ANCIENT AND RECENT DEMOGRAPHIC EVENTS

INFLUENCE MITOCHONDRIAL DNA DIVERSITY

IN AN IMMIGRANT BASQUE POPULATION

by

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The following individuals read and discussed the thesis submitted by student Michael Christopher Davis, and they also evaluated his presentation and response to questions during the final oral examination. They found that the student passed the final oral examination, and that the thesis was satisfactory for a master's degree and ready for any final modifications that they explicitly required.

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DEDICATION

To the memory of Dr. Adriel Johnson (1957-2010), my undergraduate advisor and professor of Biology at the University of Alabama in Huntsville. His life of service, teaching, and research was (and remains) an example for us all.

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ABSTRACT

The Basques are an ancient people, considered by many anthropologists to represent the oldest extant European population. Because of this, they have been the subject of numerous sociological and biological investigations. The Basque Diaspora, a relatively recent demographic expansion of the Basque population, has until now been overlooked in molecular genetic studies. Samples were taken from 53 individuals with Basque ancestry in Boise, Idaho, and the mitochondrial DNA (mtDNA) sequence variation of the first and second hypervariable regions were determined. Thirty-six mtDNA haplotypes were detected in the sample. Comparing the genetic diversity in the Idaho sample with other Basque populations, signatures of founder effects were observed, consistent with both the recent and ancient history of Basque mitochondrial lineages. There has been a marked alteration of haplogroup frequency and diversity, and there is a slight reduction in other measures of diversity in the immigrant Basque population sampled compared to the native Basque population. I have found a relatively high percentage of the Cambridge Reference Sequence (rCRS) haplotype for hypervariable regions I and II, which is absent in previous studies of Basque mtDNA, and rare in other Spanish populations. The amount of nucleotide diversity is consistent with a sample that is predominantly haplogroup H, which is especially common in the Basque regions of Europe, due to ancient migrations and expansions out of glacial refugia. This is the first report of mtDNA diversity in an immigrant Basque population, and I find that the diversity in Basques of the Northwestern U.S.A. can be explained by the recent

history of migration, as well as the phylogeography and diversity of the major European

haplogroups.

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INTRODUCTION

Migration and colonization events have genetic consequences (Dobzhansky and Wright 1943; Slatkin 1985). Colonization events can lead to an alteration and/or reduction in genetic diversity (i.e., genetic drift) through founder effects or genetic bottlenecks (Nei et al. 1975). Alternatively, sufficient gene flow (via immigration) can counter the force of genetic drift (Novak and Mack 2005). These consequences are influenced by several factors: the level and structure of genetic diversity within and among native populations, founder population size, and the number of discrete migration/colonization events that have occurred (Novak 2010). The demography of *Homo sapiens* has been characterized by recent population growth and multiple expansions out of Africa (Jobling et al. 2004) and colonization events of various magnitudes (Ramachandran et al. 2005; Richards et al. 2000). The genetic signatures of ancient migrations have been studied with phylogeographic analyses—studies of the patterns of genetic diversity and differentiation within and between major populations, and the geographic distribution of genetic lineages (Avise 2000). The genetic consequences of relatively recent migrations and colonization have been studied in cultural and religious population isolates (Adams et al. 2008; Puffenberger 2003), island communities (Santos et al. 2003), the forced migration of African slaves (Salas et al. 2004), and the European colonization of the Americas (Bedoya et al. 2006; Salas et al. 2008). The Basque are an ethnic group inhabiting northeastern Spain and southwestern France, and are considered by some researchers to have the greatest degree of genetic

continuity with the "original" Europeans, Paleolithic hunter-gatherers (Bertranpetit et al.
1995; Douglass and Bilbao 1975; Iriondo et al. 2003; Richards et al. 2000). One of the
most distinguishing characteristics of the Basque is their language (Euskara), a non-Indo-
European isolate that has resisted classification. Although once hypothesized to be
related to ancient Egyptian (Chambers 1844), and now considered by some to be
connected with Caucasian languages, Euskara is not currently considered a member of
any extant language family (Arnaiz-Villena et al. 1999). Since language families tend to
group along with ethnic categories (Cavalli-Sforza and Cavalli-Sforza 1995), it was
natural to propose a genetic discontinuity for the Basques, given such a unique language.
Studies of Basque genetic diversity began with observations of extreme frequencies of
specific blood types in Basque populations both in Europe and in America (Boyd and
Boyd 1937; Etcheverry 1945). Basque populations are characterized by the relatively
high frequency of Rh-negative blood types, among the highest in Europe (Boyd and Boyd
1937; Chalmers et al. 1949), and a relatively low frequency of type B blood (Alberdi et
al. 1957; Nijenhuis 1956). Because of these features, the Basque became one of the most
thoroughly studied populations in Europe, hypothesized to be a genetically isolated
remnant of an ancient population (Calafell and Bertranpetit 1994; Cavalli-Sforza 1988).
The results of numerous studies based on protein polymorphisms (Calderon et al. 1998;
Comas et al. 1998; Manzano et al. 1996) and molecular genetic markers (Alonso and
Armour 1998; Calderon et al. 2003; de Pancorbo et al. 2001; Iriondo et al. 1997; Zlojutro
et al. 2006) have demonstrated that the Basque are indeed genetic outliers, yet only in the
context of the European gene pool: more related to neighboring Europeans than other
world populations (Garagnani et al. 2009; Laayouni et al. 2010; Li et al. 2008). Although

the evidence does not support genetic isolation, there is evidence for an ancient (Paleolithic) origin of the Basque ancestors. Recent mitochondrial DNA (mtDNA) studies indicate that certain Basque lineages (in haplogroup U8a) have their origin in the Upper Paleolithic, approximately 30,000 years BP, with evidence for a subsequent re-expansion approximately 12,000 years BP (Gonzalez et al. 2006). Other lineages that reach their highest density in the Basque region (haplogroups H1, H3) also date from the latter period (Achilli et al. 2004). These findings, as well as the clinal distribution of H subhaplogroups across Europe, provide support for the hypothesis that the ancestral Basque population(s) originated from populations expanding their range from glacial refugia (Achilli et al. 2004). Although a recent study based on entire mtDNA sequences questions the Franco-Cantabrian refugia hypothesis (Garcia et al. 2010), there is in fact supportive evidence from studies of other species. Genetic evidence for post-glacial expansions from Iberian refugia has been reported for species across multiple taxa, including: plants (Grivet and Petit 2002), mammals (Melo-Ferreira et al. 2007; Michaux et al. 2003), birds (Griswold and Baker 2002), reptiles (Guicking et al. 2008), and amphibians (Rowe et al. 2006). In addition, there is evidence for multiple refugia within Iberia and other regions of Northwestern Europe (Vialatte et al. 2008), and the situation for many species is certainly more complex than a large-scale expansion from a single refugium (Gomez and Lunt 2007). It is likely that future molecular investigations will reveal additional complexity in human post-glacial phylogeography. Whether or not ancestral Basque populations emerged from single or multiple refugia, these results have dispelled theories of Basque origins linking them to modern Africans or Asians, Ancient Egyptians, or Neanderthals (Chambers 1844; Izagirre et al. 2001; Kurlansky 1999).

There is a long history of Basque immigration to the Americas: cod fishing settlements in 16th century Newfoundland (Lehuenen 1984), cattle ranches in 17th and 18th century South America, and sheep herding in the 19th century American West (Douglass and Bilbao 1975). Southwest Idaho, U.S.A. (including the cities of Boise, Mountain Home, Gooding, and Shoshone) is famous for its large Basque community, comprised primarily of migrants from the province of Bizkaia (Viscaya), Spain (Edlefsen 1948). According to Edlefsen (1948), approximately 90% of Southwest Idaho Basques trace their origins to this part of the Basque country. Within Bizkaia, it is the region roughly circumscribed by the cities of Gernika, Lekeitio, and Ondarroa (Figure 1), that contributed about 85% of all Bizkaian emigrants to the U.S.A., according to a 1917 survey by Silen (Douglass and Bilbao 1975). In 2000, there were 6,637 individuals (0.51 % of population) in Idaho who identified themselves as Basque in the census (Census 2000), giving Idaho the highest per capita Basque population of any state. The immigrant Basques of the Northwestern U.S.A. are thus an ideal study population for assessing the genetic consequences of immigration from a well-defined source population. It is worth noting that in 1945 one of the earliest published accounts of Basque blood type frequencies was made by Etcheverry, a physician in Argentina (Etcheverry 1945). Only two other studies have been published on the genetics of American Basque populations: another paper by Etcheverry (Etcheverry 1947), and a blood typing paper of two Basque populations in Idaho by Laughlin *et al*. (1956). The Idaho study was based upon the dissertation research of Margery Gray (Gray 1955).

Mitochondrial DNA has been used in numerous studies to explore the level and structure of genetic variation within human populations, and to shed light on the

complexities of human evolution (Achilli et al. 2004; Richards et al. 2000; Torroni et al. 2001). Mitochondrial DNA has the advantage of being non-recombining, always inherited through the maternal lineage, and containing non-coding "hypervariable" regions that accumulate mutations at an approximately ten-fold rate (on average) compared to coding regions (Brown et al. 1979; Stoneking 1994; Upholt and Dawid 1977). In addition, since the early 1990s, numerous studies on human mtDNA have been carried out, and the literature and data on human mtDNA is sizable (Parson and Dür 2007; Torroni et al. 2006). However, despite several studies of mtDNA variation in European Basque populations (Alfonso-Sanchez et al. 2008; Bertranpetit et al. 1995; Corte-Real et al. 1996; Gonzalez et al. 2006; Richard et al. 2007), no mtDNA research has previously been conducted on an immigrant population from the Basque Diaspora. The presence of a substantial population of Basque immigrants in the Northwestern U.S.A. allows specific hypotheses associated with the genetic consequences of migration and colonization events to be tested (Nei et al. 1975). In this study, I have: determined the sequence diversity for mtDNA hypervariable segments 1 and 2 (HVS-1 and HVS-2) in a representative sample of NW Basques; compared the diversity of NW Basques to that of Basques living within the historical Basque regions in Spain and France; and, determined the magnitude of founder effects in the NW Basque. I have also examined previously published genetic data in other American immigrant populations (and their putative European sources), and noted a trend in the relationships between immigration histories and founder effects.

SUBJECTS AND METHODS

Participants

Subjects for this study were selected from among volunteers using a protocol modeled

after Bertranpetit *et al.* (1995). Subjects were recruited from Basque cultural events held

in Boise, Idaho. I administered a questionnaire to determine each volunteer's place of

birth, the place of birth of their mother and maternal grandmother, and the surnames in

each volunteer's maternal lineages (see Appendix). The surname data were used to select

subjects that were maternally unrelated for at least two generations. Buccal swabs and

completed questionnaires were collected from volunteers who had identified themselves

as Basque. To be included in this study, each subject was required to have been born in

the U.S.A., and to have Basque maiden names going back at least two generations. Since

all volunteers were either born or had immediate family in Idaho, Nevada, Oregon, and

California, the sample was designated as Northwestern U.S.A. Basques (hereafter

referred to as NW Basques). The use of human subjects for this study was approved by

the Institutional Review Board of Boise State University, and the confidentiality of

personal information for each volunteer was assured (no personal identifying information

will be published). Based on these criteria, 53 subjects who met the study criteria were

included in this analysis.

DNA Extraction and Sequencing

DNA was extracted from each buccal swab using the QIAamp DNA Mini Kit (Qiagen).

Mitochondrial DNA hypervariable regions HVS-1 and HVS-2 were amplified using

published primers (Wilson et al. 1995) purchased from Integrated DNA Technologies. Dye-labeled primer sequencing was carried out on the PCR products using the Thermo Sequenase Cycle Sequencing Kit (USB) according to the manufacturer's protocol, and fragments were examined with the LI-COR 4300 DNA Analyzer (LI-COR Biosciences). To confirm sequence accuracy, every sample was sequenced at least twice, either using both strands of the PCR product (forward and reverse sequencing primers) or by re-sequencing with the same primer. All polymorphisms were confirmed by at least double-coverage of the sequencing data. Sequence analysis of gel-based data was carried out on E-Seq software (for reading gel images and sequence editing) and AlignIR software (for pairwise alignment against the reference sequence), both from LI-COR Biosciences. Multiple sequence alignments (for data analysis, see below) were performed using Clustal X as implemented in MEGA 4.1 (Kumar et al. 2008). Polymorphisms were determined by comparing experimental data with the revised Cambridge Reference Sequence (rCRS) (Andrews et al. 1999). Note that the rCRS has recently (2009) been annotated in the NCBI nucleotide database as the GenBank "reference sequence." The rCRS, used for a reference in population and forensic studies, is listed in the NCBI nucleotide database as NC_012920.1 (formerly it was listed as the "alternative Refseq" AC_000021.2). Previously, the mitochondrial sequence represented in the NCBI reference sequence (Refseq) database for the human genome project was accession number NC_001807, a mitochondrial genome from a Yoruba (African) individual. For this study, the definition of the first and second hypervariable segments (HVS-1 and HVS-2) were defined as nucleotide positions 16024-16383 and 66-370, respectively, following Alfonso-Sanchez *et al.* (2008).

Genetic Data Analysis

<u>Genetic Diversity</u>

The sequences obtained from each subject were used to infer haplogroup membership by the neighbor-joining method of Behar *et al.* (2007), which was shown to correctly predict the haplogroup for a European dataset 97% of the time. The level of mtDNA diversity within the NW Basque population was estimated by counting the number of haplotypes (k), the number of polymorphic sites (S), and calculating the haplotype (h), nucleotide (π), and haplogroup diversity over the aligned control region sequence data. These parameters were calculated using Arlequin 3.1 (Excoffier et al. 2005), and are the standard intrapopulation diversity measures for haplotypic sequence data (Nei 1987). Haplotype and haplogroup diversity are computed as Nei (1987) defines "gene diversity," representing the probability of randomly picking two sequences from non-identical haplogroups (or haplotypes) in a sample set. Likewise, nucleotide diversity is the probability that a randomly chosen nucleotide site on two randomly chosen sequences will be different (Nei and Kumar 2000).

We characterized nucleotide diversity using several estimates of θ: a measure of expected genetic diversity based on the formula $\theta = 2N_{fe}\mu$, where N_{fe} is the female effective population size and μ is the mutation rate (Helgason et al. 2000). The mean number of pairwise differences between sequences within a population is given by θ_π, which is the estimator for θ given the infinite-sites model of nucleotide evolution (Tajima 1983). The statistics θ_s and θ_k are additional estimators: θ_s is based on the number of polymorphic sites found in the set of sample sequences, and θ_k is based on the number of observed haplotypes (Watterson 1974).

Genetic Distances Between Populations

Arlequin was used to compute pairwise genetic distance values within and between populations, and generate pairwise mismatch distributions. A principal components analysis (PCA) was performed on the haplogroup frequency data, using SAS version 9.0 software, SAS System for Windows (SAS Institute). The results of the PCA were visualized on a biplot of the first two principal components. For admixed populations (with Amerindian and African maternal contributions), only frequencies of European-specific haplogroups were taken into account. Where haplogroup data was given for sub-haplogroups (such as, U1, U2, U3, etc.), those frequencies were combined to give the frequency of the entire haplogroup. A neighbor-joining (NJ) tree was generated with the program MEGA 4.1, using the pairwise distance matrix of Reynold's linearized F_{ST} (coancestry coefficients). This measure is determined by comparing frequencies of shared haplotypes (Reynolds et al. 1983).

Genetic Signatures of Population History

The signatures of demographic history for the NW Basques were evaluated using three techniques: quantitative test statistics, mismatch distributions, and time to the most recent common ancestor (TMRCA). Tajima's D (TD) and Fu's F_s are used to detect either the signature of selection, or in the case of neutral molecular markers, the signature of recent population expansion (Fu and Li 1993). Both tests assume that population expansion will produce an excess of rare singleton mutations. Tajima's D compares the average number of pairwise differences versus the number of segregating sites, while F_s tests this relationship using the distribution of haplotypes (Fu and Li 1993). Both

statistics were calculated in Arlequin 3.1 with 1000 random permutations. The pairwise

mismatch distributions calculated in Arlequin were compared to the expected

distributions (given the sequence length and number of segregating sites) for expanding

and stationary populations (Schneider and Excoffier 1999). The distributions of all

Basque populations were compared with each other; and Harpending's raggedness

statistic (rg) was calculated along with the 95% confidence interval in DnaSP 5.0

(Librado and Rozas 2009), to check for significant deviations from the expected

distributions.

Haplotype Networks

Haplotype networks for HVS-1 alone, and the combined HVS-1 and HVS-2 regions,

were constructed with Network 4.5 (Fluxus Engineering, http://fluxus-engineering.com)

(Bandelt et al. 1999). To generate reduced-median networks, the reduction threshold was

set to 2.0, the "frequency >1 criterion" was inactivated, and the relatively slowly

mutating sites (73, 263, 16208, and 16318) were given a three-fold greater weighting than

the other substitution sites. The mutation hotspots ("speedy sites") that are known to

confound phylogenetic analyses (Bandelt et al. 2002) were ignored in constructing the

network. Each distinct haplotype is represented by a node in the network, and the

mutation sites separating them are indicated on the connecting edges. Node size is

proportional to the number of individuals sharing the haplotype. The HVS-1 haplotype

network includes data from the French Basque region (Richard et al. 2007), Spanish

Basque (Alfonso-Sanchez et al. 2008), and the NW Basque (this study). The Spanish

Basque sample was differentiated by province of sample origin: Bizkaia or Gipuzkoa,

based on supplemental data from Alfonso-Sanchez *et al.* (2008); therefore, this sample

will be referred to as the "BG Basque." For the TMRCA analysis, the centrally located

and most common haplotype (263G) was set as the ancestral node, and all haplotype

nodes for a given population were selected as descendants. The rho statistic was

calculated, and was converted to an estimate of the age of the network (given an

estimated mutation rate of 1 base in the two hypervariable regions per 20,000 yrs)

(Saillard et al. 2000). The degree of divergence from the ancestral node is a time

estimate that is affected by changes in effective population size, which is expected to be

reduced following a population bottleneck (Nei et al. 1975).

C-stretch length variability and common indels such as 315.1C were observed but

not scored, because of the difficulty in sequencing through C-stretch regions, which do

not contain an intervening thymine (Bendall and Sykes 1995; Butler 2005). Since

misalignment errors have been reported because of highly variable C-stretch lengths (Yao

et al. 2009), it seemed prudent to ignore a small amount of data rather than risk systemic

errors in the analysis. Thus, for all diversity calculations and interpopulation

comparisons, HVS-2 positions 305-315 were ignored.

Besides searching the European mitochondrial population database (EMPOP, Parson

and Dür [2007]), the following reference populations were used for comparative

analyses: the Old Order Amish of Ohio and Indiana (van der Walt et al. 2005), the

Acadians and Channel Islanders of the Gaspe peninsula, Québec (Moreau et al. 2009),

three Spanish Basque populations (Alfonso-Sanchez et al. 2008; Bertranpetit et al. 1995;

Corte-Real et al. 1996), the Catalonians, English, and Swedes (Torroni et al. 2001), the

Ibiza Islanders (Picornell et al. 2005), the French (Dubut et al. 2004), French Basques

(Richard et al. 2007), the Portuguese (Pereira et al. 2004), Russians (Malyarchuk et al. 2002), Austrians and North American Hispanics (Budowle et al. 1999), Germans (Richards et al. 2000), Brazilians (Alves-Silva et al. 2000), Non-Basque Spanish (Alvarez et al. 2007), Cubans (Mendizabal et al. 2008), Puerto Ricans (Martinez-Cruzado et al. 2001), the Welsh (Piercy et al. 1993), Galicians (Salas et al. 1998), Albanians (Bosch et al. 2006), Tuscans (Francalacci et al. 1996), Icelanders (Helgason et al. 2000), Svanetian Georgians (Alfonso-Sanchez et al. 2006), and Angolans (Plaza et al. 2004). Although genetic diversity statistics have been published for most of these populations, all values were re-calculated from the sequence data in Arlequin, since C-stretch indels were ignored for this analysis.

RESULTS

I analyzed fifty-three samples that met the criteria for inclusion in the NW

Basque study. There were approximately equal numbers of maternally unrelated male

and female subjects, and the average age was 49.6 years. Of the 53 subjects, 38 (71.7%)

could trace their maternal lineages to Spain. Of those 38, twenty could specify their

grandmother's place of birth as Bizkaia, where historically most Basques of SW Idaho

trace their ancestry (Figure 1). Of the 20 with Bizkaian maternal origins, eleven reported

a birthplace for their grandmother that was in the region of NE Bizkaia. Two individuals

could trace their lineage back to the French Basque region, one from "France," and three

could trace their maternal line to California and no further. The number of individuals

with Bizkaian ancestry is lower than in previous studies focusing on Basques in Idaho

(Arrizabalaga 1986; Edlefsen 1948; Laughlin et al. 1956); however, it must be noted that

an Idaho birthplace was not a requirement for inclusion in this study.

mtDNA Diversity in Immigrant and European Basques

Among the 53 individuals analyzed in this study, 23 distinct HVS-1 region (359 bp)

haplotypes were observed, with 29 different polymorphic sites (Figure 2). Of these, 19

individuals (35.8%) shared a haplotype identical to the rCRS. Fifty percent of the NW

Basque HVS-1 haplotypes were private (private haplotypes are defined as not being

previously reported in EMPOP nor in the literature search for this paper); the other fifty

percent were observed at varying frequencies in several European populations (based on EMPOP and literature cited in the discussion). Transversions (compared to the reference sequence) accounted for 3 of the 29 polymorphic sites (10.3%).

Considering both hypervariable regions (665 bp), I observed a total of 36 distinct lineages (haplotypes), characterized by 54 polymorphic sites (Figure 3). As with HVS-1 alone, fifty percent of haplotypes were private. A relatively high percentage of individuals (four out of 53, or 7.5%) have the rCRS haplotype.

The NW Basque population has lower nucleotide and haplogroup diversity than native Basque, native European, and other European-derived populations (considering only European-specific mtDNA lineages) (Tables 1 and 2). The populations with the lowest diversity measures generally are the Basque and other Iberian populations. Considering only HVS-1, four out of the five populations with the lowest diversity (in terms of haplotype and nucleotide diversity, and θ_S and θ_k) are Basque, the exception being the Acadians of the Gaspe peninsula, Québec—a population that had relatively few initial settlers, and has experienced serial founder events (Moreau et al. 2009).

When considering both HVS-1 and HVS-2, the number of distinct haplotypes increases for all populations, and there is less of a difference between populations in terms of haplotype diversity (*h*) (Table 2). Although the two Basque populations listed here have lower diversity indices than the other populations, the NW Basques do not have significantly different values of *h* or π than the BG Basque population (Z-test for comparison of means, per Nei and Kumar (2000). The estimator θ_π also shows the NW Basques have the lowest diversity.

All European populations, and all European-derived American populations, have significantly negative values of both TD and F_s (Tables 1 and 2). The only population in this analysis that has a value not significantly different from zero ($p < 0.05$) is the Angolan population (Tables 1 and 2). There is a trend of slightly less negative values for relatively isolated populations such as the Basques, Icelanders, and the Svanetian Georgians. This trend may exist because greater isolation means an increased probability for the genetic diversity of a population to be influenced by genetic drift—which would result in a loss of low-frequency polymorphisms.

The distributions of pairwise mismatches were calculated for the NW Basque and various Spanish Basque populations, for HVS-1 alone, and HVS-1 and HVS-2 combined (Figure 4). The mean of the mismatch distribution is equivalent to the average pairwise difference between sequences (estimated by θ_π). Note that the pairwise mismatch distribution for the NW Basques for HVS-1 and HVS-2 exhibited a distinctly ragged appearance.

Haplogroup H is present at a much higher frequency in the NW Basque (~80%) than the Spanish Basque or other Iberian populations (Figure 5). In addition, there has been a loss in the NW Basques of some of the lower-frequency haplogroups, specifically V, I, W, and X. While there is no significant difference in nucleotide or haplotype frequencies between native Basque (BG Basque and French Basque) and NW Basque, the differences in haplogroup frequencies are statistically significant. The haplogroup (gene) diversities are as follows: for the NW Basque 0.3591 ± 0.0185, for the BG Basque 0.6797 ± 0.0126, and for the French Basque 0.6210 ± 0.0151. An exact test of population differentiation in Arlequin found significant differentiation between each population, $p < 0.05$.

Haplotype Networks

We used haplotype networks to show the relationship between haplotypes based on single nucleotide differences. The HVS-1 network shows that a few common haplotypes are shared between all the Basque populations (Figure 6). The Bizkaian and Gipuzkoan populations have four of the most divergent haplotypes, in terms of mutational distance from the central, common (and presumably ancestral) haplotype. The network for the combined HVS-1 and HVS-2 regions (Figure 7) has considerably more resolution, as expected with more sequence data. The majority of haplotypes along the longest branches (most divergent) are found in the BG Basque individuals. Even with speedy sites removed, the rCRS haplotype is only detected in the NW Basque population.

The mutational distance from the inferred ancestral haplotype is given by the rho statistic. For the BG Basque network, rho = 1.1927 ± 0.40179 (sd), and for the NW Basques, rho = 0.7381 ± 0.38095 (sd). With an estimated mutation rate of one site per sequence per 20,000 years, the age of the BG Basque network is 23,854 yr BP ($\pm$ 8036 yr, SD), and the age of the NW Basque network is 14,762 yr BP ($\pm$ 7619 yr, SD). This measure is not expected to correlate with the time of immigration to North America, but rather illustrates a decrease in effective population size consistent with a genetic bottleneck due to founder effects.

Genetic Differentiation Between Immigrant and Source Populations

The NJ tree based on Reynolds' genetic distance (also called the co-ancestry coefficient, Reynolds *et al.* [1983]) placed the NW Basques, the BG Basques, and non-Basque Spanish into one cluster (Figure 8).

The relationship between multiple immigrant and source populations, based upon frequencies of Caucasian-specific haplogroups, was visualized with a Principal Components Analysis (Figure 9). PC1 and PC2 account for 79.4% of the variability in these data. The immigrant populations most separated from the continental European populations are the Amish, the NW Basque, the Gaspe Acadians, and Puerto Ricans. Other immigrant populations overlap with the European group (Brazil, Argentina, and Cuba). The Spanish (BG Basque) and French Basque populations are positioned near each other in ordination space.

DISCUSSION

This is the first DNA study of an immigrant Basque population. My results almost

double the number of Basque control region (HVS-1 and HVS-2) sequences published,

and represent the first analysis of the genetic consequences of the Basque Diaspora.

Consistent with theoretical predictions for populations experiencing genetic drift via

founder events or population bottlenecks (Nei et al. 1975; Novak and Mack 2005), I have

observed both a reduction in mtDNA haplogroup diversity and an alteration of haplotype

and haplogroup frequencies in the NW Basques, compared with values previously

reported for Spanish and French Basque populations (i.e., the putative source

populations). In addition, I have compared the extent of the founder effect in the NW

Basques with other European immigrant populations. These results demonstrate that the

founder effects in the NW Basques are similar in magnitude (in terms of changes in

haplogroup frequencies) to those of the Acadians of Gaspe, and the Amish of Ohio and

Indiana (Moreau et al. 2009; van der Walt et al. 2005).

mtDNA Diversity in NW Basques: Evidence for Founder Effects

An interesting and distinguishing feature of the NW Basques is the relatively large

proportion of individuals matching the rCRS control-region haplotypes (Figure 2). For

HVS-1 only, the proportion observed (35.8 %) is higher than that reported for Bizkaian

and Gipuzkoan Basques (27.3%), which was said to be the highest percentage of the

rCRS HVS-1 haplotype ever reported (Alfonso-Sanchez et al. 2008). Betranpetit *et al.*

(1995) found 20% of their Gipuzkoan Basque samples shared this haplotype. In a study of HVS-1 diversity in French populations, a relatively high proportion (27.2%) of French Basques had the rCRS haplotypes (Richard et al. 2007). An immigrant population with an unusually high proportion of this haplotype are the Acadians of Gaspe, with 33.3% of the sampled population (n=99) matching the HVS-1 rCRS (Moreau et al. 2009).

When one considers both hypervariable regions, the observed frequency of the rCRS haplotype is exceptionally high in the NW Basque. For HVS-1 and HVS-2 combined, the rCRS haplotype was detected in four of 53 (7.5%) individuals analyzed in this study (Figure 3). The combined rCRS haplotype is rare in most European populations (0.25% of all sequences in the EMPOP database), and has never been reported in a Basque population. However, the absence of this haplotype in native Basques should be regarded as provisional, because fewer studies have been published using both HVS-1 and HVS-2, compared with HVS-1 alone (to date only Alfonso-Sanchez *et al.* (2008) have published Basque HVS-2 data). Unfortunately, HVS-2 sequence data is lacking for the French Basques. At present, EMPOP does not contain any sequences from French populations, although updates are planned which will include French data (W. Parson, personal communication).

Overall, the frequency of the rCRS haplotypes are consistent with founder effects, which usually result in the loss of rare haplotypes, but can result in an increase in the frequency of intermediate or even rare haplotypes (Moreau et al. 2009). Increased sampling on a microgeographic scale may also reveal European subpopulations which have a higher proportion of the rCRS control region haplotype. Such "microgeographic"

or "regional" sampling has already been shown to capture variation that is not seen when current national borders are used as proxies for populations (Dubut et al. 2004).

Of the 36 HVS-1 and HVS-2 haplotypes detected in the NW Basque population, 18 (50%) are private haplotypes, which is almost identical to the proportion (50.9%) found in the BG Basque sample (Alfonso-Sanchez et al. 2008). These numbers for private haplotypes should not be surprising (even in populations with relatively low nucleotide diversity), given the fact that almost all estimates of haplotype diversity for mtDNA control-region sequences are considered to be underestimates (Helgason et al. 2003; Pereira et al. 2004). The fact that similar haplotype diversity is seen in the immigrant Basque population can be explained by both the undersampled diversity in the source population, and the relatively long history of Basque migration to North America, which occurred in several waves throughout most of the 19th and 20th centuries (which would result in increased gene flow between American and Basque populations).

Considering only HVS-1, 179 out of the 189 individuals (94.7%) carry either the most common haplotype, or a sequence that is one mutational step removed from the most common haplotype (the rCRS, see Figure 6). A similar pattern is seen in the combined HVS-1, HVS-2 network. Despite the fact that only 7 out of the 30 haplotypes in the network are shared, the majority of individuals (89.8%) are one or two mutational steps from the two most common haplotypes (263G and 263G, 73G).

The fact that most Basque maternal lineages are so closely related may be the result of two factors—each relating to diversity in the native Basque range. First is the high proportion of haplogroup H and H subhaplogroups in both Spanish Basque and French Basque populations (Alfonso-Sanchez et al. 2008; Richard et al. 2007), which would

result in a sampling of more recently diverged control-region lineages, compared to a sample containing all the common European haplogroups (for example, see the haplotype networks in Herrnstadt *et al*. (2002)). Secondly, the cultural and reproductive isolation of Basques: only recently has there been limited migration within and between Spanish and French Basque regions (possibly due to the importance of familial ties for land ownership, see Calderón *et al.* (1993)). Related to this isolation, the Spanish Basque regions (especially Gipuzkoa) have had until recently some of the highest rates of consanguinity in Europe (Alfonso-Sanchez et al. 2001). Both of these factors will tend to decrease the female effective population size, lowering the number of divergent mtDNA lineages expected in that population.

Despite the relatively low female effective population size of all the Basque populations studied thus far with mtDNA, the signature of demographic expansion can still be detected in these populations (based on the neutrality statistics Tajima's D and Fu's F_s), see Tables 1 and 2.

For immigrant populations, an increase in the frequency of pairwise mismatches of zero or one difference is generally an indicator of a genetic bottleneck. For HVS-1 data alone, the NW Basque show this pattern (Figure 4A), and this is concordant with the much greater disparity in nucleotide diversity between the NW Basques and Spanish Basques (Table 1). When HVS-2 is included in the analysis, the classes are virtually identical; the difference being that the entire distribution is shifted toward smaller values, with the NW Basque curve showing a multi-modal and somewhat more "ragged" distribution (Figure 4B). However, when testing Harpending's raggedness coefficient (*rg)* using the coalescent simulation in DnaSP 5.0 (10,000 replicates), the results were

non-significant ($rg = 0.0181$, p = 0.06). Thus, a model of population growth cannot be

rejected. Despite the non-significant results of the *rg* simulation, the multi-modal

appearance of the distribution is qualitatively similar to distributions seen in empirical

studies (across a variety of species) of populations that have undergone either recent

bottlenecks or admixtures (Melton et al. 2010; Taylor and Keller 2007; Weber et al.

2004).

Co-Ancestry in Immigrant and Source Basque Populations

Despite its usefulness in population genetics, the hypervariable region of mtDNA has

long been known to be an unreliable marker for resolving intra-specific phylogenies in

humans (Templeton et al. 1992). Indeed, when phylogenetic trees (e.g., maximum

parsimony, maximum likelihood, or NJ trees) were constructed using HVS-1 and HVS-2

nucleotide data, no clear patterns among populations could be discerned (data not

shown). Conversely, when genetic distances are assessed with a measure based on

shared haplotypes (Reynolds' distance), a more accurate assessment of recent genetic co-

ancestry can be made (for population-level comparisons) (Reynolds et al. 1983). This

and similar distance measures (such as Slatkin's linearized F_{ST}) are the most appropriate

measures when comparing recent immigrant and source human populations using

control-region mtDNA data. These measures based on short-term genetic distance take

into account genetic drift, and are based on frequencies of haplotypes and their

distribution among populations, rather than the evolutionary relationships between

haplotypes. Thus, although founder effects have altered haplogroup and haplotype

frequencies, the NW and BG Basque populations retain more similarity to each other than

to the other European populations considered (Figure 8). The pattern of shared lineages

is independently validated by the historical record of Basque immigration (Bieter and Bieter 2000; Douglass and Bilbao 1975).

Haplogroup distribution in Basque populations

Mitochondrial DNA haplotype and nucleotide diversity in NW Basques has clearly been influenced by recent demographic events. This diversity has also been influenced by the diversity and geographic structure of European haplogroups, which have in turn been shaped by ancient demographic events (Achilli et al. 2004; Richards et al. 2000). The Basques are believed to be descended from some of the earliest human colonists of Europe, and their ancestors appear to have persisted within glacial refugia, remaining relatively isolated from other European populations for much of their history. Certain mtDNA haplogroups found in high frequency in the Basques (but distributed throughout much of Europe) are believed to have originated approximately 15,000 - 30,000 BP, during the Upper Paleolithic. For instance, among European populations, haplogroups H1, H3, and H5a are most prevalent in the Franco-Cantabrian area, which includes the Spanish and French Basque regions (Achilli et al. 2004; Alvarez-Iglesias et al. 2009). In the Iberian peninsula, haplogroup H and its subgroups have comparable haplotype diversity, but lower nucleotide diversity, than other European haplogroups (Alvarez-Iglesias et al. 2009). Any population that is enriched for Iberian H lineages should exhibit this same pattern in haplotype and nucleotide diversity. This pattern seems to hold for both the native BG Basque and immigrant NW Basque populations: both populations have the lowest diversity (π and θ_π) of the populations listed in Table 4. The nucleotide diversity values of the NW Basque are almost identical to those reported for

Iberian populations when only haplogroup H individuals are considered (Alvarez-Iglesias et al. 2009). Thus, both the level and distribution of diversity within the mitochondrial haplogroups in the putative source population appear to play an important role in shaping the diversity of the immigrant NW Basque population.

NW Basques and Other European Immigrant Populations

The results for the NW Basque are consistent with founder effects that have been documented in other immigrant populations. Perhaps the best example for comparison involves the inhabitants of the Gaspe peninsula (Québec), which can be partitioned into several groups with differing European ancestries, including the Acadians and Channel Islanders. The Gaspe Channel Islanders derive from settlers who migrated from the English Channel Islands, while the Acadians trace their roots to France. Compared to the English Loyalists and other English-speaking groups, the French Acadians in Québec started with a smaller founding population, and experienced a shorter period of immigration from France (due to the subsequent British Conquest, Moreau *et al.* 2009). The result of dissimilar demographic histories can be seen in the relative positions of the Gaspe sub-populations in ordination space, relative to the pooled population data (Figure 9). Moreau *et al.* (2009) also find evidence for founder effects in the loss of rare haplotypes and decreased genetic diversity.

Evidence for a founder effect can also be seen for Puerto Rican and U.S. Hispanic populations (Figure 9). That a founder effect is seen in the Puerto Rican population may seem counter-intuitive, because it has a long immigration history, and a relatively large population. However, because the first settlers were almost exclusively Spanish males,

and the subsequent Spanish immigrant population was relatively small (compared to Native American or African slaves), founder effects are believed to have contributed to its current distribution of haplogroup frequencies (Juan et al. 2005; Martinez-Cruzado et al. 2001). The same arguments can be made regarding the founder effect seen in the haplogroup distribution for North American Hispanics, which have a similar PC value as the Puerto Ricans (Figure 9). In addition, it is known that Hispanic populations in different regions of the US have different sources of maternal lineages—differing in the proportion of European versus Amerindian and African mtDNA (Merriwether et al. 1997). In general, although Hispanic populations are relatively large (compared to specific ethnic sub-populations such as Basques or Acadians), the European component of their mtDNA ancestry is comparatively small. Thus, it appears that just those American populations that have experienced serial founder effects (Acadians), multiple founder effects with relative isolation (Amish), and/or founding from relatively small European source populations (Acadians, NW Basques, and Puerto Ricans) are those that show the greatest differentiation between immigrant and source haplogroup profiles.

Some European-derived immigrant populations do not show such obvious founder effects (such as in Argentina, Brazil, and Cuba) (Figure 9). This is most likely due to the fact that these populations are descended from multiple sources and/or have been founded by larger populations closer in haplogroup composition to their European source populations. For many species, large founder populations and multiple introductions have been shown to reduce or counter genetic founder effects (Novak and Mack 2005).

Kin-structured migration (KSM) is a migration pattern seen in many immigrant groups, and is theorized to effect the genetics of populations by increasing variance

between source and migrant pools, and decreasing the effective size of immigrant

populations (Fix 2004). Historical studies of European migrations have found evidence

for KSM: Ostergren studied the records from 19th-century Sweden, and found kinship

networks in the migration patterns both within Sweden and in emigrants to America

(Ostergren 1982). Despite this and other studies documenting KSM in humans

(Gonzalez-Martin and Toja 2002; Williams-Blangero 1989), the genetic consequences of

KSM are not often addressed. However, this may change with increasing numbers of

studies of immigrant and isolated populations. In discussing the lower genetic diversity

of the Acadians compared with other Gaspesians, Moreau *et al.* mentions the fact that the

Acadian settler groups included extended families (Moreau et al. 2009). Kin-structured

migration was addressed in a recent investigation of North American Mennonite

congregations (Melton et al. 2010). Their results show that the genetic pattern of

population subdivision correlates with the known histories of kin-structured fission and

fusion. Anecdotal evidence suggests an important role for KSM in Basque migrations

(McCall 1968). For example, half of the Basque migrants to Idaho during the period

1897-1902 reported having relatives or acquaintances already in Idaho, and Basque

families often reported that half of their children were in America at one time or another

(Bieter and Bieter 2000; Douglass and Bilbao 1975). Thus, chain migration is a likely

scenario for the NW Basque population, and may have influenced founder effects in this

population; unfortunately, the mtDNA data alone cannot be used to rigorously test for

kin-structured migration.

Conclusions

By several measures of mtDNA diversity, I have shown that the NW Basque population has been influenced by both recent migrations and ancient demographic events. In addition, this is the first study comparing mtDNA diversity in an immigrant Basque population with the diversity previously reported in the native Basque region. I have found evidence to suggest that the NW Basque have experienced founder effects/population bottlenecks in association with immigration to the U.S.A. This finding should be further evaluated through the analysis of other immigrant Basque populations in North and South America. Furthermore, immigrant Basques in the American Northwest and elsewhere should be examined explicitly to assess the genetic consequences of kin-structured migration using more appropriate genetic markers (e.g., microsatellite DNA).

The historical and genetic links between the NW Basque and Spanish (especially Bizkaian and Gipuzkoan) Basque populations may prove fruitful in future studies of the genetic basis of disease, as has been the case with other populations experiencing founder effects, such as certain French Canadian (Québécois) and Amish populations (Ebermann et al. 2009; van der Walt et al. 2005). Although no increased incidence of genetic disease has been reported in American Basques, it is known that European Basque populations experience an increased incidence of certain specific forms of Alzheimer's and Parkinson's disease (Alvarez-Alvarez et al. 2003; Gonzalez-Fernandez et al. 2007; Simon-Sanchez et al. 2006). Moreover, comparisons of populations with similar genetic backgrounds in different environments are useful systems for determining genetic versus environmental factors in disease. Thus, despite recent papers that focus on the lack of

genetic distinctiveness of Basque populations (Laayouni et al. 2010), or cast doubts on

the appropriateness of using the Basque as a population isolate for medical genetics

studies (Garagnani et al. 2009), it is evident that the well-studied genetic traits of Basque

populations, and the relative ease of verifying Basque ancestry (using surnames), make

the Basque an ideal subject for migration studies. Clearly, the Basque continue to serve

as an excellent model system for addressing numerous questions in human population

genetics and microevolution.

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Table 1. mtDNA HVS-1 Sequence Diversity in the NW Basque and other Populations.

Populations arranged according to θ_π values. N is the sample size, k is the number of distinct haplotypes, S is the number of segregating sites, h is the gene (haplotype) diversity, π is the nucleotide diversity, θ values calculated according to methods in text, D is Tajima's D, and F_s is Fu's statistic.

Sample	N	k	S	$h \pm sd$	$\pi \pm sd$	$\theta_\pi \pm sd$	$\theta_s \pm sd$	θ_k (95% CI)	D	F_s
NW Basques	53	23	29	0.849 ± 0.042	0.006 ± 0.004	2.34 ± 1.44	6.39 ± 2.07	14.9 (8.6 – 25.6)	–2.09	–17.65
Acadians (Gaspe)	101	31	34	0.877 ± 0.028	0.010 ± 0.006	2.83 ± 1.66	6.55 ± 1.91	14.9 (9.5 – 22.9)	–1.75	–21.39
BA Basque	61	34	41	0.926 ± 0.027	0.011 ± 0.006	2.91 ± 1.55	8.33 ± 2.54	30.9 (18.7 – 51.1)	–2.25	–26.48
Galician	92	48	57	0.898 ± 0.029	0.012 ± 0.007	3.17 ± 1.65	10.41 ± 2.89	39.8 (26.3 – 60.2)	–2.32	–26.38
G Basque	45	27	34	0.949 ± 0.021	0.013 ± 0.007	3.51 ± 1.82	6.63 ± 2.20	27.6 (15.4 – 49.6)	–1.81	–21.15
BG Basque	55	27	34	0.914 ± 0.029	0.013 ± 0.007	3.58 ± 2.05	6.78 ± 2.16	20.3 (11.9 – 34.5)	–1.97	–17.64
French Basque	81	33	50	0.909 ± 0.024	0.013 ± 0.008	3.66 ± 2.07	9.26 ± 2.66	20.3 (12.8 – 31.8)	–2.15	–22.21
Welsh	92	45	54	0.926 ± 0.021	0.014 ± 0.008	3.85 ± 2.16	9.62 ± 2.69	34.2 (22.5 – 51.8)	–2.08	–25.99
Chan.Islanders (Gaspe)	76	43	50	0.977 ± 0.007	0.015 ± 0.008	4.07 ± 2.28	10.20 ± 2.93	40.2 (25.5 – 63.4)	–1.97	–25.90
Cuban Cauc.	53	35	41	0.978 ± 0.009	0.015 ± 0.009	4.16 ± 2.10	9.03 ± 2.80	43.8 (25.3 – 76.7)	–1.82	–25.83
Albanian	42	29	44	0.966 ± 0.018	0.015 ± 0.009	4.24 ± 2.38	9.30 ± 2.99	40.1 (21.7 – 75.9)	–2.10	–23.37
Russian	124	85	76	0.980 ± 0.007	0.016 ± 0.009	4.28 ± 2.37	14.09 ± 3.61	117.9 (81.2 – 172.9)	–2.22	–25.70
Argentinean Cauc.	58	41	45	0.981 ± 0.008	0.016 ± 0.009	4.36 ± 2.42	9.72 ± 2.94	61.0 (35.6 – 106.4)	–1.86	–25.77
Andalusia	49	37	45	0.964 ± 0.019	0.017 ± 0.009	4.67 ± 2.33	9.42 ± 2.94	67.1 (36.7 – 126.3)	–2.00	–25.65
Catalonia	46	28	40	0.918 ± 0.035	0.017 ± 0.010	4.74 ± 2.62	8.19 ± 2.63	29.4 (16.6 – 52.7)	–1.77	–17.59
Spain	295	176	104	0.974 ± 0.006	0.014 ± 0.008	4.78 ± 2.59	16.29 ± 3.63	183.0 (144.1 – 232.7)	–2.15	–25.08
Germany	139	77	67	0.965 ± 0.009	0.018 ± 0.010	5.03 ± 2.72	11.44 ± 2.94	70.2 (49.9 – 98.9)	–2.03	–25.36
Tuscany	49	39	58	0.967 ± 0.019	0.019 ± 0.010	5.15 ± 2.82	12.11 ± 3.69	86.4 (46.0 – 169.0)	–2.09	–25.50
Portugal	241	142	93	0.959 ± 0.010	0.019 ± 0.010	5.34 ± 2.86	15.02 ± 3.47	144.2 (110.9 – 187.9)	–2.12	–24.99
Iceland	394	124	73	0.978 ± 0.002	0.020 ± 0.010	5.35 ± 2.86	10.68 ± 2.40	61.9 (49.2 – 77.5)	–1.72	–24.75
Georgians	47	36	44	0.983 ± 0.009	0.020 ± 0.012	5.49 ± 2.98	9.51 ± 2.99	68.6 (36.9 – 131.9)	–1.50	–25.40
Angolan	44	37	58	0.992 ± 0.007	0.038 ± 0.020	10.55 ± 5.44	12.87 ± 3.98	107.0 (52.7 – 230.2)	–0.90*	–22.32

* not significantly different from zero

Table 2. mtDNA HVS-1 and HVS-2 combined sequence diversity in the NW Basque and other Populations. See Table 1 caption for key to symbols and abbreviations.

Sample	N	K	S	$h \pm sd$	$\pi \pm sd$	$\theta_\pi \pm sd$	$\theta_s \pm sd$	θ_k (95% CI)	D	F_S
NW Basques	53	32	45	0.958 ± 0.015	0.008 ± 0.004	3.87 ± 2.19	9.92 ± 3.04	33.2 (19.4 – 57.3)	-2.16	-25.92
BG Basque	55	31	46	0.956 ± 0.015	0.010 ± 0.005	5.07 ± 2.77	10.05 ± 3.05	28.6 (16.9 – 48.6)	-1.83	-20.22
Germany	99	69	74	0.977 ± 0.009	0.011 ± 0.006	5.37 ± 2.89	14.32 ± 3.80	99.7 (65.6 – 153.1)	-2.14	-25.52
French	50	44	66	0.990 ± 0.008	0.012 ± 0.007	6.03 ± 3.24	14.73 ± 4.39	171.9 (82.7 – 383.7)	-2.20	-25.40
Portugal	241	168	126	0.977 ± 0.006	0.014 ± 0.007	6.68 ± 3.50	20.63 ± 4.63	244.7 (185.9 – 323.8)	-2.19	-24.81
Iceland	338	131	96	0.981 ± 0.003	0.014 ± 0.007	6.72 ± 3.52	14.53 ± 3.21	78.0 (61.8 – 98.2)	-1.74	-24.62
Balkans	243	154	119	0.989 ± 0.003	0.014 ± 0.007	6.80 ± 3.56	19.45 ± 4.38	179.7 (137.9 – 235.0)	-2.11	-24.77
British	100	87	93	0.994 ± 0.003	0.014 ± 0.008	6.99 ± 3.67	17.96 ± 4.67	315.9 (186.3 – 557.9)	-2.14	-25.10
Spain	294	207	142	0.985 ± 0.004	0.014 ± 0.008	7.05 ± 3.67	22.05 ± 4.79	309.9 (241.1 – 400.3)	-2.18	-24.61
Tuscany	49	47	75	0.998 ± 0.005	0.017 ± 0.009	8.09 ± 4.24	16.82 ± 4.99	555.9 (192.6 – 1832.1)	-2.01	-25.02
Georgians	47	42	63	0.995 ± 0.005	0.017 ± 0.009	8.33 ± 4.36	14.04 ± 4.25	185.8 (85.1 – 440.2)	-1.67	-25.00
Angola	44	36	72	0.989 ± 0.008	0.033 ± 0.016	15.82 ± 7.99	16.55 ± 5.01	90.2 (45.6 – 187.4)	-0.70*	-16.17

* not significantly different from zero

Figure 1. Map of Basque regions of Europe and major Basque settlements of the Western United States.

The Basque regions of Europe (A), and important cities of Basque settlement in the Western United States (B), based on the presence of Basque Centers (those registered with NABO, the North American Basque Organizations). Boise, Idaho, is indicated with an asterisk. The three provinces of Bizkaia, Gipuzkoa, and Araba (Vizcaya, Guipúzcoa, and Álava in Spanish), together make up the autonomous Spanish Basque Region. The French Basque region, opposite the French-Spanish border (dotted line), is in the department Pyrénées-Atlantiques. The majority of original Basque settlers into SW Idaho were from the region in Bizkaia approximately encircled by the cities of Gernika, Lekeitio, and Ondarroa.
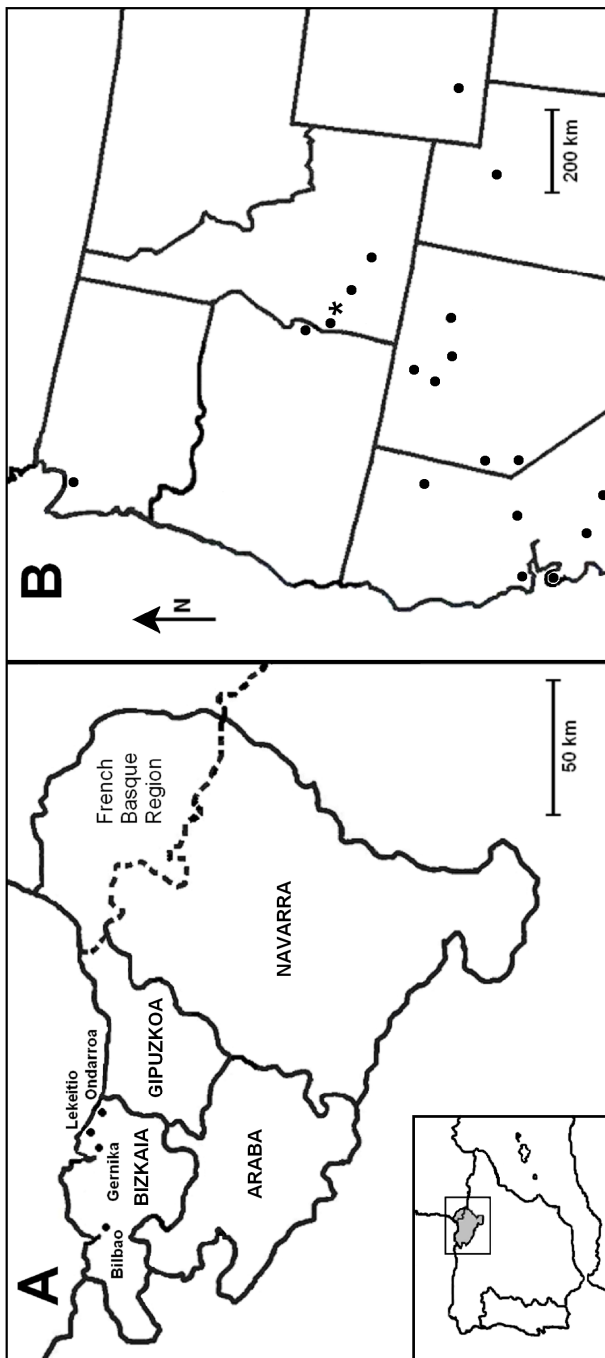
Figure 2. NW Basque Polymorphic sites for HVS-1.

Top row: rCRS. Numbers refer to position of nucleotide in rCRS. Rows refer to haplotypes seen in the NW Basque samples. Dot indicates identity with rCRS. N = number of individuals sharing each haplotype. Last column: inferred haplogroup membership using Behar's NJ method (Behar et al. 2007).

Polymorphisms in HV-1 (numbering minus 16000)

Note: the following table is printed rotated 90° on the page. Position numbers are printed vertically as stacked digits above each nucleotide column; they are transcribed here as best read. The top row of letters ("rCRS") is the reference sequence; dots (·) in the original indicate identity with the reference.

n	(sample)	C	C	T	G	C	C	T	C	T	G	T	A	C	C	C	C	A	T	T	A	G	C	T	Hg
	rCRS	C	C	T	G	C	C	T	C	T	G	T	A	C	C	C	C	A	T	T	A	G	C	T	
19	NWB HV1-1	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	H
8	NWB HV1-2	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	H
1	NWB HV1-3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	H
1	NWB HV1-4	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	H
4	NWB HV1-5	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	K
1	NWB HV1-6	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	H
1	NWB HV1-7	·	·	·	·	·	·	·	·	·	·	T	·	·	·	T	·	·	·	·	·	·	·	·	U5a
1	NWB HV1-8	·	C	·	·	·	·	·	·	T	·	·	·	·	·	G	T	·	·	·	·	·	·	·	H
3	NWB HV1-9	·	·	T	·	·	·	·	·	T	·	·	·	·	·	·	·	·	T	·	A	·	·	·	U
1	NWB HV1-10	·	·	·	·	·	·	·	·	·	·	·	·	C	C	C	·	·	·	·	·	·	·	·	H
1	NWB HV1-11	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	H
1	NWB HV1-12	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	HV
1	NWB HV1-13	·	·	·	T	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	H
1	NWB HV1-14	C	·	·	·	·	·	·	·	·	·	·	·	T	T	·	C	·	·	·	·	·	·	·	T
1	NWB HV1-15	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	T	·	·	·	·	·	·	T	H
1	NWB HV1-16	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	H
1	NWB HV1-17	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	H
1	NWB HV1-18	·	·	·	·	·	T	T	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	U
1	NWB HV1-19	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	H
1	NWB HV1-20	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	T	T	·	A	·	·	·	H
1	NWB HV1-21	T	·	C	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	J
1	NWB HV1-22	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	H
1	NWB HV1-23	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	H

Figure 3. NW Basque Polymorphic sites for HVS-1 and HVS-2.

See legend for Figure 2. Number of individuals with matching haplotypes show for (1) the BG Basque (Alfonso-Sanchez et al. 2008)and (2) the European mtDNA database (Bush et al. 2009; Parson and Dür 2007).

Polymorphisms in HV-1 (numbering minus 16000) *Polymorphisms in HV-2* HV-1 + HV-2 Haplotype matches

HV-1 reference (rCRS) bases:
`C C C T G C C T C T C T C G T A C C C A T C C A T T A G C T C`

HV-2 reference (rCRS) bases:
`A T C T A G A C T C T T G G A A T C C T C T C`

n		Hg	Basque matches[1]	EMPOP matches[2]
	rCRS			
4	AB1	H	0 (0.0)	11 (0.25)
4	AB2	H	8 (14.5)	257 (5.7)
2	AB3	H	0 (0.0)	2 (0.04)
1	AB4	H	1 (1.8)	8 (0.18)
2	AB5	H	0 (0.0)	0 (0.0)
1	AB6	H	0 (0.0)	0 (0.0)
1	AB7	H	0 (0.0)	0 (0.0)
4	AB8	H	0 (0.0)	53 (1.18)
6	AB9	H	5 (9.1)	19 (0.42)
1	AB10	H	0 (0.0)	0 (0.0)
1	AB11	H	0 (0.0)	25 (0.56)
1	AB12	H	0 (0.0)	0 (0.0)
2	AB13	K	0 (0.0)	26 (0.58)
1	AB14	K	0 (0.0)	0 (0.0)
1	AB15	U5a	0 (0.0)	27 (0.60)
1	AB16	H	0 (0.0)	2 (0.04)
1	AB17	U	0 (0.0)	0 (0.0)
1	AB18	H	0 (0.0)	0 (0.0)
1	AB19	H	0 (0.0)	0 (0.0)
1	AB20	HV	0 (0.0)	1 (0.02)
1	AB21	H	0 (0.0)	0 (0.0)
1	AB22	T	0 (0.0)	0 (0.0)
1	AB23	K	0 (0.0)	5 (0.11)
1	AB24	K	1 (1.8)	4 (0.09)
1	AB25	H	0 (0.0)	0 (0.0)
1	AB26	H	0 (0.0)	0 (0.0)
1	AB27	H	1 (1.8)	6 (0.13)
1	AB28	U	1 (1.8)	4 (0.09)
1	AB29	U	0 (0.0)	0 (0.0)
1	AB30	H	0 (0.0)	0 (0.0)
1	AB31	H	0 (0.0)	0 (0.0)
1	AB32	H	0 (0.0)	1 (0.02)
1	AB33	J	0 (0.0)	0 (0.0)
1	AB34	H	0 (0.0)	1 (0.02)
1	AB35	H	0 (0.0)	1 (0.02)
1	AB36	H	0 (0.0)	0 (0.0)

Figure 4. Pairwise mismatch distributions for HVS-1 and combined control region.

Mismatch distributions of Basque populations based on HVS-1 data (A), and combined HVS-1 and HVS-2 data (B). Population data are for: NW Basque (this study), G Basque (Gipuzkoan Basques, Bertranpetit et al. [1995]), BA Basque (Bizkaian and Alavan Basques, Corte-Real et al. [1996]), BG Basque (Bizkaian and Gipuzkoan Basques, Alfonso-Sanchez et al. [2008]).
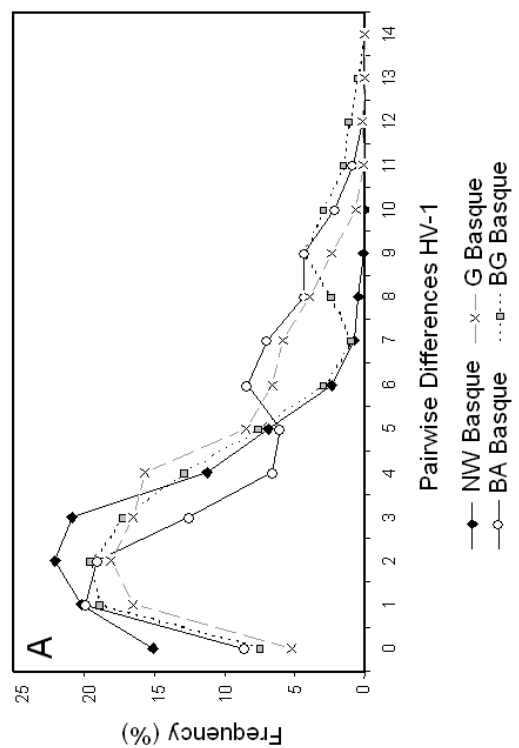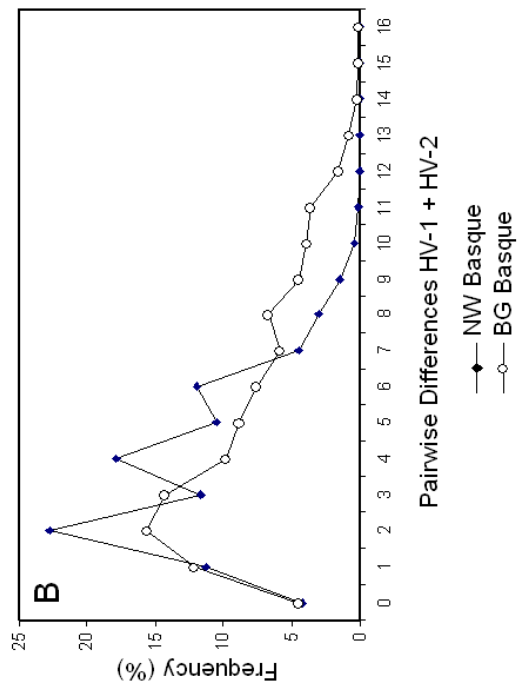
Figure 5. mtDNA haplogroup distributions in Basque and Iberian populations.

For the major European mtDNA haplogroups, in addition to others of low frequency

(OTH), the relative frequency for each population is given. See Subjects and Methods

section for sources of published data for each population.

Figure 6. Median-joining network of HVS-1 haplotypes.

Haplotype network with speedy sites ignored (see Subjects and Methods section for

details). Node size is proportional to frequency of haplotypes, the smallest nodes

represent haplotypes carried by one individual. Edges connecting the circles represent

single nucleotide mutational steps. Lineages from different Basque regions are colored

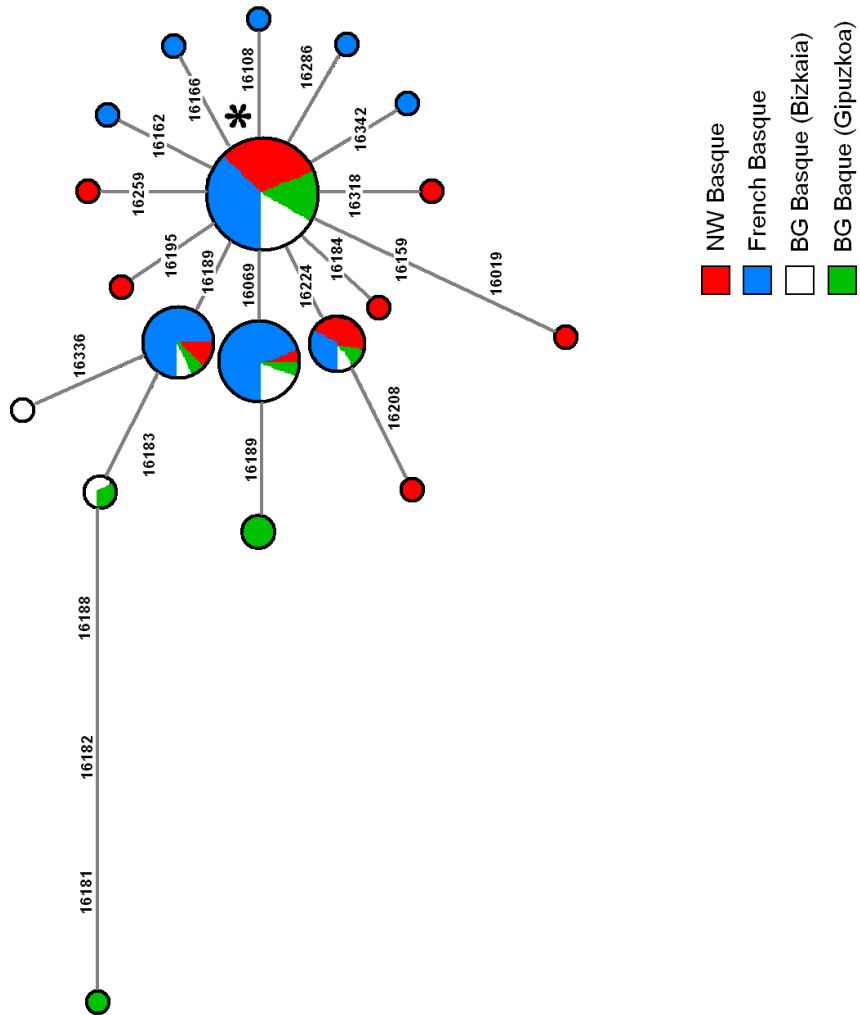according to the key. The rCRS haplotype is indicated by an asterisk (*).

Figure 7. Median-joining network of HVS-1 plus HVS-2 (control region) haplotypes.

See legend for Figure 6.

NW Basque
BG Basque (Bizkaia)
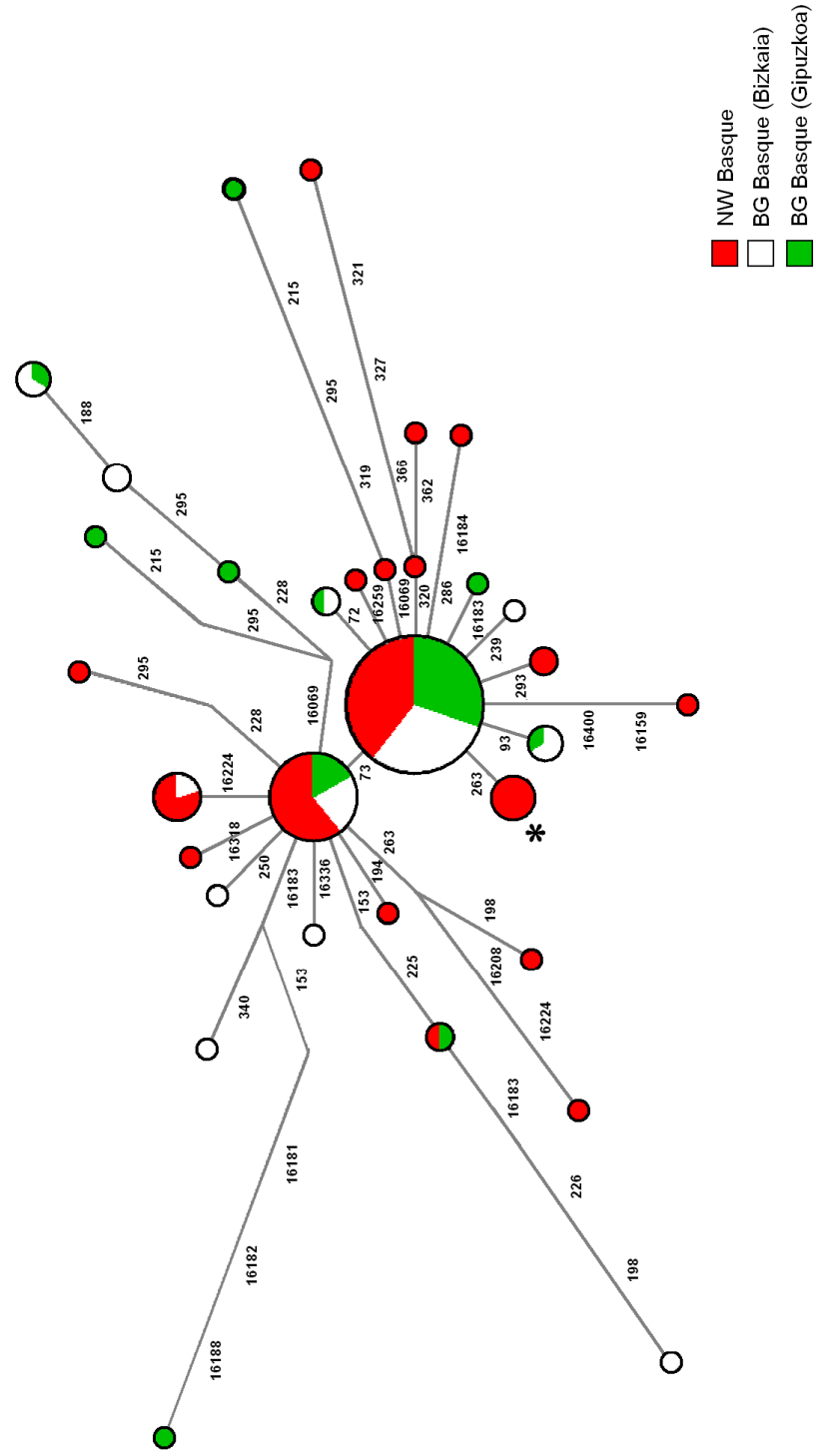BG Basque (Gipuzkoa)

Figure 8. Neighbor-joining Tree of control-region genetic distance.

Neighbor-joining tree of control-region (combined HVS-1 and HVS-2) data showing

recent coancestry relationships between populations. NJ clustering algorithm was

applied to the distance matrix. Scale bar indicates Reynolds' linearized F_{ST} distance.
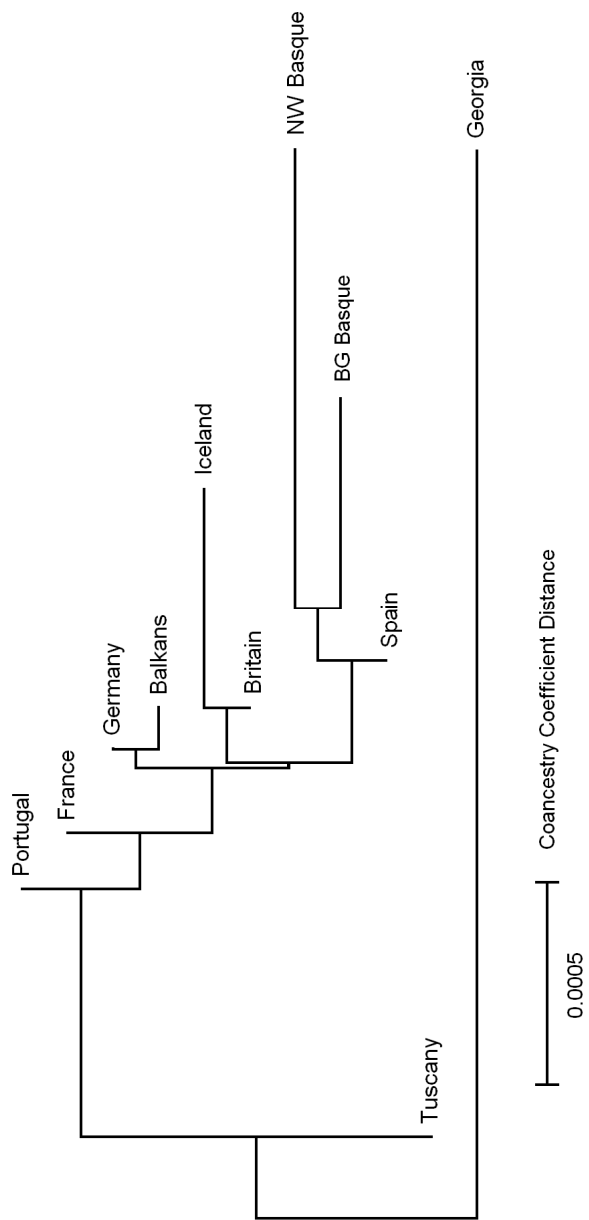
Figure 9. Principal components analysis (PCA) of European and European-derived populations based on haplogroup distribution.

PCA based on frequencies of major European haplogroups. Abbreviations of American populations are shown in boxes. Basque populations are indicated by fillled (NW Basque) or open (French and Spanish Basque) squares. Native European populations are indicated by open circles, and immigrant populations are indicated with filled circles. Percentage variation accounted for by each PC is shown. Population abbreviations: AMH: Amish. ACD: Acadian (Gaspe). NWB: NW Basque. CAT: Catalonian. IBZ: Ibiza Islanders. SWD: Swedish. GSP: All Gaspesians. CHL: Channel Islanders (Gaspe). FRA: French. POR: Portuguese. RUS: Russians. AUS: Austrians. GER: Germans. BRZ: Brazilians. AZR: Azores Islanders. NSP: Northern Spanish (non-Basque). CUB: Cubans. ENG: English. ARG: Caucasian Argentinians. FRB: French Basque. BGB: Spanish (Bizkaia, Gipuzkoa) Basque. HSP: North American Hispanics. PRC: Puerto Ricans.
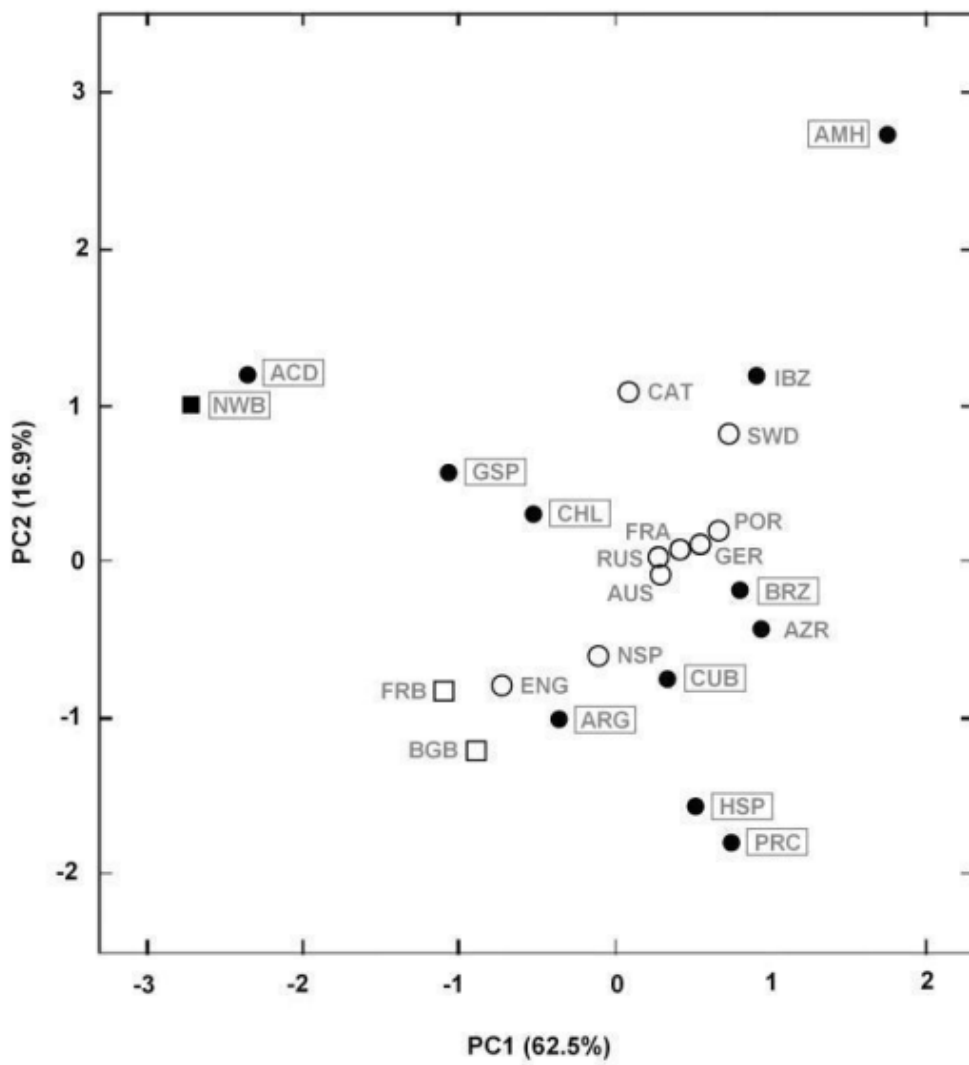
APPENDIX

The Questionnaire Distributed to Study Volunteers to Determine Mode of Basque Ancestry

Idaho DNA Study Survey

Swab Collection Data: For Research Purposes Only

A random number will be associated with your sample.

No Identifying Information will be published.

For this research project, we are requesting demographic information. Due to the make-up of Idaho's population, the combined answers to these questions may make an individual person identifiable. We will make every effort to protect participants' confidentiality. However, if you are uncomfortable answering any of these questions, you may leave them blank.

1. **Sex:** Male ☐   Female ☐

2. **Age:** ☐

3. **Your self-identified Ethnic Background**: ☐ African-American ☐ Asian ☐ Caucasian ☐ Hispanic ☐ Native American ☐ Basque

Other _____

4. **Your place of Birth (POB):** United States → City, State
   _____

                               Other → Country _____

       How long have you lived in Idaho?   _____

5. **Your Mother's POB**: City, U.S. State or Country
_____

       6. Her Mother's POB: City, U.S. State or Country
_____

       7. Her Father's POB: City, U.S. State or Country
_____

8. **Your Father's POB:** City, U.S. State or Country

_____

9. His Mother's POB: City, U.S. State or Country

_____

10. His Father's POB: City, U.S. State or Country

_____

If Basque:
11. **Mother's Maiden name**:_____

12. **Mother's Mother's Maiden name:**
_____

13. **Father's surname:** _____

14. **Father's father's surname:** _____

Any other Basque surnames in family? _____

By answering and returning this questionnaire, as well as providing a cheek swab, you consent to have the information you provide used for research purposes only, with the understanding that you may contact the researchers at Boise State University and withdraw from the study at any time. No identifying information will be published, and the information collected will be stored in a secure location, and will only be accessed by researchers for the purposes of this study.

I am 18 yrs old or older, and I give consent that my questionnaire and cheek swab (DNA) be used for research purposes.

Signature _____

Date _____