	0.2609	0.0400	0.0345	0.0526	0.1143	-	0.1818	0.0928
5	0.0973	0.0926	0.1303	0.0400	0.1379	0.1579	0.0286	0.1875	0.0455	0.1031
6	0.1838	0.2037	0.2174	0.2800	0.1724	0.4212	0.1714	0.1250	-	0.1031
7	0.2109	0.2964	-	0.2800	0.2414	0.0526	0.1429	0.3125	0.0455	0.2062
8	0.1081	0.1296	-	0.1200	0.1379	0.0526	0.2285	0.1875	0.0455	0.1753
9	0.1135	0.0370	0.2174	0.0800	0.1379	0.1053	0.1714	0.0625	0.3180	0.1134
10	0.1189	0.0185	0.0870	-	-	0.0526	0.0857	-	0.0909	0.0722
11	0.0432	-	0.0870	0.1600	0.0690	0.0526	0.0286	0.1250	0.1364	0.0412
12	0.0216	0.0556	-	-	0.0345	-	0.0286	-	0.0455	0.0206
13	0.0054	0.0370	-	-	-	-	-	-	-	-
14	0.0054	-	-	-	-	-	-	-	-	-
DXS995										
2	-	-	0.0435	0.0800	-	-	-	-	-	0.0103
3	0.6054	0.5741	0.6522	0.5600	0.5517	0.5790	0.6857	0.5000	0.5909	0.6083
4	0.0486	0.0556	-	0.0800	-	-	0.0286	-	0.0455	-
5	0.2649	0.2593	0.3043	0.2000	0.4138	0.2105	0.2286	0.1250	0.3636	0.3505
6	0.0703	0.1110	-	0.0800	0.0345	0.2105	0.0571	0.3750	-	0.0309
7	0.0108	-	-	-	-	-	-	-	-	-

Appendix IX.3 *cont.*

Markers	Populations									
	São Miguel N=185	**Santa Maria** N=22	**Terceira** N=54	**Faial** N=25	**Pico** N=29	**São Jorge** N=23	**Graciosa** N=19	**Flores** N=35	**Corvo** N=16	**m.Portugal** N=97
DXS8037										
4	-	0.0185	-	-	0.0345	-	-	-	-	-
6	0.0649	0.0741	-	0.0400	-	0.0526	0.2286	0.2500	0.0909	0.0412
8	0.0162	0.0370	-	0.0400	-	-	0.0286	-	-	0.0103
9	0.0108	-	0.0435	-	-	-	-	-	-	0.0103
10	0.1243	0.0926	0.2174	0.1600	0.1724	0.0526	0.2286	0.0625	0.1364	0.1443
11	0.4324	0.4815	0.3913	0.4800	0.4482	0.5264	0.2856	0.1875	0.5454	0.4743
12	0.2757	0.1667	0.3478	0.2400	0.2759	0.3684	0.2286	0.3750	0.2273	0.2371
13	0.0649	0.0370	-	0.0400	0.0690	-	-	0.1250	-	0.0619
14	0.0108	0.0926	-	-	-	-	-	-	-	0.0206
DXS1066										
1	0.0108	0.0370	-	0.0400	-	0.0526	-	0.0625	-	0.0206
2	0.0162	0.0185	-	0.0400	-	-	-	-	-	0.0103
3	0.7405	0.8704	0.8261	0.7200	0.7931	0.5263	0.7143	0.5625	0.7727	0.7114
4	0.1514	0.0556	0.1739	0.1200	0.1379	0.2632	0.2000	0.3125	0.1818	0.1649
5	0.0811	-	-	0.0800	0.0690	0.1579	0.0857	0.0625	0.0455	0.0722
6	-	0.0185	-	-	-	-	-	-	-	0.0206
DXS983										
1	-	0.0185	-	0.0400	0.0345	-	-	-	0.0455	0.0103
2	0.1622	0.1852	0.1304	0.2400	0.1379	0.1053	0.1143	0.1250	0.1818	0.1134
3	0.0054	-	-	-	-	-	-	-	-	0.0206
4	-	-	-	-	-	-	-	-	-	0.0309
5	0.1243	0.0741	0.3043	0.1600	0.2069	0.1579	0.0571	0.1875	0.0455	0.2165
6	0.4865	0.4815	0.3479	0.2400	0.3793	0.4210	0.5429	0.4375	0.2727	0.3506
7	0.2108	0.2407	0.1739	0.2800	0.2414	0.3158	0.2286	0.1875	0.4545	0.2165
8	0.0108	-	0.0435	0.0400	-	-	0.0571	0.0625	-	0.0309
9	-	-	-	-	-	-	-	-	-	0.0103

Appendix IX.4. HLA class I and II allele frequencies in São Miguel population (the highest values are in bold).

Alleles	Allele frequencies %	Alleles	Allele frequencies %
HLA-A (2n=212)		**HLA-B (2n=212)**	
A*01	0.151	B*07	0.066
A*02	**0.250**	B*08	0.137
A*03	0.094	B*13	0.005
A*11	0.042	B*14	0.071
A*23	0.019	B*15	0.052
A*24	0.137	B*18	0.052
A*25	0.005	B*27	0.042
A*26	0.009	B*35	0.061
A*29	0.066	B*37	0.014
A*30	0.033	B*38	0.014
A*31	0.024	B*39	0.009
A*32	0.061	B*40	0.028
A*33	0.028	B*41	0.024
A*66	0.005	**B*44**	**0.156**
A*68	0.071	B*45	0.009
A*80	0.005	B*47	0.005
HLA-Cw (2n=212)		B*49	0.052
Cw*01	0.024	B*50	0.033
Cw*02	0.066	B*51	0.066
Cw*03	0.075	B*53	0.024
Cw*04	0.104	B*55	0.019
Cw*05	0.071	B*57	0.042
Cw*06	0.090	B*58	0.014
Cw*07	**0.311**	B*78	0.005
Cw*08	0.052	**HLA-DPA1 (2n=212)**	
Cw*12	0.047	**DPA1*01**	**0.462**
Cw*14	0.019	DPA1*0103	0.255
Cw*15	0.047	DPA1*0105	0.005
Cw*16	0.071	DPA1*0201	0.226
Cw*17	0.024	DPA1*0202	0.042
		DPA1*0301	0.009

Appendix IX.4 *cont.*

Alleles	Allele frequencies %	Alleles	Allele frequencies %
HLA-DPB1 (2n=212)		**HLA- DRB1 (2n=212)**	
DPB1*0101	0.057	DRB1*01	0.085
DPB1*0201	0.212	DRB1*03	0.165
DPB1*0202	0.014	DRB1*04	0.123
DPB1*0301	0.080	**DRB1*07**	**0.170**
DPB1*0401	**0.316**	DRB1*08	0.028
DPB1*0402	0.094	DRB1*09	0.019
DPB1*0501	0.014	DRB1*10	0.019
DPB1*0601	0.005	DRB1*11	0.118
DPB1*0901	0.005	DRB1*12	0.009
DPB1*1001	0.028	DRB1*13	0.146
DPB1*1101	0.024	DRB1*14	0.019
DPB1*1301	0.052	DRB1*15	0.075
DPB1*1401	0.014	DRB1*16	0.024
DPB1*1501	0.005	**HLA- DQB1 (2n=212)**	
DPB1*1601	0.005	DQB1*02	0.302
DPB1*1701	0.038	**DQB1*03**	**0.321**
DPB1*1901	0.014	DQB1*04	0.028
DPB1*2501	0.005	DQB1*05	0.151
DPB1*3901	0.005	DQB1*06	0.198
DPB1*5101	0.005		
DPB1*6601	0.005		
DPB1*7801	0.005		

Appendix IX.5. Publications on the Azorean population (adapted from PubMed, August 27, 2007).

Authors	Title	Journal
POPULATION GENETICS		
Neto D, Montiel R, Bettencourt C, *et al*.	The African contribution to the present-day population of the Azores Islands (Portugal): Analysis of the Y-chromosome haplogroup E.	*Am J Hum Biol*. 2007. DOI: 10.1002/ajhb.20651
Service S, DeYoung J, Karayiorgou M, *et al.*	Magnitude and distribution of linkage disequilibrium in population isolates and implications for genome-wide association studies.	*Nat Genet.* 2006 38: 556 560.
Branco CC, Palla R, Lino S, *et al.*	Assessment of Azorean ancestry by *Alu* insertion polymorphisms.	*Am J Hum Biol*. 2006 18 (2): 223-6.
Santos C, Abade A, Cantons J, *et al.*	Genetic structure of Flores island (Azores, Portugal) in the 19th century and in the present day: evidence from surname analysis.	*Hum Biol*. 2005 77 (3): 317-41.
Fernando O, Mota P, Lima M, *et al.*	Peopling of the Azores Islands (Portugal): data from the Y-chromosome.	*Hum Biol*. 2005 77 (2): 189-99.
Cabral R, Branco CC, Costa S, *et al.*	Geography of surnames in the Azores: specificity and spatial distribution analysis.	*Am J Hum Biol*. 2005 17 (5): 634-45.
Branco CC, Mota-Vieira L.	Surnames in the Azores: analysis of the isonymy structure.	*Hum Biol*. 2005 77 (1): 37-44.
Spinola H, Brehm A, Bettencourt B, *et al.*	HLA class I and II polymorphisms in Azores show different settlements in Oriental and Central islands.	*Tissue Antigens*. 2005 66 (3): 217-30.
Santos C, Montiel R, Sierra B, Bettencourt C, *et al.*	Understanding differences between phylogenetic and pedigree-derived mtDNA mutation rate: a model using families from the Azores Islands (Portugal).	*Mol Biol Evol*. 2005 22 (6): 1490-505.
Pacheco PR, Branco CC, Cabral R, *et al.*	The Y-chromosomal heritage of the Azores Islands population.	*Ann Hum Genet*. 2005 69 (Pt 2): 145-56.
Montiel R, Bettencourt C, Silva C, *et al.*	Analysis of Y-chromosome variability and its comparison with mtDNA variability reveals different demographic histories between islands in the Azores Archipelago (Portugal).	*Ann Hum Genet*. 2005 69 (Pt 2): 135-44.
Santos C, Montiel R, Angles N, *et al.*	Determination of human caucasian mitochondrial DNA haplogroups by means of a hierarchical approach.	*Hum Biol*. 2004 76 (3): 431-53.
Goncalves R, Freitas A, Branco M, *et al.*	Y-chromosome lineages from Portugal, Madeira and Acores record elements of Sephardim and Berber ancestry.	*Ann Hum Genet*. 2005 69 (Pt 4): 443-54.
Branco CC, Mota-Vieira L.	Population structure of Sao Miguel Island, Azores: a surname study.	*Hum Biol*. 2003 75 (6): 929-39.
Santos C, Lima M, Montiel R, *et al.*	Genetic structure and origin of peopling in the Azores islands (Portugal): the view from mtDNA.	*Ann Hum Genet*. 2003 67 (Pt 5): 433-56.
Couto AR, Peixoto MJ, Garrett F, *et al.*	Linkage disequilibrium between S65C HFE mutation and HLA A29-B44 haplotype in Terceira Island, Azores.	*Hum Immunol*. 2003 64 (6): 625-8.
Brehm A, Pereira L, Kivisild T, *et al.*	Mitochondrial portraits of the Madeira and Acores archipelagos witness different genetic pools of its settlers.	*Hum Genet*. 2003 114 (1): 77-86.
Bruges-Armas J, Martinez-Laso J, Martins B *et al.*	HLA in the Azores Archipelago: possible presence of Mongoloid genes.	*Tissue Antigens*. 1999 54 (4): 349-59.
Smith MT, Abade A, Cunha EM.	Genetic structure of the Azores: marriage and inbreeding in Flores.	*Ann Hum Biol*. 1992 19 (6): 595-601.
FORENSIC GENETICS		
Carvalho M, Anjos MJ, Andrade L, *et al.*	Y-chromosome STR haplotypes in two population samples: Azores Islands and Central Portugal.	*Forensic Sci Int*. 2003 134 (1): 29-35.
Fernandes A, Brehm A.	Y-chromosome STR haplotypes in the Acores Archipelago (Portugal).	*Forensic Sci Int*. 2003 135 (3): 239-42.
Fernandes AT, Brehm A.	Population data of five STRs in three regions from Portugal.	*Forensic Sci Int*. 2002 129 (1): 72-4.
Velosa RG, Fernandes AT, Brehm A.	Genetic profile of the Acores Archipelago population using the new PowerPlex 16 system kit.	*Forensic Sci Int*. 2002 129 (1): 68-71.

Authors	Title	Journal
Corte-Real F, Souto L, Anjos MJ, *et al.*	Population study of HUMTH01, HUMVWA31/A, HUMF13A1, and HUMFES/FPS systems in Azores.	*J Forensic Sci*. 1999 44 (6): 1261-4.
Brito RM, Ribeiro T, Espinheira R, *et al.*	South Portuguese population data on the *loci* HLA-DQA1, LDLR, GYPA, HBGG, D7S8 and Gc.	*J Forensic Sci*. 1998 43 (5): 1031-6.
ATAXIAS		
Gonzalez C, Lima M, Kay T, *et al.*	Short-term psychological impact of predictive testing for Machado-Joseph disease: depression and anxiety levels in individuals at risk from the Azores (Portugal).	*Community Genet*. 2004 7 (4): 196-201.
Lima M, Kay T, Vasconcelos J, *et al.*	Disease knowledge and attitudes toward predictive testing and prenatal diagnosis in families with Machado-Joseph disease from the Azores Islands (Portugal).	*Community Genet*. 2001 4 (1): 36-42.
Lima M, Smith MT, Silva C, *et al.*	Natural selection at the MJD *locus*: phenotypic diversity, survival and fertility among Machado-Joseph Disease patients from the Azores.	*J Biosoc Sci*. 2001 33 (3): 361-73.
Lima M, Mayer FM, Coutinho P, *et al.*	Origins of a mutation: population genetics of Machado-Joseph disease in the Azores (Portugal).	*Hum Biol*. 1998 70 (6): 1011-23.
Lima M, Coutinho P, Abade A, *et al.*	Causes of death in Machado-Joseph disease: a case-control study in the Azores (Portugal).	*Arch Neurol.* 1998 55 (10): 1341-4.
Friedman JH.	Machado-Joseph disease/spinocerebellar ataxia 3 responsive to buspirone.	*Mov Disord.* 1997 12 (4): 613-4.
Lima M, Mayer F, Coutinho P, *et al.*	Prevalence, geographic distribution, and genealogical investigation of Machado-Joseph disease in the Azores (Portugal).	*Hum Biol*. 1997 69 (3): 383-91.
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