Curso 2009/10
CIENCIAS Y TECNOLOGÍAS/11
I.S.B.N.: 978-84-7756-945-9

ROSA IRENE FREGEL LORENZO

La evolución genética de las poblaciones humanas canarias: determinación mediante marcadores autosómicos y uniparentales

Directores
ANA MARÍA GONZÁLEZ MATILLA
JOSÉ MARÍA LARRUGA RIERA

SERVICIO DE PUBLICACIONES
UNIVERSIDAD DE LA LAGUNA

SOPORTES AUDIOVISUALES E INFORMÁTICOS
Serie Tesis Doctorales

Agradecimientos

Me gustaría dar las gracias a mis directores, José Mª Larruga y Ana Mª González, por permitirme participar en un proyecto tan interesante como el estudio de las poblaciones antiguas canarias. Igualmente, quisiera agradecer a Vicente M. Cabrera su inestimable ayuda y el entusiasmo que siempre ha puesto en este trabajo.

A Nicole Maca-Meyer por enseñarme a dar los primeros pasos en el mundo del ADN antiguo y a Pablo Hernández Carpio por ayudarme con el trabajo de post-PCR.

A todos los compañeros que compartieron conmigo los buenos y malos momentos del inicio de la carrera investigadora, especialmente a Patri por su amistad y compañerismo durante todos estos años.

Este trabajo no habría sido posible sin mi familia y amigos. Gracias a mi madre y a mi hermano por su apoyo incondicional y por entender las exigencias de este trabajo. A todos mis amigos, muy especialmente a Aída, por hacerme olvidar los malos ratos de esta tesis. A Lara y Noe por su visita a Porto y a Sergio por estar siempre dispuesto a ayudar. Gracias a Ana Tronholm por estar siempre pendiente de mi.

Al Instituto de Patología e Inmunología de la Universidad de Oporto por acogerme durante mi estancia. Especialmente, gracias a António Amorim, Leonor Gusmão y Verónica Gomes por su hospitalidad y por su ayuda con la técnica del SNaPshot.

A Jose Pestano y a todos los compañeros del Departamento de Genética de la Universidad de Las Palmas de Gran Canaria por acogerme en su laboratorio y por echarme una mano siempre que lo he necesitado.

A Ana y Jose del Servicio de Secuenciación y Genotipado de ADN (SEGAI) por su ayuda con la PCR a tiempo real.

Gracias a todas las instituciones que han hecho posible este trabajo cediendo las muestras antiguas: la Dirección General de Patrimonio Histórico del Gobierno de

Canarias, el Museo Arqueológico y la Unidad de Patrimonio del Cabildo de La Palma, la Unidad de Patrimonio del Cabildo de El Hierro, el Museo Arqueológico y la Unidad de Patrimonio del Cabildo de La Gomera, el Museo Canario y el Instituto Cabrera Pinto. También quisiera agradecer a Matilde Arnay, Alejandro Gámez, Alejandra Calderón y Juan Carlos Hernández por ayudarme a resolver mis dudas sobre la prehistoria e historia de Canarias.

Este proyecto estuvo financiado por el Ministerio de Ciencia y Tecnología (BFU2006-04490) y por una beca predoctoral concedida por la Agencia Canaria de Investigación. Igualmente, quisiera agradecer a esta institución la concesión de una beca para la realización de una estancia en el extranjero durante el año 2008.

Finalmente, quisiera agradecer muy especialmente a Romén sus ánimos, su comprensión y su paciencia, sobre todo en los difíciles inicios de este proyecto. Esta tesis no habría sido posible sin su apoyo.

A mi madre y mi hermano

A Romén

Abreviaturas:

ACP	fosfatasa ácida
ADN	ácido desoxirribonucleico
ADNa	ADN antiguo
ADNmt	ADN mitocondrial
ARN	ácido ribonucleico
DTT	ditiotreitol
G6PD	glucosa-6-fosfato deshidrogenasa
GuSCN	tiocianato de guanidina
HVR	región hipervariable
MDS	escalamiento multidimensional
NRY	región no recombinante del cromosoma Y
pb	pares de bases
PCA	análisis de componentes principales
PCR	reacción en cadena de la polimerasa
PTB	bromuro de N-fenaciltiazolio
q-PCR	PCR cuantitativa o a tiempo real
RFLP	polimorfismos de tamaño de los fragmentos de restricción
SAP	fosfatasa alcalina de camarón
SDS	sodio dodecil sulfato
SNP	mutación puntual
SSCP	polimorfismo conformacional de cadena monocatenaria
UNG	uracilo N-glicosilasa

ÍNDICE

1. INTRODUCCIÓN 1

1.1. La población canaria 3

1.1.1. Los aborígenes canarios 4
1.1.1.1. Evidencias sobre el origen geográfico de los aborígenes canarios 4
1.1.1.2. Evidencias sobre el proceso de colonización aborigen de las Islas Canarias 6
1.1.2. La conquista de las Islas Canarias 7
1.1.3. La colonización de las Islas Canarias 10
1.1.3.1. La población aborigen tras la conquista 10
1.1.3.2. La colonización europea 11
1.1.3.3. El tráfico de esclavos en Canarias 12
1.1.3.4. Influencia americana en Canarias 13
1.1.4. La población de Santa Cruz de Tenerife en los siglos XVII-XVIII: la Iglesia de La Concepción 14
1.1.5. La población canaria actual 15

1.2. Estudios genéticos en la población canaria 16

1.2.1. Primeros estudios genéticos en la población canaria: grupos sanguíneos y polimorfismos enzimáticos 17
1.2.1.1. El grupo sanguíneo AB0 17
1.2.1.2. Polimorfismos enzimáticos 19
1.2.2. Marcadores uniparentales 19
1.2.2.1. Los linajes maternos: el ADN mitocondrial 19
1.2.2.2. Los linajes paternos: el cromosoma Y 23
1.2.3. Marcadores autosómicos: el sistema CD4/Alu. 25

1.3. El ADN antiguo 26

1.3.1. Historia del ADN antiguo 26

1.3.2. Limitaciones del ADN antiguo 27
1.3.2.1. Degradación del ADN 28
1.3.2.1.1. Bloqueo de la ADN polimerasa 30
1.3.2.1.2. Rotura de la molécula de ADN 30
1.3.2.1.3. Modificación de las bases 31
1.3.2.2. Contaminación 32
1.3.2.3. Inhibición 33
1.3.3. Autentificación del ADN antiguo 34
1.3.4. Avances de las técnicas para el estudio del ADN antiguo 38
1.3.4.1. Secuenciación de alto rendimiento a partir de librerías metagenómicas 38
1.3.4.2. Genotipado de ADN antiguo mediante la técnica de SPEX 39
1.3.5. Estudios previos de ADN antiguo en Canarias 39
1.3.5.1. Estudios en la población aborigen 40
1.3.5.2. Estudios en la población histórica (S.XVII-XVIII) 41

1.4. Introduction (Summary) 43

2. OBJETIVOS 47

2.1. Objectives 51

3. MATERIAL Y MÉTODOS 53

3.1. Poblaciones actuales 55

3.1.1. Muestras 55
3.1.2. Extracción de ADN 55
3.1.3. Análisis del ADN mitocondrial 55
3.1.4. Análisis del grupo sanguíneo AB0 56

3.2. Poblaciones antiguas 57

3.2.1. Muestras históricas 57
3.2.2. Muestras aborígenes 58
3.2.3. Obtención del ADN exógeno 58
3.2.4. Limpieza del material arqueológico 59
3.2.5. Extracción del ADN 59
3.2.6. Cuantificación por PCR a tiempo real 59
3.2.7. Criterios de autenticidad 60
3.2.8. Estudio del ADN mitocondrial 62
3.2.8.1. Amplificación de fragmentos solapantes 62
3.2.8.2. Determinación de haplogrupos mediante RFLP 62
3.2.8.3. Clonaje 63
3.2.9. Estudio del gen de la amelogenina 63
3.2.10. Estudio del cromosoma Y 64
3.2.10.1. Preamplificación multiplex 65
3.2.10.2. Reamplificación semianidada 66
3.2.10.3. Análisis por RFLP 66
3.2.10.4. Análisis mediante SNaPshot 67
3.2.11. Estudio del grupo sanguíneo AB0 68
3.2.11.1. Preamplificación multiplex 69
3.2.11.2. Reamplificación semianidada 69
3.2.11.3. Análisis por RFLP 69
3.2.11.4. Análisis mediante SNaPshot 70

3.3. Análisis de datos 70

4. RESULTADOS 73

4.1. Mejoras técnicas 75

4.1.1. Puesta a punto de un protocolo de obtención del ADN contaminante en superficie de muestras dentales fósiles 75
4.1.2. Mejora de la transformación de E.coli mediante el uso de microondas 77
4.1.3. Precipitación mejorada del ADN con etanol 81

4.1.4. HaploSearch: una herramienta para la transformación de secuencias en haplotipos y viceversa..... 91

4.2. Estudio del ADN mitocondrial en la Macaronesia: variación dentro y entre archipiélagos..... 111

4.3. La colonización aborigen de la isla de La Palma..... 113

4.4. Determinación del sexo en aborígenes de las Islas Canarias, basado en parámetros mandibulares y contrastados mediante análisis del gen de la amelogenina..... 127

4.5. Historia demográfica de los linajes paternos en las Islas Canarias..... 137

4.6. Estudio del grupo sanguíneo AB0 mediante PCR-SSCP multiplex y su aplicación al estudio del poblamiento de las Islas Canarias..... 153

4.7. Evolución temporal de las frecuencias del alelo AB0 en las Islas Canarias: El impacto de la colonización europea..... 163

5. DISCUSIÓN..... 173

5.1. Evolución temporal de la composición genética de la población canaria deducida a partir de marcadores autosómicos y uniparentales..... 175

5.2. Estudio de la asimetría sexual presente en la población histórica y actual de Canarias..... 179

5.3. El proceso de colonización aborigen de las Islas Canarias deducido a partir del estudio de marcadores autosómicos y uniparentales..... 181

6. DISCUSSION..... 185

6.1. Temporal evolution of the Canary Islands genetic composition deduced from uniparental and autosomal markers 187

6.2. Admixture analysis in the historical and present-day population of the Canary Islands 190

6.3. The Canarian aboriginal settlement process deduced from autosomal and uniparental markers 192

7. CONCLUSIONES 197

8. CONCLUSIONS 203

9. BIBLIOGRAFÍA 209

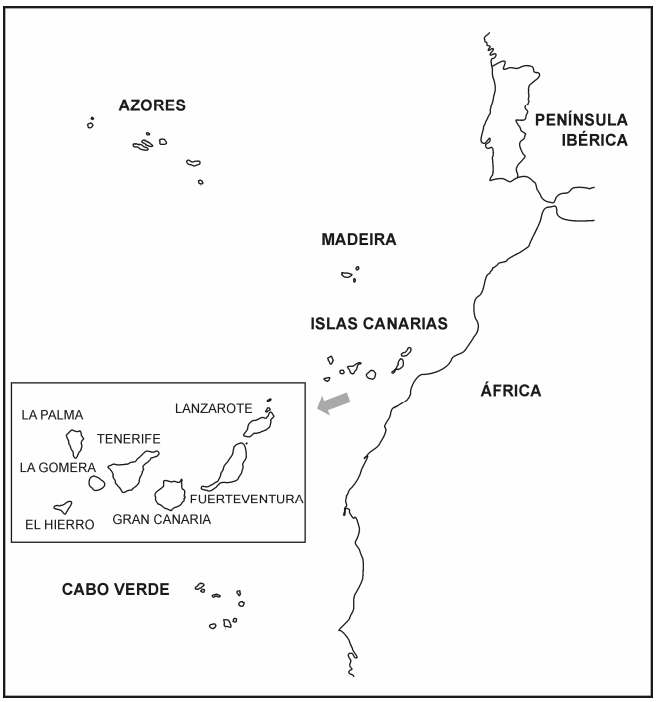
INTRODUCCIÓN

1. INTRODUCCIÓN

1. 1. La población canaria

Las Islas Canarias (Figura 1) se encuentran en el Océano Atlántico, situadas frente a la costa noroeste de África, entre las coordenadas 27º 37' y 29º 25' de latitud norte, y 13º 20' y 18º 10' de longitud oeste. Estas islas son parte de la región natural de la Macaronesia, junto con los archipiélagos de Cabo Verde, Azores, Madeira y Salvajes, y su origen es volcánico, por lo que nunca han estado conectadas al continente africano. El archipiélago canario está formado por siete islas: El Hierro, La Palma, La Gomera, Tenerife, Gran Canaria, Fuerteventura y Lanzarote. Fuerteventura, la isla más cercana al continente, se encuentra a menos de 100 Km. de la costa africana, mientras que aproximadamente 1.400 Km. separan al archipiélago del continente europeo.

Figura 1 – Situación geográfica de la Islas Canarias, dentro de la Macaronesia



1.1.1. Los aborígenes canarios

A diferencia del resto de los archipiélagos de la Macaronesia, las Islas Canarias se encontraban habitadas en el momento de ser descubiertas por los europeos (Hooton, 1916). Desde el principio de la conquista, los cronistas relacionaron las costumbres y el dialecto de los aborígenes canarios con los de las poblaciones bereberes del noroeste africano. Desde entonces hasta hoy, numerosos estudios antropológicos y arqueológicos han tratado de determinar cuando, cómo y desde donde llegaron los primeros pobladores de las islas.

1.1.1.1. Evidencias sobre el origen geográfico de los aborígenes canarios

La procedencia de la población aborigen canaria ha suscitado desde siempre un enorme interés. Desde el punto de vista histórico, para poder deducir el origen geográfico de los aborígenes, se ha recurrido tanto a la interpretación de fuentes escritas como a la de restos arqueológicos. Sin embargo, antes de llegar a cualquier conclusión, es de vital importancia que todas estas evidencias sean contrastadas empíricamente por especialistas (arqueólogos, antropólogos, historiadores, filólogos). En el caso, por ejemplo, de las antiguas fuentes escritas, que deben ser interpretadas en su contexto histórico, porque la información que aportan puede estar sesgada o incluso ser errónea. Esto es así porque, hasta fines del siglo XVI, las crónicas fueron escritas por los conquistadores, que interpretaban la sociedad y las costumbres indígenas desde una mentalidad occidental (Navarro, 2005a).

Cuando llegaron los europeos al Archipiélago Canario en los siglos XIV y XV, con intención de conquista y colonización, se encontraron que las islas estaban habitadas por poblaciones que aparentemente tenían un desarrollo cultural diferente y dialectos diversos, que desconocían la navegación y que habían permanecido aislados. Desde esos primeros contactos, el origen de la población indígena se convirtió en un tema de especial atención tanto en la literatura pre-científica como científica. El interés por conocer la procedencia de los aborígenes canarios suscitó que, a lo largo de la Historia, surgieran las más heterogéneas especulaciones sobre su origen, desde los míticos atlantes hasta los vikingos.

Muchas han sido las hipótesis sobre la procedencia de las antiguas poblaciones canarias, tantas que hoy existe una amplia bibliografía de historiografía centrada en analizar y explicar por qué surgen en cada época histórica visiones diferentes sobre el origen de los indígenas canarios y sobre el problema del poblamiento de las islas (Delgado, 2009; Estévez, 1987; Farrujía de la Rosa, 2004; Navarro, 2005b). En la actualidad, las investigaciones arqueológicas, antropológicas y filológicas apuntan hacia un indudable origen norteafricano de las poblaciones aborígenes canarias, emparentadas con el mundo protobereber y bereber (Navarro, 1997; Springer, 2001).

Desde que se iniciaron los estudios de antropología física en Canarias, sustentados en procedimientos ya superados de carácter meramente descriptivos y de base raciológica (Estévez, 1987; Farrujía de la Rosa, 2004; Farrujía de la Rosa, 2007), se establece una similitud entre los componentes físicos principales de las poblaciones antiguas canarias y las poblaciones norteafricanas prehistóricas y protohistóricas (Ferembach, 1970; Ferembach, 1986; Schwidetzky, 1963; Schwidetzky, 1975).

Por otro lado, se encuentra que determinados aspectos de la organización social y de la cultura material de los aborígenes canarios también se pueden relacionar con las poblaciones del noroeste africano, como es el caso de las viviendas semi-excavadas en el suelo (Cabrera, 1992a; Cabrera, 1992b), la forma en que enterraban a sus muertos (Tejera, 1992) o los tipos de cerámica encontrados (Cabrera, 1992a; Cabrera, 1992b; González y Tejera, 1990; León y Perera, 1996).

En trabajos más recientes, los grabados rupestres y las escrituras del tipo líbico-bereber encontradas en las distintas islas del Archipiélago, han proporcionado una prueba fehaciente de la relación de los aborígenes con poblaciones del ámbito bereber norteafricano (Springer, 2001). En algunos trabajos se ha planteado incluso que la denominación de muchas de las Islas Canarias parece corresponder con los antiguos etnónimos de tribus prerromanas norteafricanas, como la de los canarii, muy bien estudiada por Jiménez (2005) y Tejera y col., (2006).

De forma esporádica se han encontrado también evidencias de presencia de poblaciones europeas en Canarias, como son los supuestos materiales arqueológicos de origen romano encontrados en Lanzarote (Atoche y col., 1995). Sin embargo, es muy difícil determinar la intensidad de dicho contacto y si existió realmente un establecimiento de esas poblaciones en las islas, que implicara un impacto económico y cultural en los aborígenes canarios.

Se sabe que los límites de la ocupación territorial del África occidental atlántica en el caso de los fenicios, púnicos y romanos, estaban situados en lo que actualmente sería la ciudad de Rabat, con la única excepción conocida de la isla de Mogador. Por esta razón, parece poco probable que existiera una ocupación política o una explotación económica del archipiélago. Sin embargo, las Islas Canarias no eran del todo desconocidas para las poblaciones mediterráneas, por lo que podrían haberse producido algunas empresas exploratorias y arribadas fortuitas. Por otro lado, dado el aislamiento cultural de las poblaciones indígenas, parece que estos contactos debieron ser puntuales y sin consecuencias en la forma de vida de los aborígenes (Delgado, 2009).

1.1.1.2. Evidencias sobre el proceso de colonización aborigen de las Islas Canarias

Aunque se han propuesto numerosas razones que pudieron provocar la migración de los primeros pobladores a las Islas Canarias, si tenemos en cuenta la progresiva desecación del Sahara, es probable que las causas más importantes fueran de tipo económico y demográfico (Arco y Navarro, 1987; Navarro, 1997). Por otro lado, pudieron también existir razones de tipo político, como consecuencia de las diferentes influencias exteriores que actuaron sobre el Noroeste africano: la fundación de Cartago en el 814 a.C., la ocupación romana entre los siglos I a.C. y III d.C. y, las invasiones árabes en el siglo VII.

Sin embargo, la mayor incógnita probablemente no sea por qué sino cómo llegaron los primeros colonizadores a las islas, ya que todavía no existen evidencias del conocimiento de la navegación por parte de los aborígenes. Si atendemos a los documentos históricos, se tiene constancia de que los aborígenes eran buenos nadadores

y disponían de ciertos medios primitivos de navegación, como odres inflados o troncos de drago ahuecados (Arco y Navarro, 1987). También se plantean varias hipótesis respecto a la forma en la que llegaron a las islas, ya que se desconoce si llegaron por sus propios medios o si fueron traídos a las islas. Por otro lado, no se sabe si vinieron de forma voluntaria o si fueron deportados (Arco y Navarro, 1987; Jorge, 1996; Navarro, 1997).

Respecto a la fecha en que llegaron los aborígenes, existen numerosas dataciones absolutas en la actualidad, obtenidas de muestras procedentes de distintos yacimientos de todas las islas, que insisten siempre en un poblamiento tardío que corresponde con la mitad del primer milenio a. C. para la llegada de los primeros contingentes poblacionales (Arco y col., 1981; Arco y col., 1997; Martín de Guzmán, 1978; Martín, 2000; Navarro, 1997; Velasco-Vázquez y col., 2002).

Ya que en la mayoría de las islas se diferencian varias fases culturales superpuestas, se ha llegado a la conclusión de que la colonización del archipiélago se produjo probablemente por diversas migraciones desde el norte de África. La mayoría de los autores apuntan a una primera migración, procedente del Magreb, que crearía la cultura de sustrato en todas las islas, y una segunda que incorporaría elementos culturales saharianos. Estudios bioantropológicos sugieren que el poblamiento se produjo en tres momentos diferentes: dos oleadas consecutivas procedente de la zona del Magreb, a mediados del primer milenio y en torno al cambio de era, respectivamente; y una tercera de origen sahariano, en el siglo X d.C., que afectaría sólo a algunas islas (Navarro, 1997).

1.1.2. La conquista de las Islas Canarias

Aunque se sabía de su existencia desde la antigüedad clásica (Martínez, 1992; Tejera y col., 2006), las Islas Canarias fueron olvidadas durante los siglos de crisis del Imperio Romano y la Edad Media (Crosby, 1988). Durante este periodo, las poblaciones aborígenes canarias evolucionaron de manera independiente hasta que se produjo el denominado “redescubrimiento” de las islas (Cabrera, 1991).

Los primeros viajes a Canarias empezaron a realizarse a finales del siglo XIII por comerciantes europeos. También, navegantes castellanos y portugueses comenzaron a frecuentar los bancos pesqueros africanos. Estos viajes contribuyeron a mejorar el conocimiento sobre la navegación de la zona y a incrementar el interés hacia las Islas, centrado principalmente en la captura de esclavos y en la posibilidad de obtener suelo agrícola para implantar cultivos como el de la caña de azúcar (Aznar-Vallejo, 1992; Béthencourt, 1995). Existieron también fuertes motivaciones políticas, como la lucha entre las monarquías lusa y castellana por incorporar las islas a su soberanía, como paso previo a la conquista y control de las costas africanas.

Como fase inicial, se produjo en el siglo XIV una etapa de "precolonización" en la que se establecieron únicamente relaciones comerciales o evangelizadoras con la población aborigen. La etapa de colonización propiamente dicha se llevó a cabo en el siglo XV mediante el dominio militar del territorio, la creación de nuevos marcos político-administrativos, la remodelación de la población y la reordenación de las actividades económicas (Aznar-Vallejo, 1996). La conquista de las Islas duró casi un siglo y se produjo en dos fases diferenciadas. En una primera etapa, denominada conquista señorial, La Corona de Castilla cedió a Béthencourt el derecho de conquista y colonización de las islas. Esta etapa abarca desde el inicio de la conquista en 1402 hasta el 1477, año en el que los Reyes Católicos reclamaron los derechos de conquista de las islas hasta entonces insumisas (Gran Canaria, La Palma y Tenerife). Esta segunda fase se conoce con el nombre de conquista realenga y abarcó desde 1477 hasta 1496, año en el que finalmente se incorporó Tenerife a la Corona de Castilla (Béthencourt, 1995).

La conquista de las islas se inició con la llegada a Lanzarote de la expedición de navegantes normandos en 1402. Aunque inicialmente se establecieron pactos de amistad con los aborígenes de la isla, la ruptura de estos acuerdos y la esclavización de aborígenes, provocaron revueltas en la población indígena. Esta situación termina en 1404 con la llegada de nuevas tropas y con el sometimiento de la población aborigen, ya diezmada por las enfermedades y la esclavitud. La conquista de Fuerteventura es emprendida también por las nuevas tropas de normandos en 1404, en la que intervinieron aborígenes de Lanzarote, lográndose el dominio de la isla en 1405 (Suárez y col., 1988).

Tras estos acontecimientos, las fuerzas normandas tratan sin éxito la conquista de otras islas, lográndolo tan solo en la isla de El Hierro. Esto fue debido a que su población era poco numerosa, a causa de las acciones piratas y esclavistas realizadas por los navegantes europeos (Suárez y col., 1988).

La ocupación de La Gomera comenzó en 1420, cuando Maciot de Béthencourt se estableció en la isla. Esta primera etapa fue lenta y pacífica, aprovechando los pactos de amistad con algunos bandos gomeros. Fue a partir de 1455 cuando se inició realmente la conquista de la isla por parte de Fernán Peraza el Viejo. Tras la esclavización de gran número de aborígenes gomeros entre 1470 y 1480, se produjo una gran rebelión que culminó con una fuerte represión que marcaría la definitiva incorporación de La Gomera al Señorío de Canarias en 1488. Esta represión tuvo como consecuencia la muerte de un gran número de aborígenes y la esclavización de mujeres y niños, que fueron vendidos en los mercados peninsulares (Díaz y Rodríguez, 1990; Macías y Bravo de Laguna, 1997). Por otro lado, la llegada de nuevos colonos, obligó a los aborígenes que permanecieron en la isla a ocupar una situación marginal (Suárez y col., 1988).

La conquista de Gran Canaria comenzó en 1478 por orden de los Reyes Católicos. La primera fase, comprendida entre los años 1478-1480, se caracterizó por una guerra de desgaste, centrada fundamentalmente en el noreste de la isla. En la segunda etapa, a partir del año 1480, las constantes capturas de esclavos y las epidemias desembocaron finalmente en la rendición definitiva de la isla en el año 1483. Sin embargo, algunos grupos de aborígenes perduraron alzados en armas en zonas de cumbres (Suárez y col., 1988).

Aunque la isla de La Palma había estado expuesta a varios intentos de conquista a lo largo del siglo XV, la ocupación definitiva no comenzó hasta el año 1492. La empresa corrió a cargo de Alonso Fernández de Lugo, con la ayuda de hombres procedentes de Sevilla, así como de aborígenes de Gran Canaria y La Gomera. Un año después y tras diversas batallas se pactó una tregua entre los colonizadores y la población indígena de La Palma. Sin embargo, mediante engaño, el jefe de los guerreros palmeros acaba siendo apresado junto a gran parte de sus hombres, que fueron enviados a la península como

esclavos. Tras la incorporación de la isla a la Corona de Castilla, muchos de los aborígenes que quedaron en la isla también fueron esclavizados (Suárez y col., 1988).

A lo largo del siglo XV, Tenerife también había tenido una amplia presencia de comerciantes, misioneros y navegantes. La conquista de la isla, emprendida por Alonso Fernández de Lugo, fue un proceso relativamente corto (1494-1496), en el que se pueden distinguir varias etapas. En 1494 un contingente de hombres integrado por sevillanos y aborígenes de otras islas desembarcó en Tenerife, y tras sufrir una fuerte derrota se retiraron a Gran Canaria. En 1496 tuvo lugar un nuevo intento, esta vez definitivo (Suárez y col., 1988), el cual se vio facilitado por el mal estado de salud de la población aborigen, diezmada por una epidemia denominada "modorra" (Rodríguez y Hernández, 2005).

1.1.3. La colonización de las Islas Canarias

La conquista y colonización de las Islas Canarias provocó una disminución de la población aborigen (Viera y Clavijo, 1772), aunque es muy difícil estimar los datos demográficos anteriores a la llegada de los conquistadores y posteriores a la misma (Macías, 1988; Macías, 1992; Onrubia-Pintado, 2003), ya que las fuentes de las que se dispone fueron escritas por cronistas europeos y su información puede estar sesgada.

Lo que está claro es que el estilo de vida indígena se modificó drásticamente tras la conquista (Onrubia-Pintado y col., 1998; Trujillo, 2004), ya que los aborígenes que quedaban se mezclaron con los colonizadores, mientras que otros fueron trasladados a otras islas o al continente, y en algunos casos, introducidos en el mercado esclavista europeo (Crosby, 1988; Suárez y col., 1988). Sin embargo, la política de conservación del pueblo indígena de Isabel la Católica favoreció su supervivencia y mestizaje (Hernández, 1999). De los datos del Obispado de Canarias se deduce que en 1504 existían unas 1.200 familias aborígenes en todo el Archipiélago (Aznar-Vallejo, 1991).

1.1.3.1. La población aborigen tras la conquista

En cuanto al número de aborígenes que sobrevivieron a la conquista, existen grandes diferencias entre las islas. Sin embargo, en todas ellas, la población indígena

participó en el proceso repoblador; en el caso de los nobles, fusionándose con los jefes europeos, y en el resto, dedicándose a la ganadería e integrándose en otros grupos de su mismo nivel y consideración social. Dado el escaso número de mujeres europeas que había en las islas, algunas aborígenes accedieron por matrimonio a la categoría de colonizadoras (Suárez y col., 1988).

Aunque Fuerteventura y Lanzarote no tenían una población indígena numerosa, ésta se intentó conservar a pesar de la esclavización y el traslado de muchos aborígenes. Una isla más castigada en este sentido fue El Hierro que, incluso antes de la colonización europea, ya contaba con una población aborigen bastante reducida por las incursiones piratas. Tras la conquista, la isla del Hierro fue poblada por normandos, quedando tan sólo mujeres y niños aborígenes. Por otro lado, La Palma, castigada igualmente por las capturas piratas, sufre un proceso de esclavización masivo tras la conquista, quedando la población indígena relegada al sur de la isla.

En el caso de La Gomera, en un principio se conservó gran parte de la población, e incluso algunos gomeros participaron en la conquista de otras islas. Esta situación cambió tras la rebelión de los gomeros, dando como resultado la esclavización y ejecución de muchos de ellos.

En Gran Canaria, una gran parte de los aborígenes que no habían sido esclavizados fueron deportados, salvo excepciones de algunos nobles, con la intención de impedir revueltas. Algunos indígenas de Gran Canaria participaron luego en la conquista de Tenerife, quedándose posteriormente en esta isla (Betancor, 2002; Betancor, 2003). Sin embargo, el número de aborígenes, esclavos o libres, que quedaron en la isla de Gran Canaria fue considerable.

En Tenerife, tras la conquista y la esclavización de una parte de la población, los supervivientes se concentraron en la zona sur, quedando otros grupos esparcidos por el resto de la isla (Suárez y col., 1988). En 1519 se estima que la población indígena de Tenerife no sería mayor de 3.000 (Cioranescu, 1998).

1.1.3.2. La colonización europea

En un principio Lanzarote, Fuerteventura y El Hierro fueron pobladas por normandos, andaluces y moriscos, incrementándose estos últimos con el aumento de las incursiones en África en busca de esclavos, a finales del siglo XV. En el resto de las islas, los colonos procedían de diversos lugares, siendo más numerosos los portugueses, españoles, italianos y flamencos (Suárez y col., 1988).

Dentro de la población española que participó en la colonización, la mayoría procedía de Andalucía y fueron partícipes de las fases de exploración, saqueo y conquista. Posteriormente, se produjo la llegada de gallegos (Pérez, 1991), especializados en tareas agrícolas y ganaderas. También llegaron algunos extremeños, que se dedicaron a la agricultura, y burgaleses que participaron en tareas comerciales (Suárez y col., 1988).

Aunque a mitad del siglo XV hubo un corto periodo de ocupación portuguesa en La Gomera y Lanzarote, su mayor influencia se produjo tras la conquista. Los portugueses fueron los responsables de la introducción y desarrollo del cultivo de la caña de azúcar y su procesamiento. Existían, además, leyes que obligaban a los agricultores, tanto españoles como portugueses, a establecerse con sus esposas en las tierras que se les habían asignado para poder conservarlas. Algunos se instalaron en las costas como carpinteros de ribera o pescadores, pero sin lugar a dudas, una de las actividades más importantes llevadas a cabo por este colectivo de colonizadores fue la comercial, con todo tipo de mercancías incluyendo esclavos, tanto blancos como negros (Pérez, 1991).

También llegaron italianos a las islas, procedentes en su mayoría de Génova, que estuvieron relacionados con la industria azucarera. La mayoría terminó fusionándose con las familias aristocráticas y ocuparon puestos importantes en la administración. Otras minorías europeas que llegaron a las islas fueron flamencos, ingleses e irlandeses, relacionados con las exportaciones comerciales a Europa del Norte. También algunos judíos y moriscos buscaron refugio en Canarias tras ser expulsados de Castilla y Portugal a finales del siglo XV (Suárez y col., 1988).

1.1.3.3. El tráfico de esclavos en Canarias

Tras la conquista, debido al bajo número de aborígenes y la existencia de órdenes que los amparaban, se hizo necesaria la importación de mano de obra esclava procedente de las costas de Berbería (regiones costeras de Marruecos, Argelia, Túnez y Libia). Mediante cabalgadas, compras a mercaderes portugueses o expediciones a Cabo Verde y Guinea, llegaron a Canarias esclavos moriscos y negros, necesarios en todos los sectores económicos, pero sobre todo en las plantaciones e ingenios de caña de azúcar (Lobo-Cabrera, 1993).

El número de esclavos moriscos que llegaron a las diferentes islas fue variable. En Lanzarote y Fuerteventura su presencia fue mayoritaria, ejerciendo además como elemento repoblador (Suárez y col., 1988). A finales del siglo XVI, en Fuerteventura constituyeron el 15,3% de la población, dedicándose principalmente a la agricultura y pastoreo (Lobo-Cabrera, 1993).

En 1572 se prohibieron las cabalgadas, por lo que el comercio de esclavos se centró en los de raza negra. Los esclavos negros, procedentes de Berbería, Cabo Verde, Angola y Madeira, llegaron a Canarias de manos de mercaderes portugueses, siendo distribuidos de las islas mayores a las menores por comerciantes del archipiélago. En algunas islas, como Gran Canaria, llegaron a representar el 70% de la población esclava. En esta isla entraron unos 10.000 esclavos durante el siglo XVI, con un aporte regular de unos 100 individuos por año (Lobo-Cabrera, 1993).

1.1.3.4. Influencia americana en Canarias

Es necesario destacar también la continua relación existente entre Canarias y América, lo que motivó importantes movimientos de población entre ellas. Los canarios, primero como conquistadores hasta finales del siglo XVI, y luego como colonizadores y trabajadores, protagonizaron diferentes oleadas de emigración a América, principalmente a Cuba, Venezuela, Puerto Rico, Santo Domingo, Texas, Luisiana y Uruguay (Castellano y Macías, 1997). Diferentes acontecimientos económicos y políticos en los países de

destino de los canarios, motivaron posteriormente el regreso de familias enteras a las islas.

1.1.4. La población de Santa Cruz de Tenerife en los siglos XVII-XVIII: la Iglesia de La Concepción

Desde los inicios de la conquista, Santa Cruz de Tenerife ocupó un lugar importante en el Archipiélago Canario, primero como punto de arribada de las expediciones europeas y, una vez terminado el proceso de conquista, como puerto principal de las islas.

Tras la conquista, numerosos colonos europeos se asentaron en Santa Cruz de Tenerife, preferentemente en la salida de los barrancos, situación privilegiada por su cercanía al puerto. Los colonos, tras confiscar sus tierras, desplazaron a los indígenas y los dispersaron a lo largo de la isla. A los guanches que quedaron, se unieron muchos gomeros y canarios desterrados que, tras intervenir en la conquista, habían adquirido un estatus superior. Sin embargo, a pesar de la llegada de los colonos, en Santa Cruz se siguió manteniendo la base de la economía indígena, basada en la cría de ganado caprino. Durante este tiempo el desarrollo de la población fue lento y muchos abandonaron la zona debido a las malas condiciones económicas. En 1561, se estima para Santa Cruz una cifra de 57 viviendas y alrededor de 770 habitantes.

Posteriormente se empezó a desarrollar de forma gradual una nueva economía, basada en actividades agrícolas y mercantiles, además de mantener la cría de ganado caprino (Cioranescu, 1998). En el siglo XVII, la mitad de los navíos procedentes de las Américas pasaba por el puerto santacrucero, convirtiéndose en el área receptora de productos manufacturados y alimenticios de importación. Ya en 1676, el número de habitantes en Santa Cruz supera los 2000.

A principios del siglo XVIII, se produjo un aumento demográfico coincidiendo con una época de prosperidad, en la que se ve favorecido el desarrollo de centros urbanos. Santa Cruz se convirtió en el puerto principal de las islas, al que llegan multitud de navíos europeos incluyendo ingleses, holandeses, suecos y daneses. Por otro lado, en 1718 el

puerto santacrucero, según el reglamento del comercio canario-americano, se convirtió en el único autorizado para el tráfico con América y el de obligada arribada para el regreso en todo el Archipiélago Canario. En este momento, la población de Santa Cruz estaba formada por los indígenas que sobrevivieron a la conquista, los colonizadores peninsulares y europeos, y los esclavos. En las partidas de casamiento, en las que a partir del siglo XVIII se obliga a mencionar la procedencia de los contrayentes, se observa que el 98% de las mujeres que se casan en Santa Cruz han nacido en las Islas, lo que indica un papel fundamental de la mujer canaria en la formación de la sociedad insular y en su estabilización. Dentro de los españoles, los andaluces y gallegos son los más numerosos. Destaca también la presencia de población americana. Con respecto a los procedentes del extranjero, la mayoría son franceses, portugueses e italianos. Aunque, en los primeros tiempos de colonización la mayor parte de los esclavos de Tenerife eran guanches, en este momento la mayoría la constituyeron los negros y los moriscos. También, numerosos esclavos alcanzaron la libertad y vivieron en común con los españoles, mezclándose con ellos (Cioranescu, 1998).

La Iglesia de La Concepción de Santa Cruz fue fundada en 1501. Desde su inicio hasta 1823, cuando se produjo la apertura del primer cementerio civil, la Iglesia de La Concepción fue usada con fines funerarios (Larraz y González, 1995). Esta costumbre, consistente en enterrar a los muertos tanto dentro como en los alrededores del templo, fue bastante común en las iglesias católicas hasta principios del siglo XIX. En el caso de La Concepción se registraron más de 12.000 entierros en el interior del templo y más de 15.000 si se cuentan los del exterior (Arnay y Pérez, 2002; Sanz de Magallanes, 2001). Teniendo en cuenta que la Iglesia de La Concepción albergó los enterramientos tanto de hombres libres como esclavos, estos restos pueden ser un ejemplo representativo de cómo era la sociedad de Tenerife en los siglos XVII-XVIII (Sanz de Magallanes, 2001).

1.1.5. La población canaria actual

El crecimiento demográfico en las islas ha continuado desde entonces, llegándose a una población de unos 360.000 habitantes a principios del siglo XX y alcanzando los dos millones en la actualidad (INE, 2008). Las islas más pobladas son Gran Canaria y Tenerife

con más de 800.000 habitantes y, las menos habitadas son La Gomera y El Hierro con unos 20.000 y 10.000 habitantes, respectivamente.

1.2. Estudios genéticos en la población canaria

Los estudios genéticos se basan en la búsqueda y caracterización de variantes entre individuos, ya que la variabilidad que observamos en el presente es un reflejo de la historia evolutiva de nuestra especie (Relethford, 1998). Por tanto, para poder entender la evolución humana es necesario determinar el origen y evolución de dicha variación en las poblaciones. Estas variantes genéticas se originan, en la mayoría de los casos, debido a fallos en el proceso replicativo, aunque en otras ocasiones aparecen como consecuencia de otros procesos como la recombinación génica o la pérdida de segmentos de ADN. Procesos demográficos y genéticos como la migración, aislamiento o selección contribuyen a la diferenciación genética de las poblaciones.

Antes de que el material genético pudiera analizarse directamente, se usaron como marcadores de variabilidad ciertos caracteres morfológicos, como el color de la piel o el cabello, y antropométricos, como los índices craneales o la constitución corporal. El problema del uso de estos marcadores radica en que no representan la diversidad total del genoma, ya que solo tienen que ver con genes o grupos de genes relacionados con dichos caracteres fenotípicos. Además ofrecen problemas debido a su herencia compleja, a la subjetividad a la hora de analizar los datos y al hecho de que pueden verse afectados por el ambiente.

Posteriormente, los estudios poblacionales se basaron en dos tipos de marcadores: en un principio, se usaron técnicas inmunológicas para determinar diferencias poblacionales en los antígenos eritrocitarios y más tarde, se realizaron análisis de polimorfismos en proteínas, tanto estructurales como enzimáticas. Aunque los grupos sanguíneos y los polimorfismos proteínicos aportaron bastante información sobre la historia evolutiva del hombre, resultaron ser bastante homogéneos entre poblaciones (Jorde y col., 1998). Además, tal como ocurría con los marcadores morfológicos, sólo son representativos de la fracción traducida del genoma y pueden no ser selectivamente neutrales.

Finalmente, el avance en los métodos de biología molecular ha permitido realizar un análisis directo de la variación a nivel de ADN, primero con hibridaciones y enzimas de restricción, y finalmente mediante secuenciación directa del material genético. Este gran avance ha sido posible gracias al descubrimiento en 1984 de la reacción en cadena de la polimerasa (PCR, del inglés, Polimerase Chain Reaction) por Kary Mullis, que permitió la amplificación de fragmentos discretos de ADN en cantidades suficientes para un análisis más exhaustivo (Mullis y col., 1986).

El estudio de la variación a nivel de ADN se ha realizado desde diferentes enfoques: se han usado marcadores localizados en autosomas (cromosomas no sexuales), en el ADN mitocondrial y en los cromosomas sexuales, X e Y. Asimismo, estos análisis se han abordado principalmente mediante dos procedimientos diferentes: la caracterización de determinadas mutaciones puntuales o SNPs (del inglés, Single Nucleotide Polymorphisms) y la secuenciación no sesgada de fragmentos de ADN.

1.2.1. Primeros estudios genéticos en la población canaria: grupos sanguíneos y polimorfismos enzimáticos

1.2.1.1. El grupo sanguíneo AB0

El grupo sanguíneo del sistema AB0 es uno de los sistemas más importantes en trasfusiones y transplantes (Landsteiner, 1901). La destrucción y aglutinación de las células sanguíneas entre individuos de grupos incompatibles está desencadenada por el sistema inmune, que reconoce las células transfundidas como extrañas al organismo. Actualmente, las bases de este fenómeno están bien establecidas (Daniels, 1995). Las diferencias antigénicas de cada uno de los grupos sanguíneos están determinadas por la presencia o no de determinados residuos de azúcares en las glicoproteínas y glicolípidos de la membrana de los eritrocitos. Estos grupos carbohidrato se incorporan a la membrana gracias a la acción de las glicosiltranferasas A y B. Las diferencias antigénicas de los eritrocitos responden a mutaciones en el gen que codifica la glicosiltranferasa, que incorporará un tipo u otro de oligosacárido, o ninguno en el caso del alelo *O*.

Uno de los primeros análisis genéticos realizados en la población canaria fue la determinación del grupo sanguíneo AB0 en restos aborígenes mediante el uso de antígenos eritrocitarios (Schwarzfischer y Liebrich, 1963). La alta frecuencia del alelo *O*, similar a la encontrada en tribus bereberes del Atlas (Gaud, 1942; Messerlin y Lorho, 1951), corroboraron las evidencias arqueológicas y antropológicas que relacionaban la población guanche con la bereber (Navarro, 1997).

Posteriormente, numerosos estudios caracterizaron la población canaria actual para el grupo AB0, así como para otros grupos sanguíneos como Rh, MNSs, FY y P (Bravo y De las Casas, 1958; Guasch y col., 1952; Parejo, 1966; Pinto y col., 1996a; Roberts y col., 1966; Rösing, 1967; Schwidetzky, 1970). Las frecuencias alélicas de la población canaria actual se encontraron dentro del rango de las poblaciones europeas, aunque se detectó una minoritaria aportación africana. En algunas islas, como La Gomera, se encontraron frecuencias para el alelo *O* similares a las del Magreb (Gaud, 1942; Messerlin y Lorho, 1951) y a las de los estudios realizados en momias guanches (Schwarzfischer y Liebrich, 1963).

Actualmente se ha clonado y secuenciado el gen de la glicosiltranferasa (Yamamoto y col., 1990a) y se han determinado las mutaciones que caracterizan los grupos A, B y O. Las únicas diferencias existentes entre la glicosiltranferasa A y B consisten en 7 mutaciones puntuales, de las cuales sólo 4 determinan un cambio de aminoácido. La principal diferencia entre la glicosiltransferasa A y O radica en la deleción de una citosina en posición 261, que da lugar a un codón de parada prematuro y a la formación de una proteína enzimáticamente inactiva (Yamamoto y col., 1990b).

Por otro lado, los avances en la biología molecular han permitido el estudio exhaustivo de la región codificante de la glicosiltranferasa, encontrándose una gran cantidad de variantes alélicas, sobre todo dentro del grupo O (Yip, 2002). Existen algunos alelos de este grupo que son bastante abundantes, como el *O01* (*O101* según la nomenclatura de Yamamoto (2000)) y el *O02* (*O201*), que han sido detectadas en todas las poblaciones estudiadas hasta el momento (Yip, 2002). Sin embargo, también se han identificado otras variantes menos frecuentes, que muestran una distribución geográfica más restringida. En el caso de los alelos *O03* (*O303*) y *O12* (*O210*), su distribución afecta

a las dos poblaciones parentales de las Islas Canarias, ya que ambos son alelos raros compartidos por vascos y por bereberes (Roubinet y col., 2001).

1.2.1.2. Polimorfismos enzimáticos

La determinación de polimorfismos enzimáticos se basa en la caracterización de isoenzimas, o lo que es lo mismo, enzimas que catalizan la misma reacción química pero que poseen diferencias en sus secuencias aminoacídicas. El análisis de polimorfismos enzimáticos fue utilizado por primera vez para el estudio de poblaciones de *Drosophila pseudoobscura* (Hubby y Lewontin, 1966; Lewontin y Hubby, 1966), empleando métodos electroforéticos y de tinción de proteínas. Esta técnica se aplicó más tarde a la caracterización de poblaciones humanas (Harris, 1966), siendo muy usada hasta el posterior desarrollo de las técnicas basadas en el análisis directo del DNA.

Para el análisis genético de la población canaria se estudiaron ocho enzimas sanguíneas, tanto en las Islas Canarias, como en sus dos poblaciones parentales, la Península Ibérica y el noroeste africano (Afonso y col., 1989; Cabrera y col., 1996; Larruga y col., 1992; Martell y col., 1986; Morilla y col., 1988; Pérez y col., 1991; Pinto y col., 1996b). Al igual que en el caso de los grupos sanguíneos, las frecuencias de estas alozimas en Canarias resultaron similares a las típicas de poblaciones caucásicas. Sin embargo, la presencia de la variante alélica R del locus ACP (fosfatasa ácida) y del alelo G6PD A+ (glucosa-6-fosfato deshidrogrenasa), relacionados con poblaciones negroides, evidenciaron un cierto componente africano en la población canaria actual.

1.2.2. Marcadores uniparentales

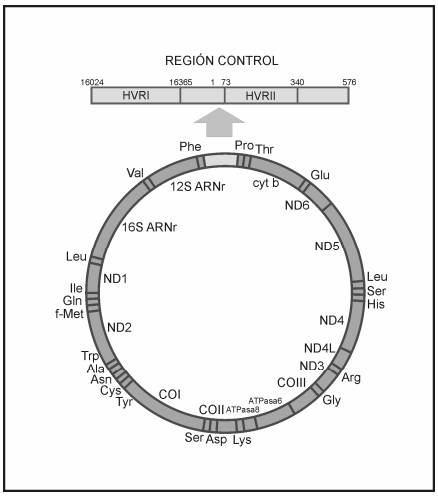
1.2.2.1. Los linajes maternos: el ADN mitocondrial

El ADN mitocondrial (ADNmt) es el material genético presente en las mitocondrias, los orgánulos en los que se genera la energía necesaria para el funcionamiento celular. Este ADN, al igual que los bacterianos, está formado por una molécula bicatenaria, circular, cerrada y sin extremos. El ADNmt contiene un pequeño

número de genes que codifican dos ARN ribosómicos, 22 ARN de transferencia y 13 proteínas que participan en la fosforilación oxidativa (Figura 2).

El ADNmt posee características que lo convierten en una excelente herramienta para estudios evolutivos basados en linajes maternos. Su pequeño tamaño (Anderson y col., 1981; Andrews y col., 1999), unas 16.500 pares de bases (pb), y su alto número de copias por célula hacen fácil su obtención y estudio. Además, el hecho de que se herede por vía materna sin sufrir recombinación, ha permitido obtener información sobre el origen y migración de las poblaciones humanas. Por otro lado, la alta tasa de mutación del ADNmt, especialmente en la región hipervariable (HVR), hace mucho más fácil la resolución de diferencias entre poblaciones y/o individuos muy relacionados (Vigilant y col., 1991).

Figura 2 – Esquema del ADN mitocondrial, mostrando un detalle de la región control.



Debido a la ausencia de recombinación y a la herencia por vía materna, el ADNmt ha permitido la reconstrucción de la historia evolutiva de nuestra especie. Al no recombinar, las diferencias existentes entre dos secuencias de ADNmt estarán debidas únicamente a nuevas mutaciones. Estas modificaciones, con el paso del tiempo, se irán acumulando y darán lugar a secuencias cada vez más diferentes, o lo que es lo mismo, a linajes cada vez más alejados. El conjunto de mutaciones acumulado en una molécula de ADNmt se denomina haplotipo. Estos haplotipos pueden relacionarse entre sí mediante el

uso de redes filogenéticas que los van clasificando en orden jerárquico, en función de las mutaciones compartidas. Al grupo de haplotipos que poseen mutaciones en común (o mutaciones basales) se le denomina haplogrupo. Estas mutaciones basales, si suponemos que no existe retromutación, relacionarán los linajes dándoles un origen común y, ya que la tasa de mutación es conocida, podemos además estimar el origen y el tiempo en el que se produjeron las migraciones humanas.

Sin embargo, debido precisamente a la alta tasa de mutación de la HVR, las relaciones filogenéticas entre linajes pueden emborronarse por retromutación, sobre todo en ciertas zonas con tasas de mutación particularmente altas ("hotspots"). Cuando existen estos problemas es necesario ampliar la región estudiada, por ejemplo, recurriendo a la secuenciación completa del ADNmt, ya que, fuera de la HVR, el número de retromutaciones y mutaciones recurrentes es mucho menor.

Cuando se analizó la variabilidad del ADNmt en distintas poblaciones, se encontró que la filogenia del ADNmt mostraba una marcada diferencia entre los linajes africanos subsaharianos y los no africanos (Cann y col., 1987). Además, uno de los haplogrupos más antiguos aparecía exclusivamente en africanos. Este resultado apoyó el origen reciente de los humanos modernos y el modelo de la salida de África ("Out of África"), según el cual todos los linajes actuales provienen de un ancestro común africano, la Eva mitocondrial. Estudios posteriores han refinado el árbol filogenético del ADNmt y han permitido una clasificación en haplogrupos y subhaplogrupos cada vez más detallada (Torroni y col., 2006; van Oven y Kayser, 2009).

Tras el estudio global de los haplogrupos del mitocondrial se encontró que éstos eran geográficamente específicos (Richards y col., 2000). Tres de ellos (L1, L2 y L3) aparecen en el área del África subsahariana. Nueve (H, I, J, K, T, U, V, W y X) incluyen la mayoría de linajes europeos, norteafricanos y caucásicos del oeste asiático. Finalmente, los haplogrupos A, B, C, D, E, F, G, M y Q están relacionados con las poblaciones de Asia, Oceanía y los nativos americanos. Por otro lado, el estudio de las edades de coalescencia de los grandes macrohaplogrupos detectó una primera expansión desde África hace unos 59.000-69.000 años aproximadamente, que independientemente colonizó el oeste asiático y la India, y siguiendo hacia el sur, alcanzó el este asiático.

Alrededor de 39.000-52.000 años atrás, la rama del oeste asiático se dispersó de forma radial llevando linajes caucásicos al norte de África y Europa, alcanzando la India y expandiéndose al norte y el este de Asia. Migraciones más recientes han desdibujado, aunque no eliminado completamente, estas huellas de las expansiones primitivas del hombre moderno (Maca-Meyer y col., 2001).

Dentro de la población canaria, un estudio preliminar de ADNmt comparó la población de la isla de Tenerife con las poblaciones más cercanas histórica y geográficamente: Península Ibérica, norte de África y el Sub-Sahara (Pinto y col., 1996c). En este trabajo se estimó que la población canaria se habría formado con una aportación del 43% de linajes norteafricanos, un 36% de peninsulares y un 21% de subsaharianos. Estos resultados mostraban una gran diferencia con aquellos obtenidos mediante marcadores autosómicos, que estimaron la contribución norteafricana en un 21%, la peninsular en un 71% y la subsahariana en un 8% (Pinto y col., 1994; Roberts y col., 1966). Estos datos apuntarían a una fuerte asimetría sexual, en la que habría comparativamente una mayor aportación norteafricana y subsahariana desde el punto de vista de los linajes maternos.

Posteriormente se realizó un análisis del ADNmt de la población canaria más amplio, incluyendo individuos de todas las islas (Rando y col., 1999). Además de la secuenciación de la HVR del ADNmt, se estudiaron varias posiciones diagnóstico por RFLP (del inglés, Restriction Fragment Length Polymorphism), que permitieron una mejor asignación dentro de haplogrupos. Aunque aparecieron linajes típicamente europeos, la presencia de un subtipo del haplogrupo bereber U6, el U6b1, detectado únicamente en Canarias, relacionó directamente la población aborigen de las islas con el noroeste africano. Sin embargo, la estima de aportación norteafricana, con tan solo un 33%, fue inferior a la obtenida por Pinto y col.(1996c) siendo mayoritaria la contribución europea con un 65%.

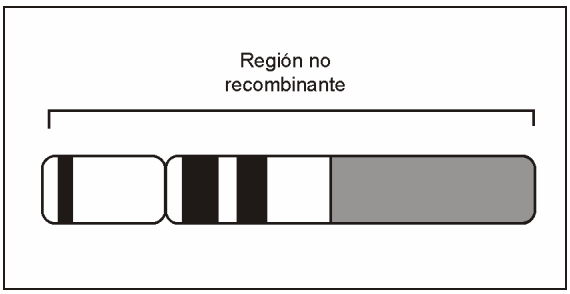
La caracterización de los linajes maternos canarios también aportó información sobre el proceso de colonización del archipiélago por parte de los aborígenes. Basándose en la composición haplotípica de las diferentes islas, se detectó una correlación negativa entre la heterocigosidad insular y la distancia al continente africano. Este hecho apuntó a

una colonización basada en un único evento migratorio de este a oeste, procedente del noroeste africano.

1.2.2.2. Los linajes paternos: el cromosoma Y

La contrapartida masculina del ADNmt está representada por la región no recombinante del cromosoma Y (Figura 3), que constituye el 95% de su longitud (Jobling y Tyler-Smith, 1995). Debido a la ausencia de recombinación y a su herencia por vía paterna, esta región se transmite de padres a hijos sin modificación, salvo por la acumulación gradual de mutaciones. A diferencia del ADNmt, el cromosoma Y es bastante grande (unas 60 x 10^6 pb en comparación con las 16 x 10^3 pb del ADNmt) y aparece en un menor número de copias (una copia por célula comparado con las 3.000 copias del mitocondrial).

Figura 3 – Esquema del cromosoma Y, mostrando la región específica del cromosoma Y (Jobling y Tyler-Smith 1995).



Uno de los problemas de la región no recombinante del cromosoma Y, en comparación con el ADNmt, es su baja tasa de mutación, unas 5-10 veces menor que su contrapartida femenina. A pesar de eso, en la actualidad se han descrito más de 600 SNPs asociados al cromosoma Y (Karafet y col., 2008). Además, debido precisamente a su baja tasa de mutación (Thomson y col., 2000), se produce un menor número de retromutaciones y mutaciones recurrentes, y, por tanto, la presencia compartida de una mutación definirá, con alta probabilidad, un grupo de cromosomas relacionados por descendencia. De esta manera, la construcción de árboles filogenéticos resulta más sencilla y exacta (Karafet y col., 2008; Underhill y col., 2001; Y-Chromosome-Consortium, 2002).

Sin embargo, esta baja tasa de mutación es un problema cuando necesitamos una división en subhaplogrupos más refinada. En estos casos, es necesario el uso de microsatélites de evolución rápida, que nos permitan identificar grupos monofiléticos dentro de haplogrupos (Cruciani y col., 2004; Semino y col., 2004). Igualmente, el uso de microsatélites es necesario para determinar las edades de coalescencia de un determinado haplogrupo, ya que el número de variantes acumuladas dentro de un determinado linaje, estará directamente relacionado con su edad (Jobling y Tyler-Smith, 2003).

Estudios recientes basados en el cromosoma Y han demostrado su eficacia para elucidar el origen, evolución y dispersión de las poblaciones humanas. Al igual que ocurrió con el ADNmt, tras el análisis global de la distribución de los haplogrupos del cromosoma Y, se encontró que éstos eran geográficamente específicos y que su filogenia apoyaba la teoría evolutiva del "Adán cromosómico Y", análogo a la "Eva mitocondrial", que habría habitado en África unos 90.000 - 130.000 años atrás (Underhill y col., 2000). En cuanto a su distribución, los haplogrupos A y B están asociados al África subsahariana; los haplogrupos E, J y T se distribuyen por África, Oriente Medio y el sur de Europa; y los haplogrupos R, G y I por Oriente Medio, el Caúcaso y el Mediterráneo. Finalmente, los haplogrupos C, D, K, F, H, L, M, O, Q y S aparecen en Asia, Australia, Oceanía y América (Karafet y col., 2008; Underhill y Kivisild, 2007).

En Canarias, una vez determinada la composición genética de los linajes maternos, se analizaron 24 marcadores bialélicos y 5 microsatélites del cromosoma Y en poblaciones de las siete islas (Flores y col., 2003), con el fin de compararlas con el noroeste africano y la Península Ibérica (Bosch y col., 2001; Flores, 2001; Flores y col., 2004; Hurles y col., 1999; Underhill y col., 2000). Los resultados indicaron que, al contrario de lo que ocurre con el ADNmt, el pool genético paterno tiene un origen europeo mayoritario (>90%), señalando de nuevo una gran asimetría sexual como resultado de la colonización europea. Sin embargo, la presencia de linajes africanos como E-M81 apunta a una pervivencia relativa de linajes aborígenes. Basándose en la distribución y la edad de estos linajes africanos, se detectó un proceso de colonización en dos fases, coincidiendo con resultados obtenidos mediante estudios antropológicos, arqueológicos y lingüísticos

(Navarro, 1997; Springer, 2001) y contrario al único evento migratorio detectado para el ADNmt (Rando y col., 1999).

1.2.3. Marcadores autosómicos: el sistema CD4/Alu.

Teniendo en cuenta que el ADNmt y el cromosoma Y sólo reflejan los linajes maternos y paternos de forma independiente, el uso de marcadores moleculares autosómicos es necesario para obtener una visión conjunta de la evolución de las poblaciones.

Entre los marcadores autosómicos usados en estudios poblacionales, el sistema de haplotipos CD4/Alu ha resultado ser una buena herramienta para trazar el origen y la expansión del hombre moderno (Tishkoff y col., 1996) y para estimar la mezcla entre poblaciones (Destro-Bisol y col., 1999). El sistema CD4/Alu está formado por dos loci asociados, un locus bialélico cuyo estado ancestral es la pérdida parcial de un elemento Alu y una repetición en tándem de un pentanucleótido (5' TTTTC 3').

Una vez analizado el sistema CD4/Alu en las poblaciones parentales de la población canaria actual (Península Ibérica, noroeste africano y África subsahariana) (Flores y col., 2000), se aplicó este marcador autosómico a la caracterización de las islas. Aunque la distribución de los haplotipos CD4/Alu en Canarias no se diferenció de la de la Península Ibérica, algunos resultados apuntaron a una influencia norteafricana (Flores y col., 2001a). Entre ellos, destacó la presencia del haplotipo 110(-), relacionado directamente con el noroeste africano (Flores y col., 2000). Esta variante apareció en la mayoría de las islas e, incluso, algunas de ellas mostraron una distribución de haplotipos que no se diferenciaba significativamente de la región norteafricana. Igualmente, se encontró una correlación inversa entre la heterocigosidad haplotípica de las islas y su distancia a la costa africana, que apoyaba los resultados obtenidos mediante el estudio de los linajes del ADNmt (Rando y col., 1999).

1.3. El ADN antiguo

1.3.1. Historia del ADN antiguo

Hasta hace unos años, las inferencias obtenidas sobre la historia evolutiva de las poblaciones se habían basado únicamente en el análisis de la diversidad de las poblaciones actuales. Sin embargo, la complejidad de los procesos genéticos que afectan a las poblaciones, y el hecho de que en muchos casos se produce la combinación de varios de ellos (deriva, cuellos de botella, efectos fundadores...), dificultan la interpretación de la variabilidad actual observada.

La posibilidad de obtener ADN de muestras arqueológicas, o ADN antiguo (ADNa), se ha impuesto como una necesidad para llegar a comprender los procesos evolutivos que han participado en la conformación de la diversidad actual. Estudios previos habían constatado la persistencia de cierta información molecular a lo largo del tiempo. Por ejemplo, se observó que el enlace peptídico se mantenía intacto en conchas y huesos fósiles de unos 10^8 años de antigüedad (Curry, 1988; Weiner y col., 1976; Wyckoff, 1972), se detectó la supervivencia de ribosomas y cromatina en insectos procedentes de ámbar con más de 40 millones de años (Poinar y Hess, 1982) e, incluso, la presencia de ácidos nucleicos en restos humanos antiguos (Wang y Lu, 1981).

Aunque existieron trabajos previos basados en clonaje molecular (Higuchi y col., 1984; Pääbo, 1985), fue el descubrimiento de la PCR el que revolucionó el campo de la investigación en ADNa (Mullis y col., 1986). La aplicación de la PCR al análisis de restos antiguos permitió la obtención de un número elevado de copias a partir de muy pocas moléculas de ADN.

El primer ensayo de PCR aplicado al estudio de restos antiguos fue la amplificación del ADNmt del quagga (*Equus quagga quagga*), una especie extinta relacionada con la cebra común (Pääbo y Wilson, 1988). A partir de ese momento, la obtención de ADNa por PCR se aplicó a otros especimenes de animales extintos en épocas históricas, como el lobo marsupial (*Thylacinus cynocephalus*) (Thomas y col., 1989) o las moas (*Dinornithidae*) (Cooper y col., 1992), e incluso, a especies del

Pleistoceno tardío, como el mamut (*Mammuthus sp.*) o el oso cavernario (*Ursus spelaeus*), llegando en la actualidad a más de 50 especies extintas analizadas para ADNa (Pääbo y col., 2004).

Como es lógico, el análisis de restos antiguos se ha usado con especial interés a la evolución de las poblaciones humanas. La obtención de ADNa se ha aplicado tanto al estudio de poblaciones históricas (Carlyle y col., 2000; Endicott y col., 2003; Lalueza y col., 1997; Maca-Meyer y col., 2004a; Stone y Stoneking, 1993; Yao y col., 2003), como al de las relaciones filogenéticas entre el hombre moderno y otros homínidos (Krings y col., 1997; Krings y col., 1999). La comparación de ADNmt de humanos modernos con el obtenido de especímenes de Neandertal, ha mostrado más divergencia que al hacerlo con el de restos antiguos de *Homo sapiens*. Estos resultados han apoyado la idea de que el Neandertal no es una ancestro directo del *Homo sapiens* (Scholz y col., 2000) y que la evolución del hombre moderno no ha seguido un modelo multirregional (Ovchinnikov y col., 2000).

Por otro lado, la aplicación del ADNa se ha ampliado a la determinación de la dieta y el comportamiento de especies extintas mediante el análisis de coprolitos (Hofreiter y col., 2000; Poinar y col., 1998), al análisis de sedimentos en el permafrost (Hofreiter y col., 2003; Willerslev y col., 2003), a la investigación del proceso de domesticación de plantas y animales (Bailey y col., 1996; Matsuoka y col., 2002; Vila y col., 2001), y a la caracterización de cepas antiguas de virus y bacterias, como *Mycobacterium tuberculosis* (Arriaza y col., 1995), *Yersinia pestis* (Drancourt y col., 1998) o el virus de la gripe de la pandemia de 1918 (Reid y col., 1999).

1.3.2. Limitaciones del ADN antiguo

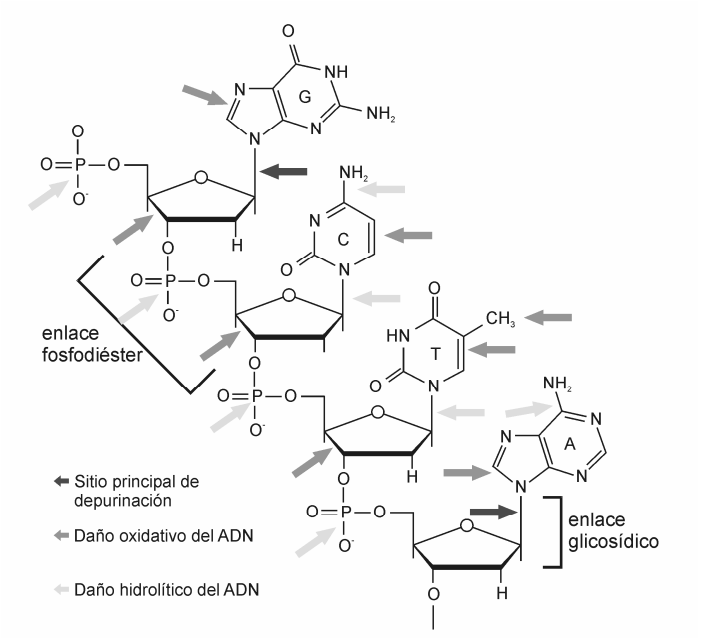
Sin embargo, a pesar de la revolución que ha causado la PCR en el campo del ADNa, existe un número elevado de limitaciones debido a las características propias del material fósil. Los primeros trabajos, basados en clonaje molecular, ya habían demostrado que el ADN recuperado de muestras antiguas se correspondía en su mayoría al material genético de hongos y microbios, y que el ADN endógeno aparecía en un bajo número de

copias, un pequeño tamaño y con una gran abundancia de loci multicopia, como es el caso de ADNmt (Higuchi y col., 1984; Pääbo, 1985).

1.3.2.1. Degradación del ADN

El optimismo suscitado por los logros obtenidos, mediante la amplificación de ADNa por PCR, se reflejó en numerosas publicaciones que aseguraban haber recuperado secuencias de ADN de millones de años de antigüedad, como ADN cloroplasmático de plantas fósiles del Mioceno (Golenberg y col., 1990) o ADNmt de dinosaurios del Cretácico (Woodward y col., 1994). Sin embargo, dado que estos estudios no pudieron replicarse, sus resultados se tomaron con bastante escepticismo (Pääbo y Wilson, 1991).

Figura 4 – Modificaciones postmortem que afectan a la molécula de ADN (Hofreiter y col., 2001a).



En la actualidad se sabe que existe un límite temporal para la supervivencia del ADNa. Mientras el organismo vive, diversos procesos enzimáticos de reparación se encargan de mantener la integridad del ADN (Lindahl, 1993a). Sin embargo, cuando el organismo muere, su material genético comienza a ser degradado por nucleasas

endógenas. En determinadas circunstancias, como una desecación rápida del cuerpo, bajas temperaturas o alta concentración de sales, las nucleasas pueden destruirse o inactivarse antes de que los ácidos nucleicos hayan sido completamente reducidos a mononucleótidos. Aunque esto ocurra y el ADN no sea degradado por las endonucleasas, se inician otra serie de procesos químicos que, aunque de manera mucho más lenta, dañan el material genético (Figura 4). Todos estos procesos de descomposición postmortem dificultan la recuperación del ADNa debido a tres razones: bloqueo de la polimerasa, degradación de la molécula de ADN y modificación de sus bases.

De esta manera, tras un tiempo suficientemente largo, el efecto acumulativo de todos estos procesos ocasionará la ausencia total de ADN amplificable. Se ha determinado que a concentraciones salinas fisiológicas, pH neutro y una temperatura de 15^{o}C, tras 100.000 años se degradaría todo el ADN de un individuo debido a procesos hidrolíticos (Lindahl, 1993a; Pääbo y Wilson, 1991). Sin embargo, como comentábamos antes, algunas condiciones ambientales, como las bajas temperaturas o la desecación del material orgánico, pueden aumentar este límite temporal. Por lo tanto, el estado de conservación de los restos antiguos estará determinado, no sólo por la antigüedad del espécimen, sino también por las condiciones ambientales a las que ha estado expuesto (Smith y col., 2003). A pesar de ello, la amplificación de moléculas de más de 1.000.000 años de antigüedad, resulta, desde el punto de vista de la degeneración postmortem, un logro imposible (Hofreiter y col., 2001b).

Por otro lado, el proceso de degradación del ADNa puede continuar incluso cuando el fósil ha sido excavado y almacenado (Burger y col., 1999; Pruvost y Geigl, 2004). El lavado del material arqueológico, por ejemplo, genera una disminución en el pH y en el contenido salino. Ésto, unido al almacenamiento durante años a temperatura ambiente, provoca que las muestras conservadas en museos tengan una menor eficiencia de amplificación que el material recién excavado. Por ello, se recomienda realizar los análisis de ADNa inmediatamente después de la excavación o conservar el material fósil en congeladores.

1.3.2.1.1. Bloqueo de la ADN polimerasa

Entre los procesos de degradación postmortem que afectan al material genético se encuentra la oxidación del ADN debido a radicales libres (Lindahl, 1993a). Éstos pueden estar generados de forma endógena, o también de forma exógena, debido a radiaciones o a la acción de bacterias y hongos (Poinar, 2002). Los efectos de la oxidación sobre el ADN causan modificaciones en los nucleótidos, bloqueando la acción de la polimerasa y, por lo tanto, impidiendo la amplificación de las moléculas de ADN mediante PCR (Hoss y col., 1996).

Otro tipo de modificación que provoca el bloqueo de la ADN polimerasa, es la creación de uniones covalentes entre bases de la misma molécula de ADN y/o con otro tipo de moléculas (Pääbo, 1989). Un ejemplo de uniones covalentes detectadas en ADNa (Poinar y col., 1998), son los productos de Maillard, que se forman por reacciones de condensación entre azúcares y grupos amino primarios.

La solución en estos casos consiste en reparar el ADN antes de ser amplificado por PCR. En el caso de los productos de Maillard, el tratamiento previo del material con bromuro de N-fenaciltiazolio (PTB), rompe los enlaces y favorece la amplificación del DNA molde (Krings y col., 2000; Poinar y col., 1998).

1.3.2.1.2. Rotura de la molécula de ADN

El ADN sufre además diversos procesos hidrolíticos que conducen a fragmentaciones y desestabilizaciones. Entre ellos se encuentra la rotura del enlace fosfodiéster y la depurinación (Lindahl, 1993a; Poinar, 2002). Cuando se rompe el enlace fosfodiéster, se producen cortes en la doble hélice que favorecen la fragmentación de la molécula de ADN en trozos más pequeños. La depurinación consiste en la rotura del enlace glicosídico, con la consiguiente pérdida de la base nitrogenada, que desemboca igualmente en el corte de la doble hélice y en la ruptura de la molécula. Estos dos procesos producen una fragmentación gradual del ADN, ocasionando al final una degradación completa de la molécula.

Por lo tanto, la reducción en tamaño de las moléculas de ADNa, generalmente entre 100 y 500 pb (Hofreiter y col., 2001b; Pääbo, 1989), hace imposible obtener productos de amplificación de tamaños superiores. Para resolver este problema, se utilizan fragmentos solapantes de pequeño tamaño (en función de la antigüedad y del estado de conservación de la muestra) que cubran la región analizada. Es importante usar amplicones pequeños, ya que al amplificar fragmentos de gran tamaño en material antiguo con un alto grado de degradación, se corre el riesgo de que se produzcan resultados quiméricos. Uno de estos procesos es la "jumping PCR", que consiste en la recombinación *in vitro* de dos moléculas de ADN con una secuencia similar durante la amplificación por PCR (Pääbo y col., 1990). Esta recombinación se puede producir entre ADN endógeno y contaminante (Wang y Wang, 1997), e incluso entre moléculas intactas y otras que presentan modificaciones postmortem, produciendo secuencias quiméricas que pueden ser tomadas, de manera errónea, como auténticas.

Por otro lado, es necesario asegurar que el proceso de degradación no ha ocasionado la destrucción total del material genético y que existe un número de copias de ADN suficientes para ser amplificadas. Si no es así y el número de moléculas de partida es extremadamente bajo, existe un alto riesgo de obtener resultados sesgados debido a la alta probabilidad de aparición de errores por modificaciones postmortem. En la actualidad, el número de copias de partida se determina usando la técnica de PCR a tiempo real (q-PCR, del inglés, Quantitative-PCR) (Alonso y col., 2004; Whelan y col., 2003). Cuando el número de moléculas de partida es superior a 1.000 copias, se espera que el resultado final esté poco afectado por modificaciones postmortem y errores de la polimerasa. Sin embargo, cuando este número es inferior sería necesario realizar varias repeticiones para confirmar los resultados.

1.3.2.1.3. Modificación de las bases

Otro proceso hidrolítico, la deaminación, se produce debido a la eliminación del grupo amino en las bases, provocando la transformación química de los nucleótidos y causando errores en la amplificación por PCR (Pääbo y col., 1989; Poinar, 2002). Este proceso, aunque no evita la recuperación del ADN, introduce modificaciones que cambian la secuencia y composición nucleotídica de la molécula de ADN, mayoritariamente

mediante cambios de tipo C → U (Brotherton y col., 2007; Gilbert y col., 2007; Stiller y col., 2006).

Para evitar que este proceso postmortem afecte a los resultados obtenidos, debemos asegurar que la PCR parte de un número de copias suficientemente elevado para impedir que una modificación quede fijada desde los primeros ciclos. Igualmente, pueden usarse enzimas que reparan estos cambios químicos, como la Uracilo N-Glicosilasa (UNG). Esta enzima elimina los uracilos, generados mediante deaminación de la citosina y fuente principal de modificaciones postmortem, reduciendo así la incorporación de errores (Hofreiter y col., 2001a).

1.3.2.2. Contaminación

El descubrimiento de la PCR ha hecho posible la amplificación rutinaria de ADN a partir de muy pocas e incluso de una única molécula, procedentes de los más diversos restos antiguos. Sin embargo, esa gran capacidad de amplificación de la PCR la hace extremadamente sensible a contaminaciones con ADN moderno. Como consecuencia de ello, se producirán falsos positivos que pueden llevar a creer que es ADNa genuino, lo que realmente es ADN exógeno que ha contaminado la muestra. Un ejemplo de ello es la demostración de que las secuencias de ADN que se creían procedentes de especímenes con millones de años de antigüedad (Cano y col., 1993; Woodward y col., 1994), eran fruto de contaminación con secuencias humanas y microbianas (Gutiérrez y Marin, 1998; Zischler y col., 1995).

Pero, sin duda, es en la especie humana donde más problemas existen con la contaminación. Esto se debe a que diferentes poblaciones comparten moléculas de ADN idénticas y a la presencia ubicua de material genético contaminante. Se ha demostrado que, usando polimerasas altamente eficientes y un número elevado de ciclos (entre 50 y 60 ciclos), se obtiene una amplificación masiva tanto en reacciones que contienen extracto de ADN como aquellas en que sólo se había añadido agua (Yang y col., 2003), debido a la existencia de ADN humano contaminante incluso bajo rigurosas medidas de control (Gill y col., 2000). Este ADN contaminante puede entrar en contacto con la muestra antigua durante la excavación arqueológica, el estudio antropológico, la extracción del ADN o la

amplificación por PCR. Aunque se sigan medidas estrictas de prevención en cada uno de estos pasos, todos los procesos que tienen lugar fuera del laboratorio, como la fabricación de reactivos, productos químicos o material plástico, pueden ser igualmente susceptibles de sufrir contaminación con ADN exógeno (Eshleman y Smith, 2001; Linderholm y col., 2008; Schmidt y col., 1995).

El hecho de que cantidades insignificantes de ADN contaminante pueden llegar a amplificarse mediante PCR se ha convertido en el mayor problema para el estudio del ADNa. Por lo tanto, es necesario identificar las fuentes principales de contaminación y establecer unas pautas que permitan diferenciar resultados derivados del ADN endógeno, de aquellos procedentes de contaminación externa.

Sampietro y col. (2006) estudiaron la contaminación derivada de la manipulación de muestras dentales neolíticas en un estudio de ADNmt. Tras el clonaje del material antiguo amplificado, llegaron a la conclusión de que la contaminación por parte de los individuos encargados de la excavación y limpieza del material, aparecía en una frecuencia superior a la de aquellos encargados de los estudios antropológicos y genéticos. Además, el ADN contaminante estaba sujeto a los mismos procesos de degradación que el ADNa, presentando igualmente modificaciones en las bases. Por lo tanto, la presencia de modificaciones postmortem en una molécula no implicaría que éste sea ADN endógeno.

Posteriormente, Malmström y col. (2007) confirmaron que existe un incremento en el número de moléculas endógenas amplificadas, proporcional a la reducción en el tamaño del amplicón. Por el contrario, la obtención de moléculas contaminantes se ve favorecida al aumentar el tamaño del fragmento amplificado por PCR. Este comportamiento asimétrico puede ser de ayuda para identificar el ADN endógeno y excluir las moléculas contaminantes.

1.3.2.3. Inhibición

Otro de los principales problemas de los estudio basados en ADNa es la inhibición de la PCR. Esto es debido a la presencia de compuestos que se copurifican con el ADN e

inhiben la acción de las polimerasas (Hanni y col., 1995). Además, este efecto inhibidor aumenta cuando se trabaja con amplicones de gran tamaño (Pusch y Bachmann, 2004). Para evitar la inhibición, es recomendable utilizar métodos de extracción que minimicen la presencia de estos compuestos. Sin embargo, sobre todo en muestras que han estado enterradas (Sutlovic y col., 2008), la existencia de sustancias inhibidoras es bastante común.

Existen dos mecanismos para superar los procesos de inhibición. Por una parte, se pueden realizar diluciones seriadas con la finalidad de reducir la concentración del inhibidor (Pääbo y col., 1989). Sin embargo, dado que la cantidad de ADN es la mayoría de veces limitante, la dilución del extracto no siempre es una buena opción. Otro mecanismo para asegurar la obtención de ADNa es la adición de albúmina del suero bovino (BSA) a la reacción de PCR (Pääbo y col., 1988). Esta proteína se une a los compuestos inhibidores, protegiendo así la acción de la polimerasa y favoreciendo la amplificación.

1.3.3. Autentificación del ADN antiguo

Los estudios de ADNa tienen el potencial de aportar contribuciones significativas a la historia evolutiva de las especies. Sin embargo, las técnicas aplicadas al ADNa tienen numerosos problemas asociados, principalmente relacionados con la generación de datos auténticos (Gilbert y col., 2005). Por lo tanto, es necesario adoptar una serie de criterios de autentificación, tanto *a priori* como *a posteriori*, que permitan asegurar la fiabilidad de los resultados obtenidos.

Los primeros criterios de autenticidad publicados se referían únicamente a tres cuestiones principales: incluir controles negativos para monitorizar la presencia de contaminación, duplicar las extracciones para confirmar la replicabilidad de los resultados, y encontrar una relación inversa entre el tamaño del amplicón y la eficiencia de la PCR (Pääbo, 1989).

A estos criterios básicos de autenticidad se han ido sumando otros (Cooper y Poinar, 2000; Handt y col., 1994; Hofreiter y col., 2001b; Lindahl, 1993b; Pääbo y col.,

2004), conforme se ha ido ampliando nuestro conocimiento en el mecanismo de degradación del ADN y en las fuentes de contaminación (Tabla 1).

TABLA 1 – Criterios de autentificación.

Areas de trabajo aisladas: Para evitar la contaminación del material antiguo con ADN moderno es imprescindible separar las zonas de trabajo relacionadas con la extracción de ADN, de aquellas en las que se manipula ADN amplificado por PCR. Además, en el laboratorio de ADNa se deben seguir los más estrictos controles para evitar la contaminación, como es el uso de guantes, bata y mascarilla, la limpieza continuada con lejía, la exposición a U.V.A. y/o el uso de presión positiva.

Amplificación en paralelo de controles de extracción y PCR: Tanto las extracciones como las amplificaciones deben incorporar múltiples controles negativos para detectar la presencia de ADN contaminantes en los reactivos, tubos,…

Correlación inversa entre la eficiencia de la amplificación y el tamaño del fragmento amplificado: Esta correlación es un indicador simple del grado de degradación y la presencia de modificaciones que puedan bloquear la amplificación por PCR. El tamaño máximo amplificable dependerá de la antigüedad del espécimen y de las condiciones de temperatura y humedad a las que ha estado sujeto (Smith y col., 2003). Si no existe una reducción de la eficiencia de la amplificación al aumentar el tamaño del fragmento, es un indicativo de que existe contaminación en la muestra.

Duplicación de la amplificación por PCR de cada muestra: La repetición de amplificaciones partiendo del mismo o diferentes extractos es importante por tres razones. Para empezar, nos puede permitir la detección de contaminación en una determinada extracción o amplificación. Además, cuando partimos de un bajo número de copias, muchas veces sólo algunas alícuotas del extracto contienen ADN molde suficiente, por lo que la repetición de los análisis puede resultar muy útil. Finalmente, para la identificación de modificaciones postmortem es necesario comparar los resultados de múltiples amplificaciones.

Clonaje de los productos amplificados por PCR: Para detectar la presencia de heterogeneidad en las secuencias amplificadas, debido a la contaminación y/o a la presencia de modificaciones postmortem, los productos de PCR deben clonarse y secuenciarse de forma rutinaria.

Reproducción de los resultados en un laboratorio independiente: Otra forma de detectar la contaminación intra-laboratorio consiste en reproducir los resultados en un laboratorio independiente. Si los resultados obtenidos en un primer momento no pueden ser duplicados en el segundo laboratorio, éstos deben ser descartados.

TABLA 1 – Continuación.

Ensayos bioquímicos de conservación de macromoléculas: Este criterio se basa en el hecho de que una mala conservación bioquímica indicará que la muestra tiene poca probabilidad de contener ADN amplificable. Además, estos ensayos pueden servir como una manera de seleccionar que especímenes pueden contener ADN y deben ser incluidos en posteriores análisis. La técnica más usada es la determinación del grado de racemización de los aminoácidos.

Cuantificación del número de moléculas de ADN amplificables: Esta medida nos permite determinar si existen pocas moléculas de partida y, por lo tanto, si nuestros resultados son susceptibles de verse afectados por modificaciones postmortem. Si existe una cantidad suficiente (en general, más de 1000 copias), es menos probable que las modificaciones postmortem puedan generar errores en los resultados. La cuantificación de ADN molde es especialmente importante cuando se estudian marcadores autosómicos en material antiguo. Si amplificamos un locus heterocigótico por PCR, se espera que se amplifique un 50% a partir de cada uno de los alelos. Sin embargo, esto sólo ocurre cuando partimos de un número alto de moléculas. En cambio, si se inicia la amplificación a partir de un número bajo de copias existe un riesgo elevado de obtener sólo uno de los alelos debido a "allelic-dropout" (amplificación sesgada de los alelos presentes en un individuo) durante los primeros ciclos. Por lo tanto, será necesario realizar múltiples amplificaciones sucesivas con el fin de diferenciar los individuos heterocigóticos de los homocigóticos.

Análisis de restos asociados: Cuando existe material orgánico asociado, como restos animales, nos pueden servir para tener un valor independiente del grado de preservación del ADN y de la presencia de ADN humano contaminante.

Exclusión de inserciones nucleares en el ADNmt: Fragmentos de ADN procedentes de genomas de orgánulos como las mitocondrias pueden estar presentes en el genoma nuclear (Bensasson y col., 2001; Timmis y col., 2004). Teniendo en cuenta que el ADNmt es la molécula más usada en estudios antiguos, estas integraciones nucleares pueden amplificarse ocasionalmente y originar errores en la interpretación de los datos. Para prevenir este problema, es necesario usar diferentes juegos de primers solapantes, ya que es poco probable que diversos pares de primers amplifiquen una inserción nuclear (Krings y col., 1997).

Sin embargo, cabe destacar que, aunque se cumplan todos los criterios de autentificación propuestos, no podemos asegurar que la secuencia de ADN obtenida sea genuinamente antigua. Por ejemplo, si un espécimen está contaminado con una cierta molécula de ADN, ésta aparecerá en ambos laboratorios aunque se cumplan todos los

criterios de autentificación. Por lo tanto, un análisis exhaustivo de los datos obtenidos es aún más necesario en este caso que en otras áreas de la Genética (Pääbo y col., 2004).

Por ello se ha destacado que, además de seguir los criterios de autentificación, hay que realizar un control *a posteriori* de los resultados (Bandelt, 2005). Para el estudio de secuencias de ADN, existen tres indicadores que pueden cuestionar la autenticidad de los datos obtenidos:

- **Principio de expectación filogenética:** si los resultados reflejan los marcadores presentes en el equipo de excavación o en el personal de laboratorio, y no lo que se podía esperar a partir del origen geográfico de las muestras antiguas, es muy probable éstos estén afectados por contaminación. Por ello, es necesario disponer de un panel de secuencias de cada uno de los investigadores que han manipulado las muestras, desde el momento de la excavación hasta su análisis molecular.

- **Estructura en mosaico:** esto ocurre cuando la secuencia obtenida se compone de varios fragmentos solapantes, que por separado tienen diferentes sentidos filogenéticos, y en conjunto, no pertenecen a ningún haplotipo conocido. Esta estructura en mosaico puede ser debida a contaminación o a la mezcla de muestras.

- **Espectro anormal de mutación:** cuando existe una aglomeración de mutaciones poco comunes a la largo de una molécula antigua, dicho resultado debe descartarse, ya que posiblemente las modificaciones postmortem hayan transformado el material genético inicial, de forma que ya es imposible conocer su haplotipo real.

1.3.4. Avances de las técnicas para el estudio del ADN antiguo

1.3.4.1. Secuenciación de alto rendimiento a partir de librerías metagenómicas

Aunque la secuenciación basada en el uso de dideoxinucleótidos (Sanger y Coulson, 1975; Sanger y col., 1977) es muy robusta, tiene ciertas limitaciones cuando se aplica al estudio del ADNa. Para empezar, la secuenciación de Sanger está diseñada para lecturas largas y es poco recomendable para fragmentos inferiores a 100 pb. Además, en la mayoría de los casos, es imposible leer los 15-20 nucleótidos posteriores al primer.

En la actualidad, el campo del ADNa está experimentando una nueva revolución debido a la introducción de técnicas de secuenciación de alto rendimiento (high-throughput sequencing), como la tecnología 454 (Margulies y col., 2005), que permiten la secuenciación del ADN genómico total extraído de una muestra. Estas técnicas están basadas en la construcción de librerías de ADN genómico (librerías metagenómicas), amplificación por PCR en emulsión y secuenciación mediante pirosecuenciación.

La secuenciación de alto rendimiento a partir de librerías metagenómicas posee numerosas ventajas. Para empezar, estas librerías permiten la inmortalización del ADN obtenido a partir de muestras antiguas muy preciosas, obviando la necesidad de repetir continuamente extracciones destructivas (Green y col., 2006; Noonan y col., 2005; Noonan y col., 2006). Por otro lado, cada molécula se amplifica de forma aislada, mediante PCR en emulsión, por lo que no es necesario clonar los fragmentos amplificados. Además, la pirosecuenciación permite la lectura de los nucleótidos situados al lado del primer, por lo que la eficacia de lectura de fragmentos pequeños es mucho mayor.

1.3.4.2. Genotipado de ADN antiguo mediante SPEX

La técnica de genotipado mediante SPEX (del inglés, Single Primer EXtension), se basa en la amplificación por PCR del ADNa usando un único primer, que hibrida específicamente en un locus determinado (Brotherton y col., 2007; Brotherton y col., 2009).

La mayor ventaja de la técnica SPEX es que no presupone un tamaño mínimo para los fragmentos amplificados, sino que la extensión de la molécula sintetizada continúa hasta que se vea interrumpida, ya sea por fragmentación del molde o, por modificaciones en las bases que bloqueen la polimerasa. Por lo tanto, al no depender del tamaño del molde, este método puede permitir la obtención de moléculas de ADN antiguas en material altamente degradado, en el que la amplificación por PCR tradicional es imposible.

Además, ya que esta técnica no exige un tamaño concreto para que se produzca la amplificación, favorece la obtención de ADN endógeno respecto al contaminante, y permite discriminar la contaminación moderna por diferencias en el tamaño del amplicón.

Otra ventaja de la técnica del SPEX es que facilita la interpretación de la naturaleza de las modificaciones postmortem en el ADNa. Tanto en la PCR tradicional como en la secuenciación a partir de librerías metagenómicas, debido a la estructura bicatenaria del ADN, es difícil discernir cual de las cadenas molde ha originado una determinada modificación. Con la técnica del SPEX, al usar un solo primer, la amplificación se realiza a partir de una sola de las cadenas del ADN molde, por lo que se puede caracterizar perfectamente el origen de estas modificaciones (Brotherton y col., 2007).

1.3.5. Estudios previos de ADN antiguo en Canarias

Un problema que no puede resolverse con el mero estudio de las poblaciones actuales canarias, es el de la correcta asignación temporal de las influencias africana y europea. Por ejemplo, la presencia del haplogrupo U6 del ADNmt en Canarias podría deberse al tráfico de esclavos procedentes del Norte de África en las islas, en vez de asumir un origen prehispánico para esos linajes. Por otro lado, aunque los linajes pertenecientes al haplogrupo U6 que se observan en Canarias tienen un origen común con los norteafricanos (Maca-Meyer y col., 2003a), el sublinaje autóctono canario U6b1 no ha sido observado en el norte de África, mientras que el subhaplogrupo más abundante en esta zona, el U6a, es muy escaso en el archipiélago (Brakez et al. 2001; Rando et al. 1998; Rando et al. 1999). Por estas razones, parece que extrapolar la composición genética de la población aborigen a partir de las poblaciones actuales del noroeste africano puede no ser demasiado correcto.

Sin embargo, el avance de las técnicas moleculares relacionadas con el material antiguo, ha permitido también el estudio de marcadores del ADNmt directamente en restos fósiles procedentes de Canarias, tanto prehistóricos como históricos (siglo XVII – XVIII).

1.3.5.1. Estudios en la población aborigen

El análisis del ADNmt en restos aborígenes procedentes de Tenerife, La Gomera, El Hierro y Gran Canaria, señaló a los bereberes como la población africana más relacionada con la guanche (Maca-Meyer y col., 2004a), confirmando así las conclusiones obtenidas mediante estudios antropológicos y culturales. Además, la detección del subhaplogrupo U6b1, presente únicamente en la población canaria actual, apoyó la pervivencia de pool genético materno aborigen en la población actual.

Al realizarse estima de mezcla, usando por primera vez a la población aborigen como población parental, se encontró que los aborígenes canarios contribuyeron con un 42-73% a los linajes maternos actuales. Este dato confirmó los obtenidos usando como población parental el norte de África (Pinto y col., 1996c; Rando y col., 1999) y está en

consonancia con los datos históricos que apuntan a que, a finales del siglo dieciséis, al menos dos tercios de la población tenían un origen indígena (Wölfel, 1930).

Por otro lado, la alta diversidad encontrada en los Guanches a nivel del ADNmt, comparable con la de las poblaciones actuales de Canarias y el norte de África, no parece estar a favor de una colonización aborigen de las islas con acusados efectos fundadores. Al contrario, es congruente con una gran diversidad de los primeros colonizadores que llegaron a las islas, o bien, a la existencia de varias oleadas migratorias.

1.3.5.2. Estudios en la población histórica (S.XVII-XVIII)

La composición genética para el ADNmt de los restos históricos (Maca-Meyer y col., 2005) no presentó diferencias significativas con la población canaria actual. La mayoría de los linajes observados en la población histórica procedían de Europa, reflejando el gran impacto que la conquista y posterior colonización tuvieron sobre las islas. Sin embargo, también aparecieron linajes asociados al norte de África, como el sub-haplogrupo canario U6b1, que podría deberse a cierta persistencia de linajes aborígenes en la población histórica.

Cabe destacar igualmente la presencia de linajes típicamente subsaharianos, probablemente debido al tráfico de esclavos desde esta área. También aparecen haplogrupos amerindios que, aunque en baja frecuencia, reflejan la íntima relación de Canarias con América y el papel que jugó el puerto de Santa Cruz en el comercio con este continente (Arnay, 2009).

1.4. Introduction (Summary)

The Canaries consist of seven main islands situated in the Atlantic Ocean, near the western Saharan coast of Africa. Owing to their oceanic volcanic origin, they have probably never been connected to the continent, even though the nearest, Fuerteventura, is only 100 km from Africa.

Although the Islands were already known to Classical cultures, they were apparently forgotten until visited by European merchants in the 13th century. During the 15th century, they were gradually conquered indirectly or directly by the Spanish kingdom of Castile, beginning with Lanzarote in 1402 and finishing with Tenerife in 1496. Around this time, Portuguese and Castilians began to settle other archipelagos such as the Azores, Madeira and Cape Verde, but only the Canary Islands were found inhabited (Hooton 1916).

In general terms, the Conquest was rather violent because many Guanches fought fiercely against the invaders. Although some islands received the first European expeditions peacefully at first, they later suffered revolts due to the enslavement of a large number of the inhabitants. In those cases, the rebels, mainly men, were killed or deported by the conquerors (Viera y Clavijo 1772).

The crushing of resistence and the subsequent European colonization had a great impact on the Guanche way of life (Crosby 1988). In spite of the aboriginal protective policy of Queen Isabel 'La Católica', who legally abolished slavery there in 1498, a large number of indigenous islanders were deported during and after the Conquest and, some of them, were introduced into the 16th century European slave trade (Suárez et al. 1988). Those that survived and stayed within the islands progressively mixed with the European colonizers. However, the better socio-economic position held by the Europeans favoured mating between indigenous females and the, at first, predominantly male conquerors. In addition, the plantation of sugar-cane in the islands and the scarcity of labour, due to male mortality among the islanders during the Conquest, soon led to slave importation from North and sub-Saharan Africa.

Since before the Conquest, Guanche culture has been documented as related to that of their N-African Berber neighbours. Nowadays, anthropological, archaeological and linguistic studies provide further support for the N-African origin of the indigenous population (Navarro 1997). From the genetic point of view, the presence in the Canary Island population of the mitochondrial DNA (mtDNA) U6 lineages (Rando et al. 1999) and the Y-chromosome E-M81 haplogroup (Flores et al. 2003), both with a clear Berber ascription (Bosch et al. 2001; Rando et al. 1998), is in accordance with a N-African origin. Furthermore, admixture analysis taking the Iberian Peninsula, Northwest and sub-Saharan W-African populations as parental sources of the present-day inhabitants, showed that the indigenous contribution was about 33% for maternal (Rando et al. 1999) and only 7% for paternal lineages (Flores et al. 2003). This strong sexual asymmetry could be explained by sex-biased matings between European males and indigenous females and the greater indigenous male mortality during the Conquest (Flores et al. 2001a).

Genetic characterization of the current population has allowed us to estimate the geographic origin of the prehispanic population and also to make inferences about the way the islands were colonized. The detection of significant correlations between insular heterozygosity values for mtDNA and geographical distances of the islands from Africa has been explained assuming only one main colonization event (Rando et al. 1999). Later, using Y-chromosome markers, two opposite correlations were found, marked by E-M81 and E-M78/J-M267 respectively (Flores et al. 2003), which suggested at least two independent waves of settlers from NW Africa.

However, an unequivocal ethnic assignation as original source of mtDNA and Y-chromosome markers is not possible if only the present-day populations of the islands are studied. For instance, the current presence of the mtDNA U6 haplogroup could be explained as a result of the later N-African slave trade in the islands, instead of assuming a pre-conquest origin for these lineages. On the other hand, although the U6 haplogroups present in the Canary islanders and in N Africa have a common origin (Maca-Meyer et al. 2003), the most abundant Canary sublineage, U6b1, is absent in NW Africa, and the most abundant U6a sublineages on that continent are very scarce in the archipelago (Brakez et al. 2001; Rando et al. 1999; Rando et al. 1998). For these reasons, it would be

inappropriate to extrapolate the unknown indigenous population from a current NW-African sample pool.

However, the Guanche origin of U6b1 haplogroup was confirmed when ancient DNA was directly obtained from prehistoric (Maca-Meyer et al. 2004a) and exhumed 17th-18th century remains from Tenerife (Maca-Meyer et al. 2005). The presence of U6b1 and other haplogroups considered as founder lineages in a previous work (Rando et al. 1999), demonstrated the survival of pre-Hispanic lineages in the current population. Besides this, the admixture estimations taking the indigenous mtDNA as a parental population, gave a greater native female component (42-73%) than those based on the present-day NW-African maternal gene-pool (33-43%).

Objetivos

2. OBJETIVOS

El objetivo general de esta tesis doctoral es el análisis genético a nivel molecular de restos humanos prehistóricos e históricos de las Islas Canarias, usando marcadores polimórficos del ADNmt, del cromosoma Y y del grupo sanguíneo AB0, y su comparación con los de las poblaciones actuales. De esta forma, podremos establecer cómo se produjo el proceso de colonización aborigen, determinar la composición genética de la población prehispánica y estimar el impacto que sobre los mismos supuso la colonización europea y el posterior tráfico de esclavos de origen africano.

En concreto, los objetivos de la presente tesis son los siguientes:

- Analizar el ADNmt de muestras actuales de todas las islas para determinar si existen patrones de variación en su distribución, que reflejen cómo se produjo el poblamiento aborigen. Se pondrá especial interés en la distribución y frecuencia de aquellos marcadores considerados como fundadores en trabajos anteriores.

- Analizar, a nivel de ADNmt, muestras de la población aborigen de la isla de La Palma, ya que junto con El Hierro, es la isla más alejada del continente africano. El análisis de su composición genética y su comparación con la población aborigen de Tenerife, previamente estudiada, nos permitirá determinar qué modelo de colonización se ajusta más a la diversidad y nivel de estructura observados.

- Analizar el gen de la amelogenina en muestras aborígenes e históricas para determinar cuales pertenecen a individuos varones, como paso previo al estudio del cromosoma Y en las mismas.

- Analizar un conjunto de dieciséis marcadores del cromosoma Y, que discriminan poblaciones europeas de norteafricanas y subsaharianas, en muestras aborígenes y en la de una población de los siglos XVII-XVIII. De esta forma,

determinaremos el origen de los linajes paternos de la población aborigen, la evolución temporal de las frecuencias haplotípicas del cromosoma Y en la población canaria y la influencia que la colonización europea y el tráfico de esclavos ejerció en este proceso.

- Analizar el locus autosómico AB0, a nivel de ADN, en aborígenes, en una población histórica y en muestras actuales de las Islas Canarias, así como en poblaciones actuales de la Península Ibérica y norte de África, al objeto de tener una visión complementaria a la obtenida por el estudio de los marcadores uniparentales.

2.1. Objectives

The overall objective of this thesis is the molecular genetic analysis of prehistoric and historic human remains from the Canary Islands, using mtDNA, Y-chromosome and AB0 locus polymorphic markers, and its comparison with current populations. We can thus clarify the early colonization process, the genetic composition of the pre-conquest population, and the impact that European colonization and the subsequent African slave-trade had on the islands.

Specifically, the objectives are:

- To analyse the mtDNA in present-day samples from all the Canary Islands with the aim of determining whether there are patterns of variation in their distribution that could reflect the aboriginal colonization process. We will specially focus on the distribution and frequency of those lineages considered founder haplotypes in previous studies.

- To analyse the indigenous population of La Palma in order to provide further information on the colonization. Since it is one of the most distant islands from the African continent, a study of its genetic composition and a comparison with previously published data on that of Tenerife could lead us to identify which colonization model best fits the observed diversity and structure levels.

- To analyse the amelogenin gene in order to determine which samples belong to male individuals, previous to the Y-chromosome study on the ancient material.

- To analyse, in the pre-Hispanic and historical samples, a set of sixteen Y-chromosome markers that discriminate between European, North-African and Sub-Saharan populations. This will determine the most probable origin of the paternal lineages, the temporal evolution of the Y-chromosome haplotype frequencies, the influence of European colonization, and the slave-trade input on the Canary population.

- To analyse, at DNA level, the AB0 autosomal locus in pre-conquest, historical and modern samples from the Canary Islands, and in the present-day populations of the Iberian Peninsula and North Africa. This will provide a complementary autosomal dataset to contrast with those obtained from the study of uniparental markers.

Material y métodos

3. MATERIAL Y MÉTODOS

3.1. Poblaciones actuales

3.1.1. Muestras

Las muestras de ADN actuales fueron obtenidas a partir de sangre y/o saliva de individuos no emparentados y con, al menos, dos generaciones de ancestros nacidos en la zona de interés. El número de muestras analizadas para cada marcador en las poblaciones actuales se detalla en la Tabla 2.

TABLA 2 – Tamaños muestrales para los estudios de ADN mitocondrial y locus AB0 en las poblaciones actuales de las Islas Canarias, el norte de África y la Península Ibérica.

	ADN mitocondrial	Locus ABO
Islas Canarias	503	508
Norte de África	-	207
Península Ibérica	-	385

3.1.2. Extracción de ADN

La extracción de ADN a partir de muestras de sangre y saliva se llevó a cabo siguiendo un protocolo basado en lisis alcalina (Rudbeck y Dissing, 1998). En algunos casos en los que la lisis alcalina no produjo una extracción de calidad suficiente, se utilizó un protocolo basado en el uso de la proteinasa K, SDS y DTT (Manak, 1993; Maniatis y col., 1982).

3.1.3. Análisis del ADN mitocondrial

El estudio de secuencias parciales del ADNmt en las poblaciones actuales se realizó mediante la amplificación de la HVR y el análisis por RFLP de ciertas posiciones diagnóstico (González y col., 2006). La HVR se amplificó usando los primers HV1 (5'

ACTTCACAACAATCCTAATCCT 3') y HV2 (5' CGGAGCGAGGAGAGTAGCAC 3'). Cuando el haplotipo obtenido fue suficientemente informativo para asignar el haplogrupo, se clasificó directamente. En aquellos casos en los que no fue posible, se clasificaron mediante el análisis de RFLPs. De la misma manera, se analizaron RFLP adicionales cuando fue necesario para una subdivisión más refinada en subhaplogrupos.

Las secuenciación completa del ADNmt se realizó mediante la amplificación de 32 fragmentos solapantes, cuyos tamaños oscilaron entre 446 y 681pb (Maca-Meyer y col., 2001).

3.1.4. Análisis del grupo sanguíneo ABO

Para el estudio de siete alelos del locus AB0 (*A101*, *A201*, *B101*, *O101*, *O201*, *O210 y O303*) en la población canaria y sus parentales, se amplificaron tres fragmentos mediante PCR Multiplex, usando los primers detallados en la Tabla 3. Dado que no existe una nomenclatura consenso para el locus AB0, en este trabajo se siguió la propuesta por Yamamoto (2000).

TABLA 3 – Primers usados en el estudio del locus AB0 en poblaciones actuales.

Primer forward (5' → 3')	Primer reverse (5' → 3')	Referencia
CTCTCTCCATGTGCAGTAGGAAGG	GAACTGCTCGTTGAGGATGTCG	(Hummel y col., 2002)
ATCAGTGACTTCTGCGACCGG	GGTGCAGGGTGCCGACCAG	este trabajo
ACCAGGCGGTCCGGAAC	GCAGCCCTCCCAGAGCC	este trabajo

La identificación de los distintos alelos se realizó mediante la técnica de SSCP (del inglés, Single-Strand Conformational Polymorphisms) (Orita y col., 1989). Esta metodología se basa en el hecho de que una cadena de ADN monocatenario se pliega de manera distinta a otra cadena que difiera en una única mutación. Esto es debido a que el cambio de una sola base afecta a la estructura terciaria del ADN. De esta forma podemos usar los SSCPs para detectar mutaciones puntuales en fragmentos amplificados mediante PCR. Además, para facilitar el reconocimiento de los diferentes alelos, se añadieron los primers usados en la amplificación para evitar la renaturalización y, a la vez, mejorar la tinción con bromuro de etidio al crear zonas bicatenarias (Almeida y col., 1998).

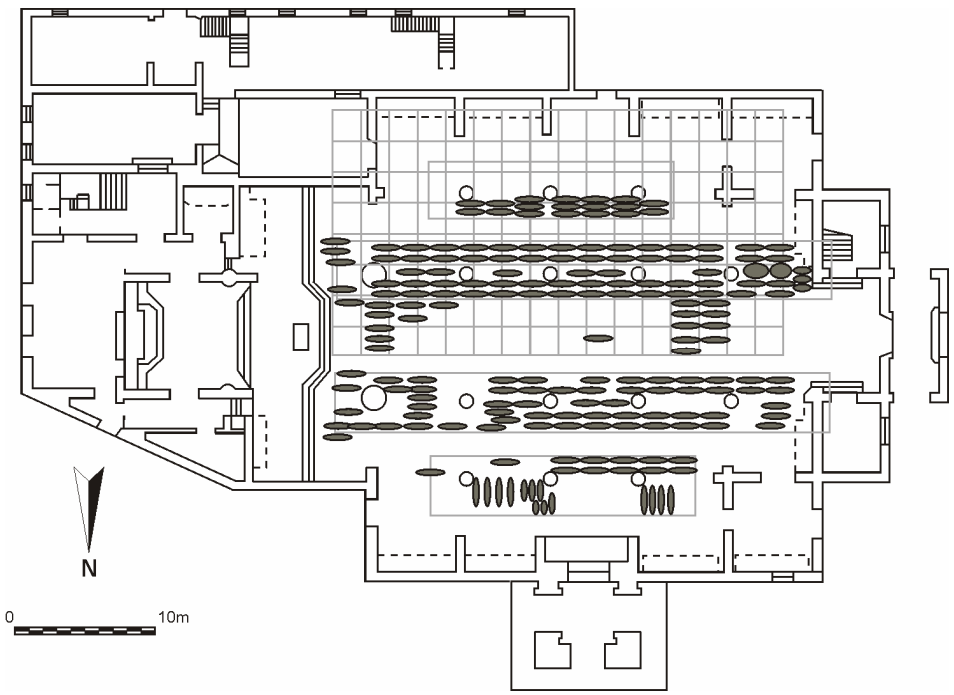
3.2. Poblaciones antiguas

Con la intención de establecer la evolución genética de la población canaria se han estudiado, tanto poblaciones aborígenes de diversas islas, como una población histórica (S.XVII – S.XVIII) procedente de Tenerife.

3.2.1. Muestras históricas

Los restos históricos, exhumados en la Iglesia de La Concepción en Santa Cruz de Tenerife (206 dientes de 195 individuos), pertenecen a los siglos XVII – XVIII. Las muestras fueron obtenidas de diversas fosas, tal como indica la figura 5. Para evitar la repetición de individuos, se procuró escoger preferentemente muestras dentales de fosas diferentes. En los casos en los que no fue posible se escogió siempre el mismo tipo dental para asegurar que no pertenecían al mismo individuo.

Figura 5 – Distribución de las fosas sepulcrales en la Iglesia de La Concepción (Arnay, 2009).



3.2.2. Muestras aborígenes

Los restos aborígenes analizados en el presente estudio consistieron, en todos los casos, en dientes sin fracturas. La elección de restos dentales de forma exclusiva se debe a que el ADN se conserva mejor en los dientes que en los huesos. Esto es así gracias a la acción protectora del esmalte y al efecto estabilizador del hidroxiapatito de la dentina (Ginther y col., 1992).

En total se analizaron 437 dientes, pertenecientes a 298 individuos diferentes. Las muestras fueron obtenidas de seis de las siete islas: Fuerteventura (13 dientes de 10 individuos), Gran Canaria (230 dientes de 115 individuos), Tenerife (45 dientes de 39 individuos), La Gomera (62 dientes de 52 individuos), El Hierro (44 dientes de 44 individuos) y La Palma (43 dientes de 38 individuos).

Las dataciones basadas en C-14 muestran que el material arqueológico de Tenerife, La Gomera, El Hierro y Gran Canaria, son anteriores a la conquista. Aunque los restos fósiles de Fuerteventura y La Palma (Martín, 1992) no fueron datados de forma directa, el material cerámico encontrado indica que no pueden tener una antigüedad mayor a 1000 años.

3.2.3. Obtención del ADN exógeno

Para determinar la existencia de ADN moderno contaminando la parte externa del diente, se realizó una extracción de ADN en superficie. Los posibles restos de ADN derivados de la manipulación previa de la muestra dental se recuperaron mediante el uso de un hisopo empapado en solución de tiocianato de guanidina. A partir de éste, el ADN contaminante fue purificado mediante un protocolo basado en el uso de sílica. Finalmente, el extracto obtenido fue amplificado por PCR para determinar la existencia de ADN contaminante y establecer su haplotipo (Ver Resultados, apartado 4.1.1 de Mejoras Técnicas).

3.2.4. Limpieza del material arqueológico

La manipulación de las muestras arqueológicas se realizó siempre con guantes y bajo estrictas condiciones de esterilidad, con el fin de evitar la contaminación con ADN moderno. Además, antes de realizar la extracción de ADN, las muestras fueron lavadas con HCl al 15%, enjuagadas con agua destilada e irradiadas con luz UV durante 10 minutos.

3.2.5. Extracción del ADN

Con la intención de reconstruir los dientes tras la extracción, las piezas dentales se cortaron con una sierra eléctrica y se obtuvo la dentina mediante pulverización con un torno de dentista. El polvo resultante se recogió en un tubo estéril y se extrajo el ADN mediante un protocolo basado en el uso de tiocianato de guanidina y columnas de sílica (Casas y col., 2006; Maca-Meyer y col., 2004a; Maca-Meyer y col., 2005).

Con aquellas muestras que fue posible realizar un proceso de extracción destructivo, la pieza dental completa fue machacada entre placas metálicas y el pulverizado extraído como en el caso anterior.

3.2.6. Cuantificación por PCR a tiempo real

Uno de los criterios de autenticidad exigidos en los estudios de ADNa es la determinación del número de moléculas amplificables por q-PCR, ya que ese valor es imprescindible para establecer el grado de fiabilidad de los resultados obtenidos. Para estimar el número de moléculas susceptibles de ser amplificadas por PCR, utilizamos el iQ™ SYBR® Green Supermix (BioRad) en un termociclador iCycler (BioRad). Se usaron además, diluciones seriadas de un control cuantificado para determinar la curva estándar y calcular así el número de moléculas iniciales de ADN.

3.2.7. Criterios de autenticidad

Con la intención de evitar la contaminación y asegurar la autenticidad de nuestros resultados, se cumplieron los estándares de prevención y control exigidos para el trabajo con material antiguo. Para ello, seguimos las recomendaciones de Pääbo y col. (2004), que incluyen las establecidas por Cooper y Poinar (2000).

- En el caso del ADNmt, los productos de amplificación fueron clonados en varias ocasiones. Asimismo, para detectar modificaciones postmortem en el ADNa, se secuenciaron un mínimo de siete clones por cada fragmento clonado.

- En las amplificaciones se incluyó siempre un control negativo de extracción por muestra y tres controles de PCR por reacción.

- Siempre que fue posible, se repitió la extracción para confirmar los resultados obtenidos.

- El número inicial de ADN amplificable fue determinado mediante PCR a tiempo real.

- Para asegurar la obtención de ADN endógeno, la región hipervariable del ADNmt se amplificó usando siete fragmentos solapantes de tamaño inferior a 150 pb. Con la misma intención, en los estudios del cromosoma Y y del grupo sanguíneo AB0 se diseñaron primers para amplificar fragmentos inferiores a 100 pb.

- La estrategia de amplificación del ADNmt mediante varios fragmentos solapantes, además de favorecer la amplificación del ADN endógeno, nos permitiría la detección de secuencias mitocondriales integradas en el genoma nuclear.

- Siempre que fue posible, los resultados fueron replicados en un laboratorio independiente. En el caso del ADNmt y el grupo sanguíneo AB0, fueron replicados en el Laboratorio de Genética Forense de la Universidad de Las Palmas de Gran

Canaria. Los resultados del cromosoma Y fueron replicados en el IPATIMUP (Instituto de Patologia e Imunologia Molecular da Universidade do Porto).

- Aunque no fue posible disponer de restos animales asociados a las muestras arqueológicas, en un trabajo previo se obtuvo ADNmt de lagartos fósiles de Canarias (Maca-Meyer y col., 2003b), excavados en cuevas con condiciones ambientales similares.

- Para los estudios de ADNmt y del cromosoma Y se obtuvo el panel de todo el personal que estuvo en contacto con las muestras dentales, desde la excavación hasta su estudio molecular. Los resultados obtenidos para las muestras antiguas fueron comparadas con dicho panel para detectar cualquier posible contaminación (Sampietro y col., 2006).

- Con el fin de determinar si el proceso de limpieza era efectivo, se contaminó de forma artificial una pieza dental con ADN genómico de pollo. Tras el proceso de descontaminación y extracción descrito anteriormente fue imposible amplificar el ADN contaminante, confirmando la eficacia de la limpieza previa del material arqueológico (Maca-Meyer y col., 2004a).

Para el ADNmt, se incluyó un paso adicional consistente en la obtención del ADN exógeno que pudiera haber contaminado la muestra dental. Para ello, se realizó una extracción del ADN superficial, previo a la descontaminación con ácido clorhídrico. El ADN obtenido se secuenció y se comparó con el panel de investigadores involucrados en el estudio y con los resultados obtenidos para el ADN endógeno.

3.2.8. Estudio del ADN mitocondrial

3.2.8.1. Amplificación de fragmentos solapantes

La región hipervariable I (HVRI) del ADNmt se amplificó usando siete fragmentos solapantes (Tabla 4), que abarcan desde la posición 15.596 hasta la 16.399 (Maca-Meyer y col., 2004a; Maca-Meyer y col., 2005), cuyos tamaños son inferiores a 150 pb.

TABLA 4 – Primers solapantes usados en la amplificación de la HVRI.

Fragmento	Primer Forward (5' → 3')	Primer Reverse (5' → 3')	Tamaño
1	CTCCACCATTAGCACCCAAAGC	AGCGGTTGTTGATGGGTGAGTC	112 pb
2	GGAAGCAGATTTGGGTACCAC	TGGTGGCTGGCAGTAATGTACG	82 pb
3	CACCCATCAACAACCGCTAT	TGATGTGGATTGGGTTTTTATGTA	112 pb
4	GGTACCATAAATACTTGACCACCTG	TTTGGAGTTGCAGTTGATGTG	117 pb
5	CAAGCAAGTACAGCAATCAACC	CTGTTAAGGGTGGGTAGGTTTG	103 pb
6	CTCCAAAGCCACCCCTCAC	GGGACGAGAAGGGATTTGAC	108 pb
7	AGCCATTTACCGTACATAGCACA	TGATTTCACGGAGGATGGTG	103 pb

Los productos de PCR fueron secuenciados usando el BigDye Sequencing kit (Applied Biosystem). Dada la gran cantidad de problemas derivados de la mala lectura de fragmentos pequeños, se añadieron al extremo 5' de los siete primers "forward" una cola, compuesta por el primer universal M13F (5' GTAAAACGACGGCCAGT 3'). Con esta modificación, se pretende mejorar la lectura en las bases próximas al primer y aumentar las zonas de solape entre los diferentes fragmentos. Además, se simplifica la secuenciación al usar un único primer común para los siete fragmentos analizados.

3.2.8.2. Determinación de haplogrupos mediante RFLP

Siguiendo a Maca-Meyer y col. (2004a), se analizaron por RFLP las posiciones diagnóstico 7025 (haplogrupo H), 12308 (haplogrupo U), 4216 (haplogrupo J/T) y 3592 (haplogrupos L0, L1, L2, L5 y L6).

TABLA 5 – Primers, enzimas de restricción y patrón de bandas observado en el estudio de las posiciones 3010 y 6776 mediante RFLP (las letras en cursiva se corresponden con bases modificadas).

Posición	Primers (5' → 3')	Enzima	Ancestral	Derivado
3010	L3005: CTCGATGTTGGATCAGGACA	*Tsp509*I	50 pb	24 + 26 pb
	H3011*: AACCTTTAATAGCGGCTGCAC*A*AT			
6776	L 6769: CTAGGGTTTATCGTGTGAGC	*Nde*I	53 pb	25 + 28 pb
	H6777*: TCTACGTCTATTCCTACTGTA*C*ATAT			

Además, se analizaron dos posiciones adicionales que caracterizan los subhaplogrupos H1 y H3 (Tabla 5). El RFLP para el H1 se realizó mediante amplificación con un primer modificado, que crea una diana para la enzima *Tsp509*I cuando el individuo posee la mutación. El RFLP diagnóstico para el haplogrupo H3 se realizó igualmente mediante amplificación con un primer modificado y determinación del estado ancestral o derivado mediante corte con la enzima *Nde*I.

3.2.8.3. Clonaje

En varias ocasiones, los productos de PCR fueron clonados usando el pGEM®-T Vector System (Promega) según las recomendaciones del fabricante. Las colonias con inserto fueron seleccionadas mediante crecimiento en placas de LB/ampicilina/IPTG/X-Gal. El inserto obtenido fue amplificado por PCR y se secuenciaron al menos siete clones de cada producto, usando el BigDye Sequencing kit (Applied Biosystem).

Debido a la necesidad de contar con un elevado número de células transformantes para los ensayos de clonaje, y a la imposibilidad de obtenerlas mediante métodos altamente eficaces como la electroporación, se ensayó el efecto de las microondas en la transformación de *E. coli* (ver apartado 4.1.2 de Mejoras Técnicas).

3.2.9. Estudio del gen de la amelogenina

Como paso previo al estudio del cromosoma Y en las poblaciones antiguas de las Islas Canarias, los individuos fueron sexados mediante la amplificación de un fragmento del gen de la amelogenina. Este gen está presente tanto en el cromosoma X como en el Y, pero posee diferencias (Salido y col., 1992) que permiten la determinación del sexo en restos antiguos mediante PCR (Faerman y col., 1995).

La amplificación del gen de la amelogenina se realizó usando los primers Amel-A (CCCTGGGCTCTGTAAAGAATAGTG) (Sullivan y col., 1993) y Amel-C (AATRYGGACCACTTGAGAAAC) (Maca-Meyer y col., 2005), que amplifican una región del intrón 1 de ambos cromosomas sexuales. El hecho de que exista una deleción de 6 pb en el cromosoma X, ocasiona que se obtenga un fragmento de 66 pb para el alelo X y uno

de 72 pb para el alelo Y. De esta forma, mientras en los machos se observan ambos fragmentos, en las hembras sólo aparece uno de ellos. La visualización del patrón de bandas se realizó mediante electroforesis en geles verticales de poliacrilamida. Para confirmar la correcta migración de los alelos del cromosoma X e Y, se usaron siempre marcadores de peso molecular específicos.

Para evitar fenómenos de "allelic-dropout", se realizaron al menos dos amplificaciones adicionales en el caso de los machos, y entre cuatro y cinco en el caso de las hembras. De esta forma se disminuye la posibilidad de sexar erróneamente a un macho como hembra por eliminación del alelo Y durante los primeros ciclos de la PCR. Además, siempre que fue posible, se llevaron a cabo dos extracciones independientes de dientes del mismo individuo, en el mismo laboratorio, pero separadas en el tiempo y ejecutadas por personas diferentes.

3.2.10. Estudio del cromosoma Y

El estudio del cromosoma Y en las poblaciones antiguas consistió en el análisis de dieciséis marcadores bialélicos (M2, M9, M33, M34, M45, M60, M78, M81, M89, M96, M170, M172, M173, M201, M267, M269), que representan los linajes paternos más abundantes en el noroeste africano, el África subsahariana y Europa (Arredi y col., 2004; Bosch y col., 2001; Flores y col., 2003; Flores y col., 2004; Semino y col., 2000; Semino y col., 2004; Underhill y col., 2000). La nomenclatura de los haplogrupos del cromosoma Y se estableció siguiendo la propuesta por Karafet y col., (2008).

3.2.10.1. Preamplificación multiplex

La gran cantidad de extracto de ADN necesario para amplificar el cromosoma Y en las muestras antiguas ocasionó varios problemas. Por un lado, el estudio previo del ADNmt y del gen de la amelogenina, redujo considerablemente la cantidad de extracto disponible para continuar los análisis. Por otro lado, la necesidad de usar grandes volúmenes de extracto en las amplificaciones del cromosoma Y, provocó problemas de inhibición de la polimerasa.

Por esas razones, fue necesario introducir un paso previo de preamplificación. Aunque se probaron varias aproximaciones para resolver el problema, sólo la división de los dieciséis marcadores en tres reacciones de PCR multiplex dio resultados satisfactorios. Los primers usados y las condiciones de las tres reacciones de multiplex se muestran en la Tabla 6.

TABLA 6 – Primers usados en la preamplificación de los marcadores del cromosoma Y y tamaño del producto de PCR (la letra en cursiva se corresponde con una posición modificada).

Marcador	Primer Forward (5' → 3')	Primer Reverse (5' → 3')	Producto
Multiplex 1			
M78	GTGGTTTCTGCATTACTCCGT	TTTTGAAATATTTGGAAGGGC	92
M81	TGTAAGACTTTTAAGCACTATC	TGTTTCTTCTTGGTTTGTGTGA	112
M89	CCTGGATTCAGCTCTCTTCC	CTGCAACTCAGGCAAAGTGA	67
M267	TCGATGGAAGCATTTTTGTAA***C***TA	TTCCAAAGGGCTGGGATT	89
M269	AGGGGAATGATCAGGGTTTGG	TCTTTTGTGTGCCTTCTGAGG	87
Multiplex 2 (M89-ancestral)			
M2	GCTCCCCTGTTTAAAAATGTAGG	TACAGCTCCCCCTTTATCCTC	90
M33	GACCGTCATAGGCTGAGACA	TGGGAGTCACTCAGTTACAAAAG	96
M34	CATGTTAATGCCTGGCTTCC	GGGGGACCCCAATAATCATA	80
M60	CATTTCAAAATGCATGACTTAAAGA	CACAGACCAACATTGAGTAACCA	60
M96	CTTGGAAAACAGGTCTCTCATAA	CACCCACTTTGTTGCTTTGT	100
Multiplex 3 (M89-derivado)			
M9	GGACCCTGAAATACAGAACTGC	CGTTTGAACATGTCTAAATTAAAGAA[1]	85
M45	TGGACTTTACGAACCAACCTTT	CCTCAGAAGGAGCTTTTTGC[1]	99
M170	TGTTTTCATATTCTGTGCATTATAC	TGAGACACAACCCACACTGAA[1]	87
M172	TTTTATCCCCCAAACCCATT	GCCAGGTACAGAGAAAGTTTGG[1]	97
M173	TTTCTTACAATTCAAGGGCATTT	GCAGTTTTCCCAGATCCTGA[1]	96
M201	TTCTCAGATCTAATAATCCAGTATCAA	CATCCAACACTAAGTACCTATTACGAA[1]	63

3.2.10.2. Reamplificación semianidada

Cada marcador fue reamplificado en una reacción de PCR singleplex anidada. Para ello, se diseñó un tercer primer anidado para cada marcador y se usó con uno de los primers usados previamente en la PCR multiplex (Tabla 7). La amplificación de cada uno de los marcadores fue comprobada mediante geles de poliacrilamida, tinción con bromuro de etidio y visualización con luz U.V.

TABLA 7 – Primers usados en la reamplificación de los marcadores del cromosoma Y y tamaño del producto de PCR (la letra en cursiva se corresponde con una posición modificada).

Marcador	Primer Forward (5' → 3')	Primer Reverse (5' → 3')	Producto
M78	ACATGAACACAAATTGATACA[1]	igual que en la PCR multiplex	64 pb
M81	CTCAGCTACACATCTCTTAACAA[1]	igual que en la PCR multiplex	87 pb
M89	igual que en la PCR multiplex	GCAACTCAGGCAAAGTGAGA***C***AT[2]	65 pb
M267	igual que en la PCR multiplex	CAGGCATCAGCTAGATTGTGTT[1]	70 pb
M269	igual que en la PCR multiplex	TGCCTTCTGAGGCACATATG[1]	78 pb
M2	igual que en la PCR multiplex	CCCCCTTTATCCTCCACAGATC[1]	83 pb
M33	GACAAGATCTGTTCAGTTTATCTCA[1]	igual que en la PCR multiplex	80 pb
M34	CTGGCTTCCACCCAGGAG[1]	igual que en la PCR multiplex	69 pb
M60	igual que en la PCR multiplex	AACATTGAGTAACCACTGTGTGC[1]	52 pb
M96	igual que en la PCR multiplex	AAGGACCATATATTTTGCCATAGG[1]	79 pb
M9	CTGCAAAGAAACGGCCTAAG[1]	igual que en la PCR multiplex	67 pb
M45	GAGAGGATATCAAAAATTGGCAGT[1]	igual que en la PCR multiplex	60 pb
M170	TTCATATTCTGTGCATTATACAAATTAC[1]	igual que en la PCR multiplex	83 pb
M172	igual que en la PCR multiplex	CAGAGAAAGTTTGGACTTTCAAAA[1]	89 pb
M173	igual que en la PCR multiplex	ATTTCTGAATATTAACAGATCACAAAG[1]	56 pb
M201	GATCTAATAATCCAGTATCAACTGAGG[1]	igual que en la PCR multiplex	57 pb

Referencias: [1]Este trabajo; [2]Flores y col., 2003

3.2.10.3. Análisis por RFLP

Una vez comprobada la amplificación, los productos de PCR fueron cortados con la enzima de restricción correspondiente, tal como indica la Tabla 8.

TABLA 8 – Enzimas de restricción usadas en el estudio del cromosoma Y y patrón de bandas observado.

Marcador	Enzima	Ancestral	Derivado
M78	*Aci*I	42 + 22 pb	64 pb
M81	*HpyCH4*IV	50 + 37 pb	87 pb
M89	*Nla*III	45 + 20 pb	65 pb
M267	*Mae*I	57 + 13 pb	35 + 22 + 13 pb
M269	*Mva*I	78 pb	49 + 29 pb
M2	*Nla*III	60 + 23 pb	83 pb
M33	*Mse*I	43 + 37 pb	80 pb
M34	*Hinf*I	69 pb	53 + 16 pb
M60	*Hinf*I	29 + 24 pb	52 pb
M96	-	secuenciación directa	
M9	*Hinf*I	40 + 27 pb	67 pb
M45	-	secuenciación directa	
M170	-	secuenciación directa	
M172	*Hinf*I	89 pb	59 + 30 pb
M173	*Dra*III	33 + 23 pb	56 pb
M201	-	secuenciación directa	

En aquellos casos en los que no fue posible diseñar un ensayo por RFLP, debido a la ausencia de enzimas de restricción que reconozcan la mutación y a la imposibilidad de usar primers modificados, los fragmentos de PCR fueron secuenciados.

3.2.10.4. Análisis mediante SNaPshot

Para realizar el análisis mediante SNaPshot, los productos de la reamplificación semianidada de cada PCR multiplex fueron reunidos en un tubo y se precipitaron para purificar y concentrar el ADN amplificado. Una vez resuspendidos se trataron además con Exo-SAP-it (USB), que contiene exonucleasa I y fosfatasa alcalina, para eliminar cualquier traza de primers o nucleótidos que pudiera haber quedado tras la precipitación.

Las reacciones de secuenciación se llevaron a cabo en multiplex usando el SNaPshot™ Multiplex Kit (Applied Biosystem) y las cantidades correspondientes de cada primer SBE (del inglés, Single Base Extension) (Tabla 9). Los productos de SNaPshot fueron tratados posteriormente con SAP (USB) para eliminar los nucleótidos marcados con fluorescencia que quedaron tras la secuenciación.

TABLA 9 – Secuencia, tamaño, concentración de los primers SBE y mutaciones detectadas en el análisis mediante SNaPshot del locus ABO.

Marcador	SBE primer (5' → 3')	pb	[Primer]	Mutación	Detección
M78	CCCCCCCCCCCACACTTAACAAAGATACTTCTTTC[1]	34	1.25 µM	C → T	C → T
M81	CCCCCCTAAATTTTGTCCTTTTTTGAA[1]	27	0.7 µM	C → T	C → T
M89	CAACTCAGGCAAAGTGAGAGAT[2]	22	0.10 µM	C → T	G → A
M267	TCGATGGAAGCATTTTTGTAA***C***TA[2]	24	0.15 µM	T → G	T → G
M269	GGAATGATCAGGGTTTGGTTAAT[2]	23	0.20 µM	T → C	T → C
M2	secuenciación directa	-	-	A → G	-
M33	TCAGTTACAAAAGTATAATATGTCTGAGAT[2]	30	0.10 µM	A → C	T → G
M34	CTGGCTTCCACCCAGGAG[1]	18	0.15 µM	G → T	G → T
M60	secuenciación directa	-	-	insT	-
M96	CCCCCCCCCCCGTAACTTGGAAAACAGGTCTCTCATAATA[1]	40	0.15 µM	G → C	G → C
M9	CCCCCCCCCCCCCCCCCCCCCCCCCGAAACGGCCTAAGATGGTTGAAT[1]	48	0.15 µM	G → C	G → C
M45	CCTCAGAAGGAGCTTTTTGC[2]	20	0.37 µM	G → A	C → T
M170	ACACAACCCACACTGAAAAAAA[1]	22	0.75 µM	A → C	T → G
M172	CCCCCAAACCCATTTTGATGCTT[1]	23	0.70 µM	T → G	T → G
M173	ATTTCTGAATATTAACAGATCACAAAG[2]	27	0.37 µM	A → C	T → G
M201	GATCTAATAATCCAGTATCAACTGAGG[1]	27	0.20 µM	G → T	G → T

Referencias: [1]Brión y col., 2005; [2]Este trabajo

Las reacciones de SNaPshot fueron corridas en un ABI PRISM 3130 Genetic Analyzer (Applied Biosystem) usando POP-7® (Applied Biosystem). Los resultados fueron analizados usando GeneMapper 4.0 software (Applied Biosystem).

3.2.11. Estudio del grupo sanguíneo ABO

Dado que no existe una nomenclatura consenso para los alelos del grupo sanguíneo AB0, en este trabajo, y a lo largo de la discusión y conclusiones, usaremos la propuesta por la Blood Group Antigen Gene Mutation Database (http://www.ncbi.nlm.nih.gov/gv/mhc/xslcgi.cgi?cmd=bgmut/home). Siempre que esta nomenclatura no sea usada, se indicará entre paréntesis su correspondencia con ella.

Basándonos en un trabajo publicado previamente, se amplificaron dos fragmentos pertenecientes al exón 6 y 7, respectivamente, que contienen las mutaciones que permiten diferenciar los alelos *A101*, *B101*, *O01*, *O02* y *O03* (Hummel y col., 2002).

Para la amplificación de ambos fragmentos se siguió un protocolo similar al usado para el análisis del cromosoma Y, consistente en preamplificación mediante PCR multiplex y reamplificación usando una PCR semi-anidada.

3.2.11.1. Preamplificación multiplex

Los dos fragmentos de los exones 6 y 7 fueron amplificados mediante PCR multiplex, usando los primers detallados en la Tabla 10 (Hummel y col., 2002).

TABLA 10 – Primers usados en la preamplificación del locus AB0 y tamaño del producto de PCR.

Exón	Primer forward (5' → 3')	Primer reverse (5' → 3')	Tamaño
6	CTCTCTCCATGTGCAGTAGGAAGG	GAACTGCTCGTTGAGGATGTCG	104 pb
7	AAGGACGAGGGCGATTTCTA	GCTGCAACTCTTGCACCGACC	68 pb

3.2.11.2. Reamplificación semianidada

Al igual que en el caso del cromosoma Y, cada fragmento fue reamplificado en singleplex mediante una PCR semianidada (Tabla 11). Para ello, se diseñó un nuevo primer anidado para cada fragmento, que se utilizó junto a uno de los usados para la preamplificación.

TABLA 11 – Primers usados en la reamplificación del locus AB0 y tamaño del producto de PCR.

Exón	Primer forward (5' → 3')	Primer reverse anidado (5' → 3')	Tamaño
6	igual que en la PCR multiplex	CTCGTTGAGGATGTCGATGTTGA	98 pb
7	igual que en la PCR multiplex	ACCGACCCCCCGAAGAAC	49 pb

3.2.11.3. Análisis por RFLP

Los productos de amplificación de cada exón fueron cortados con la enzima de restricción correspondiente, siguiendo el protocolo publicado por Hummel y col. (2002). Los patrones de bandas correspondientes a cada genotipo se detallan en la Tabla 12.

TABLA 12– Patrones de digestión para los diferentes genotipos.

Fenotipo	Genotipo	*Rsa*I	*HpyCH4*IV	*Nla*III	*Mnl*I
A	A101/A101	-/-	-/-	-/-	-/-
A	A101/O01	+/-	-/-	-/-	-/-
A	A101/O02	+/-	+/-	-/-	-/-
A	A101/O03	-/-	+/-	-/-	+/-
AB	A101/B101	-/-	+/-	+/-	-/-
B	B101/B101	-/-	+/+	+/+	-/-
B	B101/O01	+/-	+/-	+/-	-/-
B	B101/O02	+/-	+/+	+/-	-/-
B	B101/O03	-/-	+/+	+/-	+/-
O	O01/O01	+/+	-/-	-/-	-/-
O	O01/O02	+/+	+/-	-/-	-/-
O	O01/O03	+/-	+/-	-/-	+/-
O	O02/O02	+/+	+/+	-/-	-/-
O	O02/O03	+/-	+/+	-/-	+/-
O	O03/O03	-/-	+/+	-/-	+/+

3.2.11.4. Análisis mediante SNaPshot

La reacción de SNaPshot para la determinación del grupo sanguíneo se realizó de forma similar a la usada para el cromosoma Y, siguiendo las condiciones detalladas en la Tabla 13.

TABLA 13 – Secuencia y concentración de los primers SBE usados en las reacciones de SNaPshot y mutaciones detectadas en cada alelo.

Mutación	SBE primer (5' → 3')	[Primer]	*A101*	*B101*	*O01*	*O02*	*O03*	pb
261	TTAGGAAGGATGTCCTCGTGGT	0.20 µM	G	G	A	A	G	22
297	TTCGTTGAGGATGTCGATGTTGAA	0.25 µM	T	C	T	C	C	24
796	ACGAGGGCGATTTCTACTAC	0.10 µM	C	A	C	C	C	20
802	CCGACCCCCCGAAGAACC	0.10 µM	C	C	C	C	T	18

3.3. Análisis de datos

El análisis molecular de la varianza (AMOVA) y el cálculo de los F_{ST}s (Slatkin, 1995) se realizó en todos los casos usando el programa ARLEQUIN 2000 (Schneider y col., 2000). Igualmente se utilizó este programa para estimar la diversidad genética (Nei, 1987) y el número de migrantes intercambiados entre poblaciones en los análisis del ADNmt. En el caso del locus AB0, también se usó este programa para detectar posibles desviaciones con respecto a las proporciones de Hardy-Weinberg, aplicando el test exacto descrito por Guo y Thompson (1992).

Los gráficos de escalamiento multidimensional (en inglés, MDS) y el análisis de componentes principales (en inglés, PCA) se realizaron usando el paquete estadístico SSCP ver. 11 (SPSS, Inc.).

Para calcular la contribución de las tres poblaciones parentales (Península Ibérica, norte de África y África subsahariana) en la población canaria se utilizó el programa ADMIX 2.0 (Dupanloup y Bertorelle, 2001). En el caso del estudio de las poblaciones actuales para el locus AB0 se usó además el programa ADMIX.PAS, amablemente cedido por el Dr. Jeffrey Long.

Las relaciones filogenéticas en las secuencias de ADNmt fueron establecidas mediante redes abreviadas promedio (reduced median network) (Bandelt y col., 1999), usando el Network (http://www.fluxus-engineering.com/netwinfo.htm). Las edades de coalescencia, basadas en la región HVRI, fueron calculadas usando el estadístico rho (Morral y col., 1994) y una calibración de una transición cada 20.180 años (Forster y col., 1996). El error estándar de rho se calculó según Saillard y col. (2000)

Para determinar si existían diferencias significativas en las proporciones de varones y hembras, usamos un test de significación para diferencias de proporciones independientes (Zar, 1984). Para determinar si existen diferencias en las frecuencias de haplogrupos en el estudio del cromosoma Y, se utilizaron el test exacto de Fisher y el test de contingencia.

Los resultados obtenidos en la presente tesis fueron comparados con los de las poblaciones de interés que habían sido publicados previamente. En el caso del ADNmt, esta comparación es a menudo muy complicada, ya que estos datos pueden encontrarse tanto en formato de secuencia nucleotídica, como en forma de haplotipo. Para solucionar este problema, hemos puesto a punto el software HaploSearch (www.haplosite.com/haplosearch) que, de manera simple y rápida, permite la transformación de haplotipos a secuencia y viceversa (ver apartado 4.1.4 de Mejoras Técnicas).

RESULTADOS

4. RESULTADOS

4.1. Mejoras técnicas

4.1.1. Puesta a punto de un protocolo de obtención del ADN contaminante en superficie de muestras dentales fósiles.

Para determinar si las muestras dentales presentaban contaminación en su parte externa, se puso a punto un protocolo para la obtención de ADN en superficie.

Para ello, se usó un hisopo estéril empapado en una solución de tiocianato de guanidina (6M GuSCN, 0.1M Tris-HCl, pH 6.4) para recuperar el ADN contaminante de la superficie del diente. Posteriormente el ADN fue extraído del hisopo usando una solución de extracción (6M GuSCN, 0.1M Tris-HCl, pH 6.4, 0.2M EDTA, 2% Triton X-100) y purificado mediante un protocolo basado en el uso de sílica (Ver apartado 4.3 de la sección de Resultados).

A partir del extracto se amplificó la región hipervariable del ADNmt en dos fragmentos, utilizando las parejas de primers HV1-H3R y L3F-HV2 descritas anteriormente. Aquellas muestras que pudieron ser amplificadas, se secuenciaron y se compararon con el panel de investigadores involucrados en el estudio.

De las 38 muestras analizadas sólo en 21 de ellas no fue posible obtener ADN en superficie. En tres casos, el extracto externo estuvo contaminado por el investigador encargado del análisis. En las restantes, la secuencia obtenida perteneció a los dos investigadores encargados de realizar los estudios antropológicos del material. En dos casos, se obtuvo incluso mezcla de las secuencias de los dos investigadores, que pudieron ser identificadas tras el clonaje del producto de PCR (Ver anexos). Sin embargo, la secuencia del arqueólogo encargado de la excavación y de la conservación del material, no fue recuperada en ningún caso.

Teniendo en cuenta que, cuando las muestras han sido contaminadas de forma previa, la replicación en dos laboratorios independientes no garantiza la validez de los resultados (Pääbo y col., 2004), la obtención del ADN contaminante en superficie puede ser una herramienta útil para demostrar la autenticidad del ADN endógeno. Aunque previamente se había demostrado que el material arqueológico es más susceptible de ser contaminado durante la excavación (Sampietro y col., 2006) y su conservación en museos (Pruvost y Geigl, 2004), nuestros resultados indican que las muestras dentales bien conservadas son menos sensibles a estas rutas de contaminación. Esto es así porque la secuencia del arqueólogo encargado de la excavación y conservación de la muestras no fue obtenida en ningún caso, mientras que las secuencias de los antropólogos involucrados en los estudios posteriores, aparecieron en el 50% de los extractos externos y, en pocos casos, como contaminantes en los extractos endógenos.

4.1.2. Mejora de la transformación de E.coli mediante el uso de microondas

En la actualidad, a pesar de la existencia de métodos más eficaces como la electroporación, la transformación de células competentes mediante tratamiento con cloruro de calcio y choque térmico sigue siendo el más usado.

En el presente trabajo, hemos comparado las eficiencias de transformación del choque térmico clásico a 42ºC durante dos minutos, con la exposición a microondas a diferentes intensidades y tiempos.

Al comparar las eficiencias de los diferentes tratamientos, observamos que una exposición de un minuto a 180 W, comparado con el tratamiento a 42ºC, aumenta la eficiencia de trasformación unas tres veces (3,3 ± 0,5). Además, cuando se combinan ambos métodos, sometiendo las células a un choque térmico, seguido de exposición a microondas, se consigue una cantidad cinco veces mayor de células transformantes.

Los resultados del presente trabajo han sido publicados en la revista *Letter on Applied Microbiology* en un artículo titulado “Microwave improved E. coli transformation” (a continuación).

SHORT NOTE

Microwave improved *Escherichia coli* transformation

R. Fregel[1], V. Rodríguez[2] and V.M. Cabrera[1]

1 Department of Genetics, University of La Laguna, Tenerife, Canary Islands, Spain
2 Laboratory of Genetics, Department of Biology, University Institute of Health Sciences (IUNICS), and Juan March Hospital, Palma de Mallorca, Spain

Keywords
cloning, *Escherichia coli*, microwave, plasmid DNA, transformation.

Correspondence
Rosa Fregel, Department of Genetics, Faculty of Biology, University of La Laguna, La Laguna, 38271, Tenerife, Canary Islands, Spain. E-mail: rfregel@ull.es

2007/2027: received 17 December 2007, revised 9 January 2008 and accepted 10 January 2008

doi:10.1111/j.1472-765X.2008.02333.x

Abstract

Aims: The calcium chloride chemical transformation of *Escherichia coli* is still the most widley used cloning method in small laboratories. Therefore, any practicable improvement in its transformation efficiency seems to be of general interest.

Methods and Results: We found that giving calcium chloride competent cells a 1 min microwave pulse at the lowest power setting (180 W), instead of the classic 1–2 min 42°C heat-shock step, increases the transformation efficiency around threefold (3.3 ± 0.5). Moreover, when both treatments were given in a 2-min 42°C − 5 min on ice − 1 min microwave pluse sequence, an additional improvement of 1.6 was obtained, resulting in an overall increase in efficiency of approximately 5.3-fold compared to classical heat shock.

Conclusions: This transformation method significantly improves the classical heat shock treatment.

Significance and Impact of the Study: This method might be useful to those laboratories that cannot afford an electroporation apparatus.

Chemical transformation and electroporation are the two methods used to transform *Escherichia coli* cells with plasmid DNA. Electroporation gives transformation efficiencies at least tenfold greater than chemical methods, but it requires an electroporation apparatus. High-efficiency competent cells are commercially available, but they are expensive and have to be kept frozen at −80°C. For those laboratories that cannot afford these options, the classical method, using calcium chloride and a short heat pulse at 42°C (Mandel and Higa 1970; Dagert and Ehrlich 1974), is the usual choice. Several improvements of this technique have been carried out to obtain better competent cells (Huff *et al.* 1990; Nakata *et al.* 1997; Chen *et al.* 2001). With this method, routinely transformation efficiencies are about 10^6–10^7 transformants per mg DNA and they are at least tenfold lower when the input DNA is from ligation reactions, which sensibly reduces the number of recombinant clones obtained per plate. For these reasons, any additional improvement to augment the transformation efficiency of the classical protocol should be welcome. We have found that substituting the 42°C heat-shock treatment by a microwave pulse at the lowest setting power (180 W), significantly ($P < 0{\cdot}01$) improves the transformation efficiency. Even more, when 42°C heat-shock treated cells are subsequently exposed to the microwave treatment, an additional improvement of 1·6 was obtained.

For cloning experiments, we routinely used the JM109 *E. coli* strain and pUC18 or pGEM (Promega, Madrid, Spain) plasmids. We have experimentally confirmed that for 100 μl of calcium chloride competent cells, 10 ng of DNA and 2 min of 42°C heat-shock, give the best transformation efficiencies (around 10^7 transformants per mg DNA). However, this efficiency increases threefold when the competent cells are held 1 min at 180 W in a commercial microwave oven instead of the classical 42°C heat-shock step (Fig. 1a). Nevertheless, this comparatively higher efficiency steady drops when, keeping the time pulse, the microwave power is augmented (Fig. 1a), or when, keeping the minimum microwave power, the exposition time is increased (Fig. 1b). It seems that this improvement is in part due to a more efficient microwave heat-shock treatment because, when the calcium chloride competent cells were kept on ice during the microwave

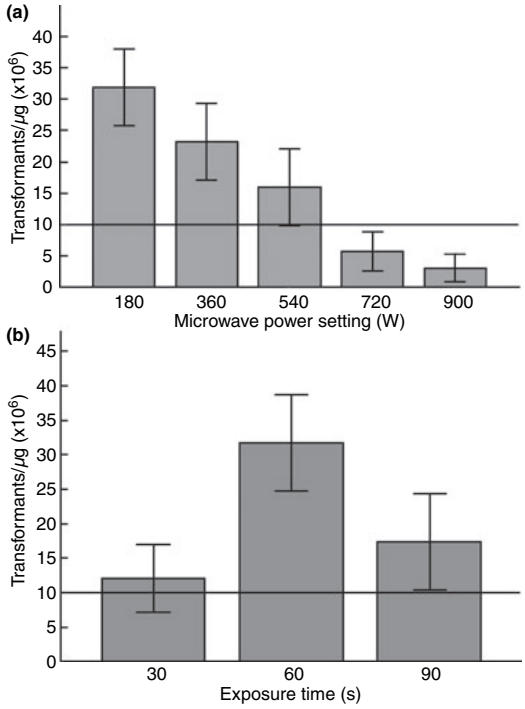


Figure 1 Transformation efficiency following microwave treatment. (a) Microwave transformation efficiencies using five different power settings. (b) Microwave transformation efficiencies at the lowest power setting (180 W), using different exposure times (seconds). Data are averaged ± SD of four repetitions per treatment, excepting the lowest setting, which was based on 19 repetitions. Horizontal line represents the 42°C heat-shock efficiency mean value ($1{\cdot}0 \times 10^7 \pm 0{\cdot}45$ transformant per mg DNA).

treatment, the number of transformants recovered was very low. Furthermore, when we used cells that were not calcium chloride treated, as used in electroporation, no transformants were obtained at all. However, this threefold improvement of the microwave treatment compared to the classic heat-shock treatment, subsists when using other *E. coli* strains as XL1 blue or other plasmids, as pBR322 (results not shown). To test whether both treatments affect the cellular DNA intake by the same mechanism, we applied each method twice and in combination, allowing a 5-min interval on ice between treatments for cellular recovery. The results of the duplicate experiments were as follows: (i) double heat-shock treatment diminishes the transformation efficiency by about 0·40, (ii) double microwave treatment improves the transformation efficiency by about 1·20, (iii) the sequential heat shock – microwave treatment improves the transformation efficiency respect to the simple microwave treatment by about 1·60, and (iv) the sequential microwave – heat shock treatment diminishes the transformation efficiency with respect to the simple microwave treatment by about 0·50. It seems that both treatments further potentiate transformation efficiency when the heat-shock pulse is applied first. In this case, the overall transformation efficiency is augmented about 5·3-fold with respect to the classical 42°C heat-shock.

Finally, we think that, to accurately reproduce this method in other laboratories, the optimal microwave setting and exposure time should be previously determined.

Acknowledgements

The authors thank handling editor (Dr Trudy Wassenaar) for suggesting the possibility that a successive application of the 42°C and microwave treatments could improve the transformation efficiency even more. This research was supported by grants of the Spain Ministry of Science and Technology BMC2001-3511 and Gobierno de Canarias COF2002/015 to V.M. Cabrera and R. Fregel is supported by a Gobierno de Canarias predoctoral fellowship.

References

Chen, X., Guo, P., Xie, Z. and Shen, P. (2001) A convenient and rapid method for genetic transformation of *E. coli* with plasmids. *Antonie Van Leeuwenhoek* **80**, 297–300.

Dagert, M. and Ehrlich, S.D. (1974) Prolonged incubation in calcium chloride improves competence of *Escherichia coli* cells. *Gene* **6**, 23–28.

Huff, J.P., Grant, B.J., Penning, C.A. and Sullivan, K.F. (1990) Optimization of routine transformation of *Escherichia coli* with plasmid DNA. *Biotechniques* **9**, 570–572, 574, 576–577.

Mandel, M. and Higa, A. (1970) Calcium-dependent bacteriophage DNA infection. *J Mol Biol* **53**, 159–162.

Nakata, Y., Tang, X. and Yokoyama, K.K. (1997) Preparation of competent cells for high-efficiency plasmid transformation of *Escherichia coli*. *Methods Mol Biol* **69**, 129–137.

4.1.3. Precipitación mejorada del ADN con etanol

En la actualidad, la precipitación con etanol absoluto continúa siendo el método más usado para la concentración y purificación de ADN. Sin embargo, este procedimiento requiere numerosos pasos consecutivos: ajuste de la concentración de sal, adición del volumen adecuado del alcohol y de un carrier, cuando es necesario, y después de la precipitación, lavado del pellet con etanol al 70%. En este trabajo, presentamos un método de precipitación más rápido basado en el uso de tres soluciones opcionales que funcionan en único paso.

Los diferentes cócteles de precipitación estuvieron compuestos por un 30% de solución acuosa y un 70% de etanol absoluto. La solución acuosa de cada cóctel contenía una sal volátil, que puede ser evaporada al final del protocolo mediante una breve incubación a 37ºC y una concentración salina (75 mM NH_4Ac), que es tres veces inferior a la recomendada para la precipitación de ADN. Además se probó el efecto de la adición de tres carriers diferentes a distintas concentraciones: acrilamida lineal (Solución A), glicógeno (Solución G) y polietilenglicol (Solución P).

Las concentraciones óptimas para los distintos carriers en cada uno de los cócteles fue: 0,025% de acrilamida lineal para la Solución A, 5 mg/ml de glicógeno para la Solución G y 50% (peso:volumen) de PEG para la Solución P. Una vez obtenidos estos valores, probamos si este método más rápido de precipitación era compatible con las manipulaciones posteriores de ADN. En resumen, para el uso de enzimas de restricción, las soluciones A y G funcionaron tan bien como el protocolo estándar. En el caso de la precipitación de reacciones de secuenciación, los resultados óptimos se obtuvieron con la Solución A. Finalmente, usando las Soluciones G y P para el clonaje se consiguió un número de transformantes significativamente mayor que utilizando el protocolo estándar de precipitación con etanol.

Los resultados obtenidos en este trabajo han sido publicados recientemente en la revista *Electrophoresis*, con el título “Improved ethanol precipitation of DNA”.

29-Dec-2009

Dear Dr. Fregel,

I am pleased to inform you that your manuscript, "Improved ethanol precipitation of DNA", is now acceptable for publication in ELECTROPHORESIS.

With the acceptance of your manuscript the final uploaded version in MS-Central will be used for copy-editing and the subsequent production process, so please do not send a new text file.

Please find below a list of required material for production:

- Please complete and return the attached "Copyright Transfer Agreement" and the "Color and Page Charge Agreement". Note: even if you do not wish to have any figures printed in color, we require this form to be completed to cover any page charges that might accrue (see Instructions to Authors on the journal homepage).
- If you would like to submit any color illustration as potential cover artwork (free of charge) please feel free to do so.
- Any permission statements, where applicable.

Please send this material to the Managing Editor at:

Fax: +49-6201-606-172

E-mail: elpho@wiley-vch.de

After we have received this material, your paper will go into production and you will receive a galley proof by e-mail in due course. Once you have corrected the proof, the paper will then be published on-line via Wiley InterScience ahead of its appearance in the printed journal.

Thank you for supporting ELECTROPHORESIS. I look forward to seeing more of your work in the future.

Sincerely,

Prof. Ziad El Rassi

Editor-in-Chief, ELECTROPHORESIS

TITLE:

Improved ethanol precipitation of DNA

AUTHORS:

Rosa Fregel[1#], Ana González[2] and Vicente M. Cabrera[1]

INSTITUTIONS:

1. Área de Genética. Departamento de Parasitología, Ecología y Genética. Facultad de Biología, Universidad de La Laguna (ULL). Avenida Astrofísico Francisco Sánchez, s/n. 38206 La Laguna (Tenerife), España.
2. Servicio de Genómica. Servicios Generales de Apoyo a la Investigación, Universidad de La Laguna (ULL). Avenida Astrofísico Francisco Sánchez, s/n. 38206 La Laguna (Tenerife), España.

 # CORRESPONDING AUTHOR:

Rosa Fregel
Departamento de Genética
Facultad de Biología
Universidad de La Laguna
La Laguna, 38271. Tenerife
SPAIN
e-mail: rfregel@gmail.com
Telephone: (34) 922 318350
Fax: (34) 922 318311

KEYWORDS:

DNA precipitation; DNA purification; linear acrylamide; glycogen; polyethylene glycol

WORD COUNT: 1,433

MAIN TEXT:

Even today, the most widely used method for concentration and purification of DNA is precipitation with absolute ethanol and washing with 70% ethanol [1]. However, this procedure requires several tedious consecutive steps such as: adjusting for salt concentration, adding a carrier when necessary, adding appropriate volumes of an alcohol and, after precipitation, washing the pellet with 70% ethanol solution. We present here a faster improved method based on three optional solutions that work in just one step.

The principle rests on the assumption that a 70% ethanol solution, including a moderate concentration of a monovalent salt, could be used to precipitate DNA and elute higher concentrations of salts in one step. Furthermore, the addition of a DNA carrier to this solution could facilitate precipitation even in cases of dilute DNA solutions or small DNA fragments. With this aim, after several assays, we chose an aqueous ammonium acetate (NH_4Ac) solution at 75 mM as basis. This salt concentration is three times lower than that recommended as final solution for DNA precipitation. We selected NH_4Ac because it is a volatile salt that can be evaporated at the end of the protocol by a brief incubation at 37º C. After that, maintaining this salt concentration in all cases, we prepared three different cocktails by adding three different carriers at different concentrations. Solution A included linear polyacrylamide from a 0.5% concentrated stock, prepared as previously published [2], but using distilled water to prepare the 5% acrylamide stock solution, instead of a 40 mM Tris-HCl, 20 mM Na-acetate, 1 mM EDTA buffer. Solution G included different concentrations of a 100 mg/ml glycogen stock solution [3]. Finally, solution P consisted of different concentrations of polyethylene glycol 8000 (PEG) [4]. Complete cocktails contain 30% of each aqueous solution and 70% absolute ethanol. Since the linear acrylamide and glycogen carriers slowly precipitate in presence of ethanol, we recommend adding it immediately before use.

Precipitation efficiency

We optimized the efficiency of each solution using different concentrations of their respective carriers, precipitating 5 µl of a solution (100 ng/µl) of a 50bp ladder DNA marker (Promega). In addition, we tested different volume ratios of solution : DNA and determined that precipitations were optimal within ratios 4:1 to 8:1. Outside this range, losses of the smallest bands or insufficient DNA salt purification were apparent. Precipitation with 70% ethanol was used as a negative control, and a standard absolute ethanol precipitation and direct loading of the marker as positive controls. Some comparative results can be seen in Figure 1. After that, we chose the following final carrier concentrations in the aqueous solution of each cocktail: 0.025% linear polyacrylamide for solution A, 5 mg/ml glycogen for solution G and 50% (w:v) PEG for solution P. Using the directly loaded marker

as 100% value, the recovery efficiency of the different solutions was quantified by Quantity One software (BioRad), measuring the relative concentration of the smallest 50 bp marker fragment. The mean results of seven independent assays gave a 69.6 ± 5% efficiency for the standard protocol, 73.1 ± 5% for solution A, 78.4 ± 6 for solution G and 62.3 ± 6 for solution P. Finally, we tested whether this shortened ethanol DNA precipitation method was compatible with subsequent DNA manipulations.

Compatibility with DNA restriction

Aliquots of PCR-amplified hypervariable segment I (HVSI) of human mitochondrial DNA control region were precipitated with solutions A, G, and P and digested with low, medium and high salt restrictases following manufacturer recommendations. Some of the results can be seen in Figure 2. Incomplete restriction was detected only when DNA was precipitated with solution P, but solutions A and G worked as efficiently as the standard ethanol precipitation.

Compatibility with PCR DNA amplification

Aliquots of a genomic DNA solution were precipitated with A, G and P solutions and the pellets used as DNA template for PCR amplification of the HVSI fragment. The same aliquot was precipitated with the standard ethanol protocol and loaded directly into the reaction as positive controls. In the three cases, results were optimal when using a 4:1 solution:DNA ratio v/v. Furthermore, precipitation with solution G yielded more PCR final product than both positive controls.

Compatibility with DNA sequencing

PCR-amplified sequencing reactions, using control M13mp18 DNA, were precipitated with A, G and P solutions, the resulting pellets resuspended in formamide and run in a MEGAbace DNA analyser (Amersham Biosciences). The standard ethanol protocol was used as a positive control. In the three experimental cases results were optimal when using an 8:1 solution:DNA . ratio v/v. Sequences precipitated with solution P always gave electropherograms with higher background than controls, sequences precipitated with solution G were of the same quality as controls but, on occasions, showing moderately compressed profiles at the beginning of the run. However, sequences precipitated with solution A gave better results than controls, usually at the beginning and end of the electropherogram (Supporting information).

Compatibility with DNA cloning

PCR-amplified DNA fragments of 500 bp, 125 bp and 83 bp were co-precipitated in equimolar quantities with pGEMT vector using A, G and P solutions and the standard ethanol precipitation protocol and the Quiagen PCR purification kit as positive controls. In the three experimental cases, results were optimal when using 8:1 solution:DNA . Pellets were resuspended in the ligation solution using volumes and conditions recommended by the manufacturer. Transformations were carried out always using the same batch of competent cells and following the calcium chloride [5] and 42º heat-shock standard protocol. Comparative transformation efficiencies were estimated as the mean of three independent assays (Table 1). In this case, solution A performed only as well as controls. However, when using the 500 pb fragment, ligation efficiencies were significantly higher ($p<0.05$ in all cases) using solutions G and P, than using Quiagen and standard ethanol protocols. Ligation efficiencies using the 125 bp and 83 bp fragments were significantly better than controls only with the G solution ($p<0.05$ in both cases).

Advantages of this shorter ethanol precipitation protocol

Although there is no optimal precipitation solution for all subsequent DNA manipulations, in all cases a shorter general protocol than the standard is achieved. Briefly, instead of adding the appropriate salt concentration, the carrier, and the 2 volumes of ethanol to the DNA solution in sequential steps and mixing several times (particularly tedious if precipitation is performed in microplaques), it is sufficient to add 4 or 8 volumes of the appropriate A, G or P solution in just one step. Incubation at –20ºC or at room temperature, centrifugation and discarding the supernatant are similar processes in both methods. However, washing the DNA pellet with 70% ethanol and subsequent centrifugation are again avoided in our shorter protocol. Moreover, using solution A to precipitate sequences, and solutions G and P for cloning, gives significantly better results than using the standard ethanol protocol.

ACKNOWLEDGEMENTS

R.F. was supported by a predoctoral fellowship from the Canary Government (TES2005/097).

All authors declare there are no conflicts of interest.

REFERENCES

[1] Maniatis, T., Fritsch, E. F., Sambrook, J., *Molecular cloning: A laboratory manual.*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 1982.

[2] Gaillard, C., Strauss, F., *Nucleic Acids Res* 1990, *18*, 378.
[3] Tracy, S., *Prep Biochem* 1981, *11*, 251-268.
[4] Lis, J. T., *Methods Enzymol* 1980, *65*, 347-353.
[5] Mandel, M., Higa, A., *J Mol Biol* 1970, *53*, 159-162.

TABLES

Table 1 – Mean number of cells transformed with DNA precipitated by different methods, compared with the optimized conditions for A, G and P solution.

	500 bp fragment	**125 bp fragment**	**83 bp fragment**
QUIAGEN	270 ± 78	211 ± 71	152 ± 20
Standard protocol	235 ± 74	195 ± 77	138 ± 68
Solution A (0.025%)	287 ± 88	321 ± 65	254 ± 129
Solution G (5 mg/ml)	495 ± 102	927 ± 325	612 ± 168
Solution P (50% w/v)	505 ± 111	498 ± 173	157 ± 50

FIGURE LEGENDS

Figure 1 – Ethanol precipitation of a 50 bp DNA ladder marker with different conditions. Lanes: 1: 70% ethanol with 75 mM $AcNH_4$ (EA-70%) in its aqueous fraction; 2: directly loaded sample; 3: solution G with 10 mg/ml glycogen; 4: solution G with 5 mg/ml glycogen; 5: solution P with 50% w/v of PEG; 6: solution A with 0.025% linear polyacrylamide; 7: sample precipitated with the standard 0.3 M sodium acetate and two vols. of ethanol.

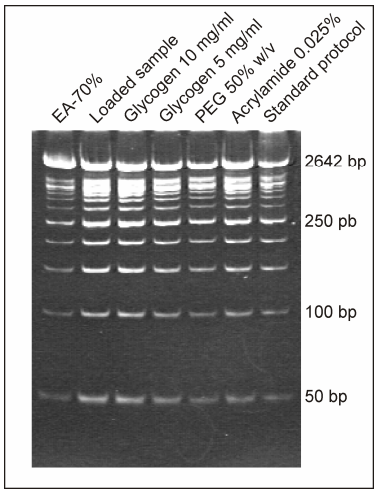
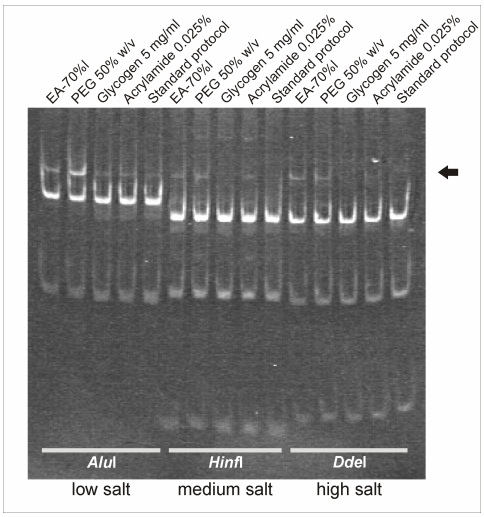
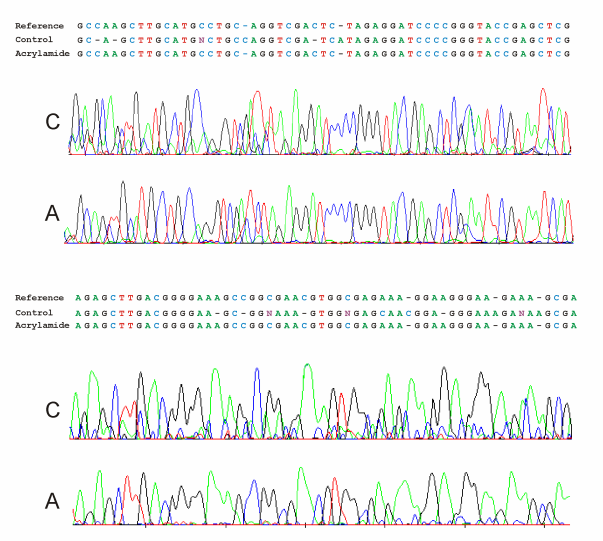


Figure 2 – Digestion with low, medium and high salt restrictases of DNA precipitated in different conditions, as detailed in Figure 1.



SUPPORTING INFORMATION

Supporting figure 1 – Comparative beginning (bases 15 to 69) and end (bases 670 to 724) eletropherogram segments obtained by control and solution A sequence-reaction precipitation.



4.1.3. HaploSearch: una herramienta para la transformación de secuencias en haplotipos y viceversa

En la actualidad los estudios de ADNmt han abordado prácticamente todas las poblaciones humanas. La comparación de los datos obtenidos en cada una de ellas ha permitido conocer el origen y la asignación geográfica de cada linaje de ADNmt.

Sin embargo, en muchos casos, la comparación de los datos obtenidos con los publicados resulta muy complicada. Esto es así, porque estos datos pueden aparecer en dos formatos: secuencias nucleotídicas o haplotipos, en los que sólo se indican las posiciones mutadas respecto a una referencia. La transformación manual entre ambos formatos es un proceso bastante largo, que la mayoría de las veces, ocasiona la introducción de numerosos errores.

HaploSearch es un programa de interfaz muy simple que permite la transformación de haplotipos a secuencia y viceversa, de una forma sencilla y muy rápida. Además, su implementación en una página web (www.haplosite.com/haplosearch) evita la necesidad de usarlo en ningún sistema operativo en particular.

El programa HaploSearch ha sido enviado a la revista *Human Heredity*, con el título “HaploSearch: a tool for haplotype sequence two-ways transformation” (a continuación).

20-Feb-2010

Dear Dr. Fregel:

Thank you for submitting your manuscript to "Human Heredity"; the submission number is: 1393. Your submission will now be checked by the editorial office, and you will receive a confirmation mail from the editorial office soon. This step will also activate your personal user-id and password, enabling you to login to the system to check the status of your manuscript.

If you have any queries please send an email to: katherine.montague@mail.rockefeller.edu.

With kind regards,

Editorial Office

HaploSearch: a tool for haplotype-sequence two-ways transformation

ROSA FREGEL[1], & SERGIO DELGADO[2]

[1]Department of Genetics, Faculty of Biology, University of La Laguna, La Laguna, Tenerife, Spain, and [2]Department of Statistics, Operations Research and Computing, Faculty of Mathematics, University of La Laguna, La Laguna, Tenerife, Spain.

Running title: HaploSearch: haplotype-sequence transformation

Keywords: Software, mitochondrial DNA , sequence, haplotype

Correspondence: Rosa Fregel, Department of Genetics, Faculty of Biology, University of La Laguna, Avda. Astrofísico Fco. Sánchez, s/n, 38271 La Laguna, Tenerife, Spain. FAX: +34 922 318 311. E-mail: rfregel@gmail.com

ABSTRACT:

Comparison of available mitochondrial DNA data is some times hindered for data presentation format. HaploSearch is a simple tool for transforming DNA sequences in haplotype data and vice versa, speeding up the manipulation of large datasets. Although designed for mitochondrial DNA, HaploSearch could be used with any kind of DNA type. HaploSearch program, detailed software instructions and example files are freely available on the web at http://www.haplosite.com/haplosearch/.

MAIN TEXT:

In 1981, the sequence and organization of the human mitochondrial DNA (mtDNA) was published for the first time (Anderson et al. 1981). Since that, populations from almost everywhere have been studied from the mtDNA point of view. The comparison of these large sets of mtDNA data have allowed to know the origin and global distribution of each human mtDNA lineage.
However, published data comparison is frequently complicate as results could appear in two different formats: haplotype (detected mutations respect to a reference sequence) and nucleotide sequence data. Moreover, some statistical analyses require haplotype data, whereas others are designed for nucleotide sequences. Manual transformation between formats is time-consuming, complex and likely to introduce mistakes.
HaploSeach program transforms haplotype and sequence data between them in an easy and rapid way. This program admits both partial and complete mtDNA sequences, and recognizes substitution mutation (transitions and transversions) and indels (insertions and deletions).
Although HaploSearch was designed to analyze mtDNA sequences, it is suitable for transforming haplotypes and sequences in any kind of DNA sources. The program only requires a reference sequence from which extract the information, as occurs with the revised Cambridge Reference sequence (CRS) for mtDNA (Andrews et al. 1999).

Data format
Sequences must be introduced in the commonly used fasta format. The use of that format in HaploSearch permits to obtain the complete and partial mtDNA data directly from the main databases (GeneBank, mtDB...). All sequences have to be equal in length, so they have to be aligned, previously to HaploSearch analysis, with the reference sequence.
Mutations into haplotypes must to be ordered from smaller to higher position. Mutations have to be written following this rules, as it is commonly accepted: a) transitions indicated with the nucleotide position of the mutation; b) tranversions, indicated with the nucleotide position and the mutated base;

c) delections, with the the first deleted base position, followed by letter “d” and the deleted bases; d) insertions, with the base number before the gap, followed by letter “i” and the inserted bases. If no mutations exist, the haplotype would be “CRS” or the corresponding designation for other DNA types. For more information, see “HaploSearch Instructions” at http://www.haplosite.com/haplosearch/help/.

Input data file

Input data files have to be unformatted text files (txt files) with the following indications:

1. Transforming sequences to haplotypes:The input file for transforming sequences to haplotypes has to be a txt file containing the sequences in fasta format. Moreover, we have to indicate what is the reference sequence and the position number for the first nucleotide in the sequence.

```
START:16056
>REFERENCE
CCCTCCCC-GCTGACGTCATG
>Sequence1
CCCCCCCCCGCTGCCGTCATG
>Sequence2
CCCTCCCC-GCCGACGTCATG
```

2. Transforming haplotypes to sequences:The input file for transforming haplotypes to sequences is similar to the previous file, but using haplotype data.

```
START:16056
>REFERENCE
CCCTCCCCGCTGACGTCATG
>Sequence1
16059 16063iC 16068C
>Sequence2
16066
```

Examples of these files, for complete and partial mtDNA sequences, and for non-mtDNA data, are available at HaploSearch site.

Output data file

HaploSearch output data files have the same format than the input data file for the opposite transformation. This feature allows the obtention of the original data from the output file and check if a mistake was introduced.

HaploSearch interface

Using HaploSearch interface is simple and intuitive. To run HaploSearch, you must only browse the file containing the data and select between the two transformation options: get the haplotype or get the sequence.

HaploSearch has been developed as a web application in order to be very easy to access since it does not need any special operative system nor additional libraries. The only requirement is to have a web browser in the computer.

The application has been written using a web framework called Django (www.djangoproject.org). This framework lets you use a high-level programming language called Python (www.python.org) to build the code.

Inside the source-code we have used an Object Oriented Programming (OOP) approach. Because of that, we read each sequence from the input file and build an object class representing it. Afterwards we are able to call special methods defined in the class to manipulate the object and get the haplotype. It can be done backwards.

All the input data have string format. In other words, they are not well structured so a big part of processing is addressed to slice, split and work with patterns to get a more organized information. Regarding this, we have also built a syntax checking system which validates the input files format. Once we have performed the syntax checking, processing can go on.

To get the haplotype from the sequence, we have developed a method which iterates over all the nucleotides comparing them with the ones we have in the reference sequence. Each time we detect a change, it is notified and stored in a complex data structure where we keep transitions, tranversions, delections and insertions.

To get the sequence from the haplotype, we analyze each mutation using a method which isolates the changes and takes into account the reference sequence. In this way, we rebuild the original sequence through storing information in fast-read data lists.

Our computing methods can process a big amount of input data in a short time since they are implemented in a modular way and exploding all the facilities of high-level programming languages such Python. Because of this, the application is highly scalable as well.

ACKNOWLEDGEMENTS

We acknowledge V.M. Cabrera and N. Pérez for their helpful comments. R.F. is supported for a Gobierno de Canarias predoctoral fellowship.

REFERENCES

Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG. 1981. Sequence and organization of the human mitochondrial genome. Nature 290: 457-465.

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23: 147.

4.2. Estudio del ADN mitocondrial en la Macaronesia: variación dentro y entre archipiélagos

La Región Macaronésica incluye cinco archipiélagos habitados: Azores, Madeira, Cabo Verde y Canarias. Sin embargo, en el momento de ser descubiertos por los europeos, sólo las Islas Canarias se encontraban habitadas.

Tras la conquista de Canarias y el descubrimiento de los otros archipiélagos, se produjo un proceso de colonización europea, predominantemente llevada a cabo por colonos portugueses y españoles. Además, numerosos esclavos norteafricanos y subsaharianos fueron llevados a estos archipiélagos debido a la falta de mano de obra. En el caso de Cabo Verde, las islas fueron pobladas básicamente por esclavos traídos desde la costa oeste de África y colonizadores europeos, dando lugar a una población mestiza denominada Mulatos o Criollos.

Aunque los archipiélagos de la Macaronesia se han estudiado desde el punto de vista del ADNmt de forma independiente, no existe ningún trabajo que lo haya hecho de forma global. En el presente estudio se ha analizado el ADNmt de 120 individuos de Azores y 503 de las Islas Canarias que, junto a los resultados publicados con anterioridad, suman un total de 1.542 de la Macaronesia y 1.068 de la Península Ibérica.

Los resultados obtenidos indican que Cabo Verde es el archipiélago más discrepante, debido a que casi la totalidad de sus linajes maternos son de asignación africana. Por el contrario, la composición genética de Azores, Madeira y Canarias para el ADNmt, presenta una mayor abundancia de haplogrupos característicos del oeste de Eurasia y, en menor medida, linajes africanos. Además de Cabo Verde, existen dos islas en Azores (Flores y Corvo) y una en Canarias (La Gomera) que se diferencian significativamente del resto. En el caso de las islas de Flores y Corvo, ésto puede explicarse mediante la acción de efectos fundadores y deriva genética. Por otro lado, La Gomera se diferencia del resto debido a que posee la mayor frecuencia del haplogrupo autóctono canario U6b1.

El análisis de los haplotipos presentes en la Macaronesia muestra que existe un bajo porcentaje de linajes compartidos entre archipiélagos, lo que podría reflejar que la variación genética de Azores, Madeira, Cabo Verde y las Islas Canarias estuvo determinada principalmente por el proceso inicial de colonización y una posterior microdiferenciación debida al pequeño tamaño poblacional de algunas islas. Por otro lado, el contacto entre archipiélagos parece haber tenido un escaso impacto en su composición genética, al menos desde el punto de vista del ADNmt.

Al estudiar las frecuencias de marcadores del ADNmt y el cromosoma Y con asignación geográfica norteafricana y subsahariana, en los archipiélagos de la Macaronesia y en la Península Ibérica, se observa que en general, los linajes maternos de estas áreas contribuyeron en mayor medida a las poblaciones actuales, a pesar de que el tráfico de esclavos afectaba principalmente a individuos varones. En el caso de Canarias, la mayor aportación norteafricana detectada para el ADNmt, se explica además por una asimetría sexual debida al desplazamiento de los varones aborígenes por parte de los europeos.

Los resultados obtenidos en el estudio del ADNmt en la Macaronesia han sido publicados en la revista *American Journal of Physical Anthropology* con el título "Mitochondrial DNA patterns in the Macaronesia Islands: variation within and among archipelagos".

AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 000:000–000 (2010)

Mitochondrial DNA Patterns in the Macaronesia Islands: Variation Within and Among Archipelagos

Cristina Santos,[1,2]* Rosa Fregel,[3] Vincente M. Cabrera,[3] Ana M. González,[3] José M. Larruga,[3] and Manuela Lima[2]

[1]*Unitat Antropologia Biològica, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain*
[2]*Department of Biology, Center for Research in Natural Resources (CIRN), University of the Azores, 9500 Ponta Delgada, S. Miguel, Azores, Portugal*
[3]*Department of Genetics, Faculty of Biology, University of La Laguna, 38271 Tenerife, Canary Islands, Spain*

KEY WORDS mtDNA; haplogroup; Y-chromosome; African lineages

ABSTRACT Macaronesia covers four Atlantic archipelagos: the Azores, Madeira, the Canary Islands, and the Cape Verde islands. When discovered by Europeans in the 15th century, only the Canaries were inhabited. Historical reports highlight the impact of Iberians on settlement in Macaronesia. Although important differences in their settlement are documented, its influence on their genetic structures and relationships has yet to be ascertained. In this study, the hypervariable region I (HVRI) sequence and coding region polymorphisms of mitochondrial DNA (mtDNA) in 623 individuals from the Azores (120) and Canary Islands (503) were analyzed. Combined with published data, these give a total of 1,542 haplotypes from Macaronesia and 1,067 from the Iberian Peninsula. The results obtained indicate that Cape Verde is the most distinctive archipelago, with an mtDNA pool composed almost exclusively of African lineages. However, the other archipelagos present an mtDNA profile dominated by the presence of West-Eurasian mtDNA haplogroups with African lineages present in varying proportions. Moreover, no signs of integration of typical Canarian U6 lineages in the other archipelagos were detected. The four Macaronesia archipelagos currently have differentiated genetic profiles, and the Azores present the highest intra-archipelago differentiation and the lowest values of diversity. The analyses performed show that the present-day genetic profile of the Macaronesian archipelagos was mainly determined by the initial process of settlement and further microdifferentiation probably as a consequence of the small population size of some islands. Moreover, contacts between archipelagos seem to have had a low impact on the mtDNA genetic pool of each archipelago. Am J Phys Anthropol 000:000–000, 2010.

The Macaronesia region includes four inhabited archipelagos located in the Atlantic Ocean (see Fig. 1): the Portuguese archipelagos of the Azores and Madeira, the Spanish archipelago of the Canary Islands, and the Autonomous Republic of Cape Verde. The Azores are located in the north-west and have 241,763 inhabitants (INE, 2003a) distributed very unevenly across nine islands (Santa Maria, São Miguel, Terceira, Graciosa, São Jorge, Pico, Faial, Corvo, and Flores). Madeira has 245,011 inhabitants (INE, 2003a) and consists of two islands (Madeira and Porto Santo). There are seven Canary Islands (Lanzarote, Fuerteventura, Gran Canaria, Tenerife, La Gomera, La Palma, and El Hierro), and they currently have 1,694,477 inhabitants (INE, 2004). Finally, Cape Verde has 434,263 inhabitants (INE, 2003b) distributed across nine islands (Brava, Fogo, Santiago, Maio, Boavista, Sal, São Nicolau, São Vicente, and Santo Antão).

The islands of Azores and Madeira were uninhabited when they were discovered by Portuguese sailors in the early-15th century, whereas Cape Verde, also uninhabited, was discovered later in the 15th century (Brandão, 1995). By contrast, the Canary Islands were already inhabited, probably since Neolithic times, by *Guanches*, an aboriginal group that appears to have been culturally and genetically related to the North African Berbers (Fructuoso, 2004; Maca-Meyer et al., 2004), when the Europeans, mainly vassals of the Portuguese and Castilian Kings, began the conquest of the archipelago in the early-15th century.

After the discovery of Madeira and the Azores and the conquest of the Canary Islands, these archipelagos were peopled essentially by Portuguese and Spaniard individuals as well as by individuals of various European origins (such as French, Italian, English, German, and Flemish) (Mendonça, 1996; Vieira, 2001; Fructuoso, 2004; Matos, 2005). Furthermore, North African and sub-Saharan slaves also contributed to peopling these archipelagos, albeit with a differential representation

Additional Supporting Information may be found in the online version of this article.

Grant sponsor: Fundação para a Ciência e a Tecnologia; Grant number: SFRH/BPD/20944/2004; Grant sponsor: Canary Islands Government; Grant sponsor: Spain Ministry of Education and Science; Grant numbers: BFU2006-04490/BMC.

Cristina Santos and Rosa Fregel contributed equally to this work.

*Correspondence to: Cristina Santos, Unitat Antropologia Biològica, Edifici C, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain. E-mail: cristina.santos@uab.es

Received 25 February 2009; accepted 7 August 2009

DOI 10.1002/ajpa.21180
Published online in Wiley InterScience (www.interscience.wiley.com).

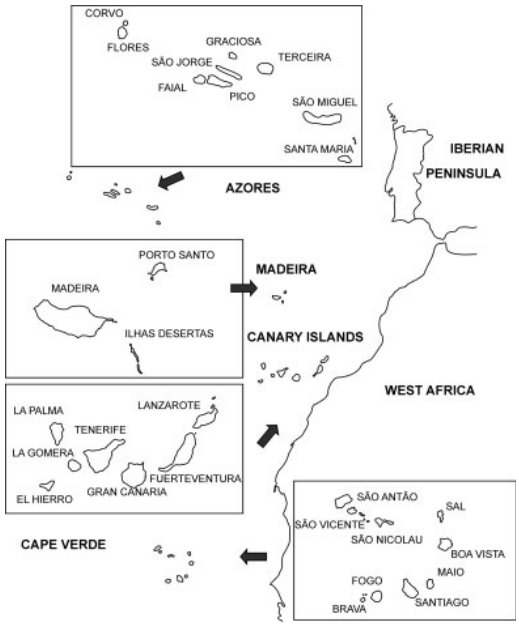


Fig. 1. Geographic location of the four Macaronesia archipelagos.

(Mendonça, 1996; Vieira, 2001; Fructuoso, 2004; Matos, 2005; Gregório, in press; Mesquita, 2005).

The Cape Verde islands were peopled essentially with African slaves brought by the Portuguese from the West African coast and by European men. This combination between European males and African females gave rise to an admixed population called Mulattos or Crioulos (Ferreira, 2001).

Links between the Macaronesia archipelagos were established after their discovery. There are references to the transportation of Guanches as slaves to Madeira and historical evidence of individuals from Madeira actively participating in the peopling of the Azores. Moreover, politics and mainly the dispute over the Canary Islands between the Portuguese and Castilian Kings, as well as economic reasons, encouraged contact between the archipelagos, particularly between the Canary Islands and Madeira (Mendonça, 1996; Vieira, 2001; Fructuoso, 2004; Matos, 2005). After the discovery of Cape Verde, this archipelago acted as a platform connecting Africa to Europe, America, and India, from where slaves were taken away to various regions, including the Canary Islands, Madeira, and the Azores. Moreover, there are references to the transportation of Guanches to Cape Verde (Ferreira, 2001; Vieira, 2001; Matos, 2005).

Mitochondrial DNA (mtDNA) variation in each of the Macaronesia archipelagos has been described by various groups of researchers (Rando et al., 1999; Brehm et al., 2002, 2003; Santos et al., 2003, 2006). However, no integrative work on the genetic relationship between the four archipelagos, to determine whether the political and commercial contacts between archipelagos have a significant impact on the genetic composition of the islands, has been carried out.

In this context, the main goals of this work are to (a) increase the mtDNA data for the Azores and the Canary Island archipelagos; (b) analyze the genetic relationship between the Macaronesia islands; and (c) infer the impact of evolutionary forces on the islands and archipelagos of Macaronesia.

MATERIALS AND METHODS

Samples and data

mtDNA data [hypervariable region I (HVRI) sequences and coding region informative polymorphisms for haplogroup assignment] were analyzed in 623 individuals from the Azores (120) and the Canary Islands (503), which when added to published data give a total of 1,542 individuals from Macaronesia (292 from the Azores, 155 from Madeira, 803 from the Canary Islands, and 292 from the Cape Verde islands) and 1,068 from the Iberian Peninsula (540 from mainland Portugal and 528 from mainland Spain). Voluntary donors were asked for informed consent, and the birthplaces of all their known ancestors were anonymously facilitated. Detailed geographic origins and references for the samples analyzed are listed in Table 1.

DNA extraction and mtDNA analysis

Total DNA from blood or buccal cell samples was extracted using standard protocols (Rando et al., 1999; Santos et al., 2003). mtDNA amplification, HVRI sequencing, and RFLP analysis were carried out, as described previously, in CIRN (Santos et al., 2003) and in La Laguna (Gonzalez et al., 2006). The following strategy was used for haplogroup assignation. Sequences with unequivocal HVSI haplogroup motifs were directly assigned to their respective haplogroup. The rest were tentatively sorted into their most probable haplogroups; this classification was confirmed by RFLP analysis of diagnostic positions. Additional RFLPs were carried out when the HVSI-based assignation proved to be incorrect or when further subgroup assignation was necessary. The sequence range obtained in CIRN was 16,050–16,399 and in La Laguna it was 15,850–16,430. However, we used the positions between 16,050 and 16,399 for the integrated analysis.

Data analysis

Samples were assigned to haplogroups according to the phylogenetic classification proposed by Torroni et al. (2006) and updated by van Oven and Kayser (2009).

Haplogroup and haplotype-sequence variation were used to perform AMOVA analysis (Excoffier et al., 1992) and Slatkin's linearized F_{ST} pairwise genetic distance matrices between population (Slatkin, 1995). Five groups, represented by the four archipelagos and the Iberian Peninsula, were considered for AMOVA structure. Gene diversity (Nei, 1987) was estimated for each population using the HVRI sequences between positions 16,050 and 16,399. All statistics were computed using the Arlequin 3.1 software (Excoffier et al., 2005). Multidimensional scaling (MDS) was used to represent genetic distances in a two-dimensional space using SPSS ver. 17.0 (SPSS Inc.).

Phylogenetic networks (Bandelt et al., 1999) among haplotypes were constructed using the program Network 4.5.1.0 (www.fluxus-engineering.com). Positions of HVRI were weighted according to the site-specific rate summarized by Santos et al. (2008). Mutational hotspots

TABLE 1. Number of samples analyzed, number of inhabitants, number, and percentage of different haplotypes and gene diversity calculated according to Nei (1987) for each archipelago (sequences 16,050–16399) and island and for Mainland Portugal and Spain (sequences 16,069–16,370)

Population	No. of samples (this study/previously published)	No. of inhabitants	No. of different haplotypes (%)	Gene diversity
Azores archipelago (AZO)	292 (120/172[a,b])	241,763	120 (41.1)	0.9510
Flores (FLO)	40 (4/36[a])	3,995	17 (42.5)	0.9080
Corvo (COR)	17 (17/0)	425	8 (47.1)	0.8530
Faial (FAI)	31 (7/24[a])	15,063	18 (58.1)	0.9480
Pico (PIC)	23 (20/3[a])	14,806	14 (60.9)	0.9370
São Jorge (SJ)	25 (24/1[a])	9,674	16 (64.0)	0.9170
Graciosa (GRA)	29 (29/0)	4,780	17 (58.6)	0.9160
Terceira (TER)	50 (18/32[a])	55,833	36 (72.0)	0.9650
São Miguel (SM)	51 (1/50[a])	131,609	34 (66.7)	0.9510
Santa Maria (SMA)	26 (0/26[b])	5,578	13 (50.0)	0.9290
Madeira archipelago (MAD_T)	155 (0/155[c])	245,011	88 (56.8)	0.9650
Madeira (MAD)	124 (0/124[c])	240,537	80 (64.5)	0.9680
Porto Santo (PS)	31 (0/31[c])	4,474	18 (58.1)	0.9460
Canary archipelago (CAN)	803 (503/300[d])	1,694,477	242 (30.1)	0.9616
El Hierro (HIE)	65 (35/30[d])	8,682	35 (53.8)	0.9660
La Palma (PAL)	87 (37/50[d])	78,800	46 (52.9)	0.9520
La Gomera (GOM)	71 (38/33[d])	18,285	32 (45.1)	0.9030
Tenerife (TEN)	295 (222/73[d])	701,034	128 (43.4)	0.9628
Gran Canaria (GC)	134 (89/45[d])	730,622	63 (47.0)	0.9140
Fuertaventura (FUE)	67 (31/36[d])	60,273	34 (50.7)	0.9670
Lanzarote (LAN)	84 (51/33[d])	96,781	44 (52.4)	0.9640
Cape Verde archipelago (CV)	292 (0/292[e])	341,491	120 (41.1)	0.9748
Northwestern Islands (CV_NW)	108 (0/108[e])	108,787	29 (26.9)	0.9080
Southeastern Islands (CV_SE)	184 (0/184[e])	232,704	106 (57.6)	0.9877
Mainland Portugal (MPT)	540 (0/540[f])	10,126,880	249 (46.1)	0.9530
Mainland Spain (MSP)	528 (0/528[g])	38,173,309	237 (44.9)	0.9542

[a] Santos et al. (2003).
[b] Santos et al. (2006).
[c] Brehm et al. (2003).
[d] Rando et al. (1999).
[e] Brehm et al. (2002).
[f] Two hundred and forty-one from Pereira et al. (2000) and 299 from Gonzalez et al. (2003).
[g] One hundred and ninety-eight from Larruga et al. (2001), 132 from Côrte-Real et al. (1996), 18 from Pinto et al. (1996), 92 from Salas et al. (1998), 43 from Gonzalez et al. (2003), and 45 from Bertranpetit et al. (1995).

were weighted as one, and the remaining positions were weighted from two to five depending on the site-specific mutation rate (for a detailed weighting scheme, see Supporting Information Table 1).

The significant test of independent proportions was used to assess asymmetry in the male and female contribution of North African and sub-Saharan African lineages in each archipelago and mainland Portuguese and Spanish populations (Zar, 1984). As the male and female lineages chosen to estimate the sub-Saharan African input practically represent the whole sub-Saharan African pool (Plaza et al., 2004; Rosa et al., 2004, 2007; Gonzalez et al., 2006), the normalized female:male lineage ratio used was 50%:50%. However, as the North African-specific mtDNA lineages (U6 + M1) only represent 24% of the female lineages in the region (Rando et al., 1998; Maca-Meyer et al., 2003; Olivieri et al., 2006; Gonzalez et al., 2007), whereas the authoctonous male lineage (M81) reaches a mean frequency of 56% (Bosch et al., 2001; Arredi et al., 2004; Alonso et al., 2005) the normalized female:male lineage ratio used was 30%:70%.

RESULTS AND DISCUSSION

Interarchipelago and mainland relationships

At archipelago level, the number of different haplotypes found in the Azores (59) and the Canary Islands (178) are 49.2% and 35.4%, respectively, of the total sample analyzed in this work, and 46.6% and 62.9% of these were not detected in previous screenings (Rando et al., 1999; Brehm et al., 2003; Santos et al., 2003). However, at island level, the mean number of different haplotypes was 62.5% ± 4.3% in the Azores and 55.2% ± 2.6% in the Canary Islands. On the other hand, the mean number of haplotypes not previously detected per island was 72.0% ± 14.5% in the Azores and 69.5% ± 3.3% in the Canary Islands. Interestingly, the two new haplotypes found in the small island of Flores had already been detected in previous sampling. The haplotypes of the newly analyzed samples are listed in Supporting Information Table 2. Detailed island-by-island haplotype and haplogroup frequencies for all the samples used in the present analysis are listed in Supporting Information Tables 3 and 4, respectively.

Grouped frequencies of mtDNA haplogroups for the four archipelagos and mainland Portugal and Spain are shown in Table 2. Haplogroup-based AMOVA analysis (Table 3) showed significant structure among groups and among populations within groups, with the percentage of variation among groups (5.6%) being greater than within groups (2.3%). However, when haplotypic frequencies were used (Table 3), the percentage of variation within groups (1.4%) resulted greater than among groups (0.8%). These results show that as well as haplogroup frequency differences, there are significant differences

TABLE 2. Percentage of each haplogroup in the four Macaronesia archipelagos and in Mainland Portugal and Spain

Haplogroup	CAN (N = 803)	AZO (N = 292)	MAD_T (N = 155)	CV (N = 292)	MPT (N = 540)	MSP (N = 528)
H/HV/U/R*	36.2	37.0	38.7	0.3	47.4	56.3
HV0	3.1	11.6	7.1	0.7	4.6	5.7
R*	0.1	0.7	–	0.7	0.7	0.6
K	4.5	6.2	6.5	–	5.9	5.3
U2e	1.4	0.7	4.5	–	0.9	1.9
U3	0.5	0.7	0.6	–	1.3	0.2
U4	0.4	0.3	1.9	–	1.7	1.1
U5*	1.5	3.4	1.3	–	1.9	3.0
U5a	2.1	2.4	2.6	–	2.0	2.5
U5b1	2.1	2.4	3.9	0.3	2.0	1.1
U6a	0.7	1.0	2.6	0.7	0.7	0.4
U6a1/a2/a3	0.6	0.3	0.6	2.4	0.7	0.2
U6b	0.4	1.7	0.6	–	0.6	1.1
U6b1	12.0	–	–	–	–	0.2
U6c1	2.2	–	–	–	–	–
J*	5.9	4.8	1.3	–	3.9	4.2
J1	0.7	1.7	0.6	–	1.3	0.6
J2b	0.4	1.7	–	–	1.3	1.7
J2a1	0.5	1.4	0.6	–	–	1.3
T*	1.2	–	–	–	2.6	1.5
T1a	3.2	2.1	4.5	–	3.3	1.5
T2b	4.0	4.8	2.6	0.3	3.9	2.3
T2c	4.4	3.4	0.6	–	0.4	0.6
N1/I	1.0	2.7	0.6	1.4	1.7	0.6
W	1.1	2.4	2.6	–	2.2	2.5
X	1.5	1.4	0.6	1.0	2.0	0.9
A/C	0.5	–	–	–	–	–
M1	0.5	2.4	1.9	–	0.6	0.6
L3*	0.2	–	2.6	2.7	0.9	0.6
L3b	1.4	–	–	10.6	0.4	0.6
L3d	0.9	–	0.6	7.5	–	–
L3e	0.4	0.7	2.6	15.1	1.1	0.2
L2/L2c	0.2	0.3	–	13.0	–	–
L2a	2.7	0.3	1.9	20.5	2.2	0.2
L2b/c/d/e	0.2	–	0.6	7.2	0.4	0.2
L1b	1.0	0.7	4.5	7.9	1.1	0.6
L1c	0.1	0.7	–	6.8	0.2	–
L0	–	–	–	0.7	–	–

TABLE 3. Analysis of molecular variance (AMOVA) results based on haplogroups and haplotypes

Source of variation	Percentage of variation	
	Haplogroups	Haplotypes
Among groups	5.6**	0.8*
Among populations within groups	2.3***	1.4***
Within populations	92.1***	97.8***

Groups represent archipelagos and populations represent islands.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.0001$.

among islands within archipelagos as regards their haplotypic composition within haplogroups. Detailed haplogroup- and haplotype-based pairwise genetic distances (Supporting Information Table 5) show the Cape Verde islands as the most differentiated archipelago, with highly significant differences from the others. Furthermore, two islands in the Azores (Flores and Corvo) and one in the Canary Islands (La Gomera) also showed major discrepancies in all comparisons. By contrast, the rest of the intra- and interarchipelago and mainland comparisons were of moderate significance or not significant at all. These results are displayed graphically in Figure 2. In both haplogroup and haplotypic-based bidimensional plots, the two samples from Cape Verde (CV_SE and CV_NW), the two westernmost and smallest islands in the Azores (Flores and Corvo), and the Canary island of La Gomera are the main outliers in a rather homogenous group that includes the other islands and the two mainland samples. The difference on the Cape Verde islands can be explained by the fact that around 96% of the mtDNAs are from African lineages, while the non-African lineages are only about 4%. However, in the other three archipelagos (the Azores, Madeira, and the Canary Islands), most mtDNA lineages belong to west Eurasian haplogroups. Founder effects and a strong genetic drift seem to be the main causes explaining the eccentric position of Flores and Corvo. However, some specific gene flow between these two islands can be deduced, as they share the lowest haplogroup H frequencies ($P = 0.03$) and the highest haplogroup HV0 ($P < 0.0001$) and T2c frequencies ($P = 0.027$) compared to other Azores islands. The high differentiation of La Gomera is mainly due to it having the highest frequency of the Canarian autochthonous U6b1 subgroup (Rando et al., 1999) in all the Canary Islands ($P < 0.0001$). The key roles played by founder effects and genetic drift in the genetic differentiation of these islands are further confirmed, because they show the lowest haplogroup and haplotype diversity values of their respective archipelagos (Table 1 and Supporting Information Tables 3 and 4). In spite of their central position in MDS, there is a distinction between the two mainland populations and

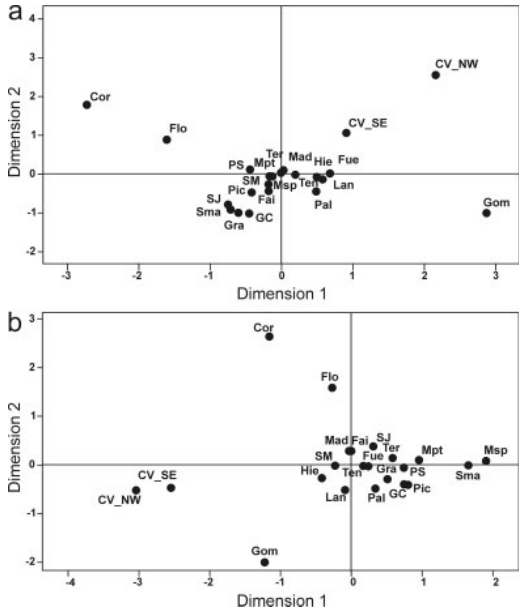


Fig. 2. Multidimensional scaling representation of the Slatkin's linearized F_{ST} pairwise genetic distance matrices between populations (Reynolds et al., 1983) based on haplotype (**a**) and haplogroup (**b**) variation.

all the archipelagos because of their significantly higher frequencies for haplogroup H lineages ($P < 0.0001$, even excluding the Cape Verde islands), lower frequencies of lineages of sub-Saharan African origin (L0, L1, L2, and L3) even when Cape Verde is excluded ($P = 0.008$) and lower frequencies of North African lineages (U6 and M1) even when the Canary Islands are excluded ($P = 0.017$). In addition to the Cape Verde islands, there are also striking peculiarities among the archipelagos. In the Canary Islands, the most notable feature is their autochthonous subhaplogroups U6b1 and U6c1, which appear to represent signs of the indigenous population living in the islands before colonization, and is only detected in this archipelago. No signs of integration of Guanche typical lineages in the other archipelagos can be deduced. Madeira presents the highest frequencies for the North African subclades U6a (Maca-Meyer et al., 2003) and M1 (Gonzalez et al., 2007) ($P = 0.010$) and for sub-Saharan African lineages, ($P = 0.001$, excluding the Cape Verde islands) compared to the other archipelagos. The existence of typical North and sub-Saharan African lineages in Azores, Madeira, and Canary Islands, according to historical records, has been related to the slave trade and could represent signs of the survival of slave genes until the present day. The interarchipelago differences in the amount of African lineages may be due to various factors. The first, and simplest, could be that the number of slaves introduced in each archipelago was different. However, this is not entirely clear, because there is no accurate estimation of the number and sex of slaves introduced in the islands. There is only the idea, supported by the economy base system, that there were more slaves on Madeira than in the Canary Islands and the Azores, because sugar cane production was a key factor in the Madeira Island economy until the 17th century, and it would require a large number of slaves to work in the plantations and production (Vieira, 2001). However, the first slaves brought to Madeira were Guanches, and no genetic signs (based on mtDNA) of this group were found in the present-day population. Furthermore, the presence only in the Canary Islands of Amerindian lineages A2 and C1 (Perego et al., 2009) and in Cape Verde of a south-east Asian B4a1a1a lineage ascription (Tanaka et al., 2004) highlights the importance of these archipelagos in the colonial expansion of Portuguese and Spanish empires. In overall terms, significant differences in the mtDNA haplogroup composition of the four Macaronesia archipelagos were detected (exact test of population differentiation: for all comparison pairs $P < 0.00005$).

TABLE 4. Percentage of shared haplotypes between archipelagos and mainland populations

	Azores (%)	Madeira (%)	Canary (%)	Cape Verde (%)	Mainland Portugal and Spain (%)
Azores	42.15	24.14	17.50	5.88	13.95
Madeira	17.36	40.23	13.33	6.72	8.75
Canary	34.71	36.78	55.83	10.08	20.57
Cape Verde	5.79	9.20	5.00	81.51	2.36
Mainland Portugal and Spain	48.76	42.53	36.25	8.40	69.98

Diagonal values represent percentage of non-shared haplotypes.

As regards HVRI haplotypes, the Canary Islands present the lowest percentage of different haplotypes, followed by the Cape Verde islands and the Azores (Table 1). Analysis of shared haplotypes shows that all the archipelagos present a low percentage of haplotypes shared with the other archipelagos (Table 4). Moreover, this idea is reinforced taking into account the percentage of shared haplotypes between the Azores and the Iberian peninsula (Table 4), which is the main parental population, and it can be deduced that there was a reduced transfer of lineages between archipelagos. To clarify this issue, we created Median Joining Networks (Bandelt et al., 1999) for haplogroups that would highlight the contact between archipelagos as U6* and L* haplogroups.

The U6* haplogroup Network is shown in Figure 3. As can be seen, there are few terminal nodes shared between archipelagos. Shared haplotypes are mostly founder mtDNA motifs, and it is impossible to ascertain if they are shared as a consequence of the contact between archipelagos, or if they were introduced independently from the parental populations in each archipelago. Detailed consideration of the U6b1 and U6c1 clades, typical of the autochthonous population of the Canary Islands, shows that they are only present in this archipelago. These results lead us to hypothesize that genetic variation in Azores, Madeira, and Canary Islands was mainly determined by the initial peopling process and that subsequent contacts between archipelagos have a reduced impact on their genetic composition.

As regards macrohaplogroup L* (see Median Joining Networks for L1* and L3* haplogroups in Figures 4 and 5, respectively), it seems that a significant propor-

tion of the L sequences found in the Canary Islands (40%), Madeira (45%), and the Azores (25%) exactly match those found in the Cape Verde islands, which is a comparatively high percentage when compared with the total number of intra-archipelago matches (Table 4). This is consistent with historical records that place the Cape Verde islands in a central position in the slave trade (Ferreira, 2001; Vieira, 2001; Matos, 2005). However, when a more-detailed haplotypic match distribution of sub-Saharan African lineages was carried out on each archipelago (Table 5), it was evident that future extensive sampling is necessary to have a more complete mtDNA landscape of the African continent as only 57% of the Cape Verde L haplotypes were already sampled in mainland Africa (Supporting Information 6). Percentages of L haplotypes that are not shared with Cape Verde or subsequently with mainland Africa in the other archipelagos are also important. Moreover, the fact that the number of matches with Cape Verde and with mainland Africa in Madeira and the Canary Islands are roughly similar points to that, aside of Cape Verde, other direct continental connections brought slaves to these archipelagos. Finally, it should be mentioned that some interinsular interchange seems evident as minor percentages of L matches were found exclusively between archipelagos (Table 5).

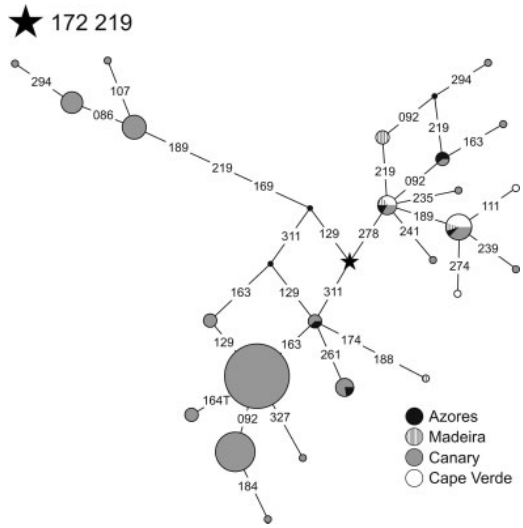


Fig. 3. Median Joining Network for U6 lineages found in the Macaronesia archipelagos.

Interisland relationships

As mentioned earlier, the settlement process of the Cape Verde islands had major differences compared to the other Macaronesia archipelagos. Moreover, as regards mtDNA variation, the archipelago was described as a typical Western African population (Brehm et al., 2002), having only minor differences between the two groups of islands. However, although all the sub-Saharan African lineages that arrived on the Cape Verde islands were of west-central African origin (Salas et al., 2002), haplogroups L3b and L2b/c/d/e are predominant in the CV_SE group of islands, and haplogroups L1c3b1 and L3e4 are in the majority in the CV_NW group. In addition, gene diversity values are significantly higher in CV_SE (Supporting Information Tables 3 and 4), suggesting more important founder effects and genetic drift for the northwestern group of islands. On the other hand, for the other three Macaronesian archipelagos, island-by-island analysis showed further differences, which were most probably the result of the settlement

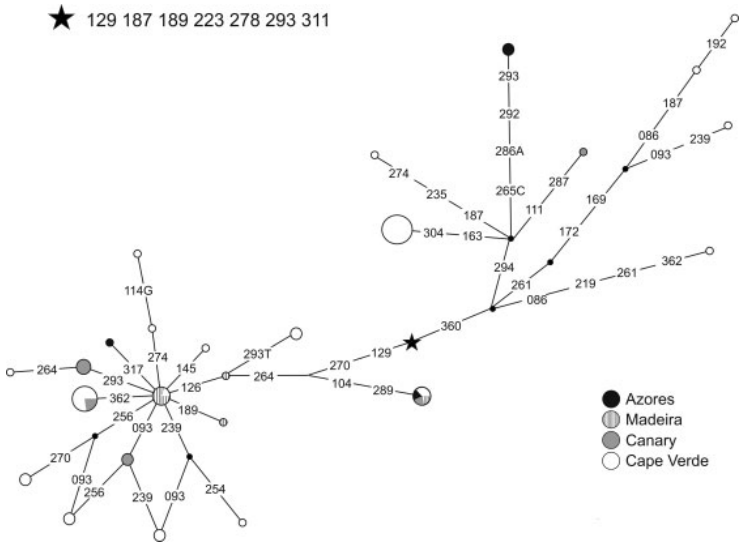


Fig. 4. Median Joining Network for L1* lineages found in the Macaronesia archipelagos.

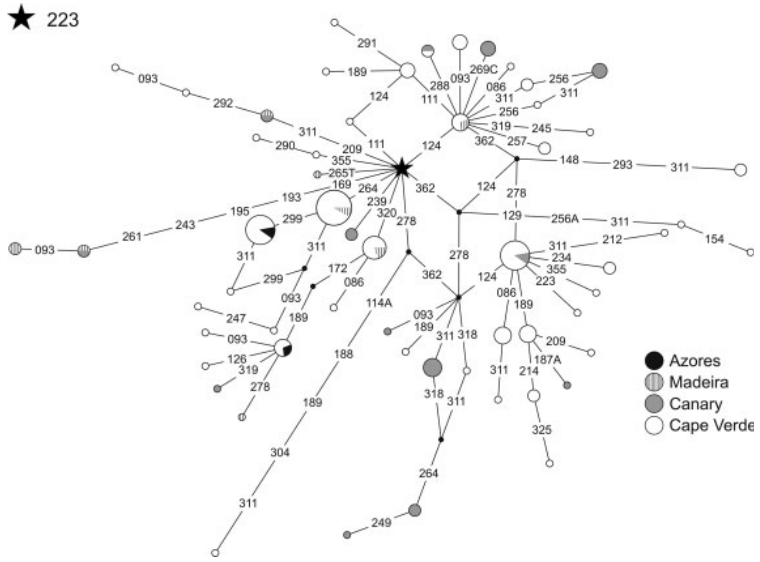


Fig. 5. Median Joining Network for L3* lineages found in the Macaronesia archipelagos.

TABLE 5. Hierarchical match distribution (%) of sub-Saharan African lineages in the different archipelagos

Archipelago	Cape Verde	Mainland Africa	Other archipelagos	Not shared
Azores	3 (42.9)	0	0	4 (57.1)
Madeira	6 (37.5)	5 (31.3)	1 (6.2)	4 (25.0)
Canary	8 (29.6)	7 (26.0)	2 (7.4)	10 (37.0)
Cape Verde	–	58 (56.9)	1 (1.0)	43 (42.1)

process as well as commercial contact between islands. In the Azores, in addition to Flores and Corvo, all the islands present their own peculiar features in lineage frequencies and, in some cases, particular relationships between islands can be deduced. São Miguel thus shows the highest frequencies for haplogroups U3, U5b1, U6a, and J*, sharing a high frequency of J* with Terceira, and U3 and U5b1 frequencies with Porto Santo island in the Madeira archipelago. Pico and Graciosa show particular affinities, because both lack of HV0 representatives and present the highest frequencies for the *K* (P = 0.0002) and U5* (P = 0.003) haplogroups. Curiously, Porto Santo again shares a high frequency in haplogroup *K* with these islands. The frequencies of J2b and L3e are of interest in Pico. Faial presents frequency peaks for J1, I, and L1c, Sao Jorge for W and M1, and Santa Maria for U5a and U6b. In addition to La Gomera, in the Canary Islands, the affinities found on the easternmost islands of Lanzarote and Fuerteventura give clues about possible secondary prehispanic colonization waves from North Africa, mainly affecting these islands, which are the nearest to continental Africa. Unlike the homogenous distribution of the autochthonous U6b1 lineage, they share the highest frequencies for the U6c1 lineage (also autochthonous) and for the T2c subgroup in the archipelago. Interestingly, mainly due to their wide distribution throughout the archipelago, several T2c lineages were considered founder types of prehispanic ascendance (Rando et al., 1999). Indeed, these lineages were later directly detected in Guanche remains, confirming their presence in the aboriginal genetic pool (Maca-Meyer et al., 2004). Although this asymmetrical distribution of haplogroups U6c1 and T2c, contrasting with the uniformity of U6b1, could be attributed to isolation and drift effects, the fact that they resemble similar asymmetrical distributions found for Y chromosome haplogroups (Flores et al., 2003) reinforces the two waves colonization hypothesis.

Finally, gene diversity values (Table 1 and Supporting Information Tables 3 and 4) are quite similar between archipelagos, with the lowest value observed in the Azores. However, when an island-by-island analysis is performed, it is possible to identify islands with very low-diversity values, particularly in the Azores Archipelago. These low-diversity values may partly be related to founder effect events as well as to the small population size of some of the islands. In fact, a significant relationship (R = 0.626; P = 0.001) between diversity and population size was observed (see Fig. 6), and with the exception of the islands of Corvo, La Gomera, and Gran Canaria (which present low diversity values taking into account their population size), the diversity values cannot be considered low if the population size is taken into account.

Asymmetry in the male and female contribution of North African and sub-Saharan African lineages

The significant predominance of aboriginal matrilineages compared to aboriginal patrilineages in the modern Canarian population has been explained by the asymmetrical migration of the Iberian conquerors, which

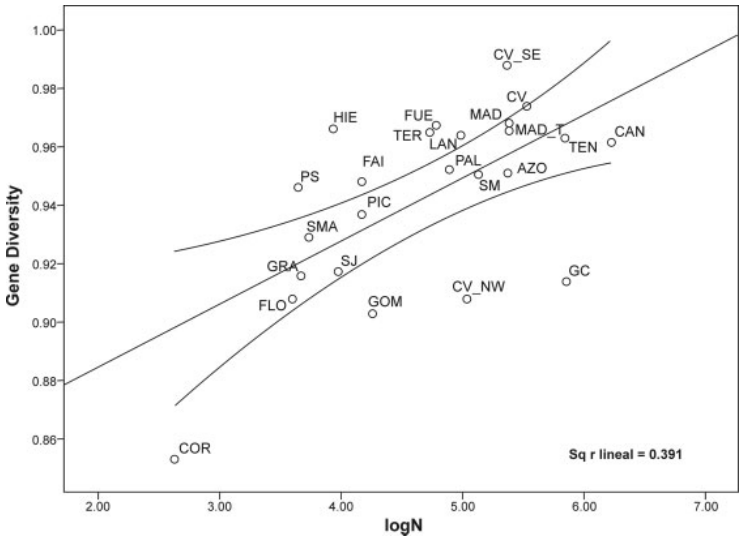


Fig. 6. Scatterplot representing the relation between gene diversity (Nei 1987) and the logarithm of population size.

TABLE 6. Maternal and paternal contributions to the Macaronesia archipelagos and to the Iberian Peninsula of sub-Saharan and North African monoparental lineages

Geographic region and marker		Azores	Madeira	Canary Islands	Cape Verde	Mainland Portugal	Mainland Spain
North Africa	mtDNA	5.5%	5.8%	16.4%	3.1%	2.6%	2.5%
	Y chromosome	5.0%	5.4%	8.3%	3.0%	5.6%	4.6%
	Probability	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	n.s.	$P < 0.0001$
Sub-Saharan Africa	mtDNA	2.7%	12.9%	7.2%	92.1%	6.3%	2.3%
	Y chromosome	1.6%	3.9%	3.9%	43.8%	3.9%	2.0%
	Probability	$P = 0.730$	$P = 0.007$	$P = 0.006$	$P < 0.0001$	$P = 0.032$	$P = 0.708$
Sample size	mtDNA	292	155	803	292	540	528
	Y chromosome	440	129	652	201	1,098	1,694

Statistical significance was determined by significance tests of independent proportions.

consisted mainly of males that displaced indigenous males, but which mixed with indigenous females (Flores et al., 2001). The existence of different sex ratios in the slave groups imported to the Macaronesia archipelagos or sex differences to obtain better social status has also been suggested elsewhere to justify the high percentage of Y-chromosome African lineages in the Azores (Neto et al., 2007). To assess the relative importance of the male and female contribution of sub-Saharan and North African lineages in each archipelago and in mainland populations, we compared the female input, as measured by mtDNA lineages with clear geographical assignation analyzed in this study, with the male input also obtained using geographically assigned Y-chromosome lineages, taken from previous studies carried out in the Cape Verde islands (Goncalves et al., 2003), the Canary Islands (Flores et al., 2003), the Azores (Goncalves et al., 2005; Neto et al., 2007), Madeira (Goncalves et al., 2005), and in mainland Spain (Flores et al., 2004; Alonso et al., 2005; Adams et al., 2008) and Portugal (Goncalves et al., 2005; Beleza et al., 2006; Adams et al., 2008). To that end, we considered all L mtDNA lineages as being of sub-Saharan African origin and compared them with Y-chromosome lineages belonging to haplogroups A, B, E-M96, E1-P2, E1-M2, and E1-M35 of sub-Saharan African origin. Likewise, mtDNA lineages U6 and M1, of North African origin, were compared to Y-chromosome E1-M81 lineages, which were also of unequivocal North African origin. The summarized results are presented in Table 6. Compared to male lineages, female sub-Saharan African lineages have been significantly favored in the Madeira, Canary Islands, and Cape Verde archipelagos and in mainland Portugal. It seems that regardless of the sex-ratio introduced by slavery, overtime sub-Saharan African matrilineages had fewer problems in contributing to the modern genetic pool of these populations than sub-Saharan African patrilineages. Likewise, the North African female contribution exceeds the male input in all the archipelagos with a high significance. For the Canary Islands, this sex bias has already been explained by the different behavior of the Iberian conquerors depending on the sex of the Canarian aborigines (Flores et al., 2001). In addition, female-mating preference to obtain higher social status could be the reason behind this gender bias in all the archipelagos. By contrast, in mainland Iberia, North African matrilineages are not significantly favored in Portugal, and their increase in Spain is less significant than in all the archi-

pelagos. This could be explained by the fact that a significant portion of male lineages reached the Iberian Peninsula as a consequence of its historic Islamic occupation, which, at least initially, as a military undertaking, brought mainly North African patrilineages to the Iberian Peninsula.

In conclusion, the four Macaronesia archipelagos today appear to have different genetic profiles, and the Azores have the highest level of intra-archipelago differentiation, which is also associated with the lowest values of diversity.

LITERATURE CITED

Adams SM, Bosch E, Balaresque PL, Ballereau SJ, Lee AC, Arroyo E, Lopez-Parra AM, Aler M, Grifo MS, Brion M, Carracedo A, Lavinha J, Martinez-Jarreta B, Quintana-Murci L, Picornell A, Ramon M, Skorecki K, Behar DM, Calafell F, Jobling MA. 2008. The genetic legacy of religious diversity and intolerance: paternal lineages of Christians, Jews, and Muslims in the Iberian Peninsula. Am J Hum Genet 83:725–736.

Alonso S, Flores C, Cabrera V, Alonso A, Martin P, Albarran C, Izagirre N, de la Rua C, Garcia O. 2005. The place of the Basques in the European Y-chromosome diversity landscape. Eur J Hum Genet 13:1293–1302.

Arredi B, Poloni ES, Paracchini S, Zerjal T, Fathallah DM, Makrelouf M, Pascali VL, Novelletto A, Tyler-Smith C. 2004. A predominantly neolithic origin for Y-chromosomal DNA variation in North Africa. Am J Hum Genet 75:338–345.

Bandelt HJ, Forster P, Rohl A. 1999. Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48.

Beleza S, Gusmao L, Lopes A, Alves C, Gomes I, Giouzeli M, Calafell F, Carracedo A, Amorim A. 2006. Micro-phylogeographic and demographic history of Portuguese male lineages. Ann Hum Genet 70:181–194.

Bertranpetit J, Sala J, Calafell F, Underhill PA, Moral P, Comas D. 1995. Human mitochondrial DNA variation and the origin of Basques. Ann Hum Genet 59 (Pt 1):63–81.

Bosch E, Calafell F, Comas D, Oefner PJ, Underhill PA, Bertranpetit J. 2001. High-resolution analysis of human Y-chromosome variation shows a sharp discontinuity and limited gene flow between northwestern Africa and the Iberian Peninsula. Am J Hum Genet 68:1019–1029.

Brandão F. 1995. História da Expansão Portuguesa 1367–1580 uma cronologia. Odivelas, Portugal: Europress. 175 p.

Brehm A, Pereira L, Bandelt HJ, Prata MJ, Amorim A. 2002. Mitochondrial portrait of the Cabo Verde archipelago: the Senegambian outpost of Atlantic slave trade. Ann Hum Genet 66 (Pt 1):49–60.

Brehm A, Pereira L, Kivisild T, Amorim A. 2003. Mitochondrial portraits of the Madeira and Acores archipelagos witness different genetic pools of its settlers. Hum Genet 114:77–86.

Côrte-Real HB, Macaulay VA, Richards MB, Hariti G, Issad MS, Cambon-Thomsen A, Papiha S, Bertranpetit J, Sykes BC. 1996. Genetic diversity in the Iberian Peninsula determined from mitochondrial sequence analysis. Ann Hum Genet 60 (Pt 4):331–350.

Excoffier L, Laval G, Schneider S. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Ferreira LE. 2001. A Diáspora Cabo-Verdiana. In: CEHA, editor. Imigração e emigração nas ilhas. Funchal, Portugal: Centro de Estudos de História do Atlântico, Secretaria Regional do Turismo e Cultura. p 235–247.

Flores C, Larruga JM, Gonzalez AM, Hernandez M, Pinto FM, Cabrera VM. 2001. The origin of the Canary Island aborigines and their contribution to the modern population: a molecular genetics perspective. Curr Anthropol 42:749–755.

Flores C, Maca-Meyer N, Gonzalez AM, Oefner PJ, Shen P, Perez JA, Rojas A, Larruga JM, Underhill PA. 2004. Reduced genetic structure of the Iberian peninsula revealed by Y-chromosome analysis: implications for population demography. Eur J Hum Genet 12:855–863.

Flores C, Maca-Meyer N, Perez JA, Gonzalez AM, Larruga JM, Cabrera VM. 2003. A predominant European ancestry of paternal lineages from Canary Islanders. Ann Hum Genet 67:138–152.

Fructuoso G. 2004. Descripción de las Islas Canarias: Capítulos IX al XX del libro I de saudades da terra. Zamudio, Spain: G. Z. Printek, S. A. L.

Goncalves R, Freitas A, Branco M, Rosa A, Fernandes AT, Zhivotovsky LA, Underhill PA, Kivisild T, Brehm A. 2005. Y-chromosome lineages from Portugal. Madeira and Acores record elements of sephardim and berber ancestry. Ann Hum Genet 69:443–454.

Goncalves R, Rosa A, Freitas A, Fernandes A, Kivisild T, Villems R, Brehm A. 2003. Y-chromosome lineages in Cabo Verde Islands witness the diverse geographic origin of its first male settlers. Hum Genet 113:467–472.

Gonzalez AM, Brehm A, Perez JA, Maca-Meyer N, Flores C, Cabrera VM. 2003. Mitochondrial DNA affinities at the Atlantic fringe of Europe. Am J Phys Anthropol 120:391–404.

Gonzalez AM, Cabrera VM, Larruga JM, Tounkara A, Noumsi G, Thomas BN, Moulds JM. 2006. Mitochondrial DNA variation in Mauritania and Mali and their genetic relationship to other western Africa populations. Ann Hum Genet 70:631–657.

Gonzalez AM, Larruga JM, Abu-Amero KK, Shi Y, Pestano J, Cabrera VM. 2007. Mitochondrial lineage M1 traces an early human backflow to Africa. BMC Genom 8:223.

Gregório R. In press. Escravos e libertos da ilha Terceira na primeira metade do século XVI. Livro de Homenagem ao Prof. Doutor Artur Teodoro de Matos. Lisboa: Centro de História de Além-Mar. Faculdade de Ciências Sociais e Humanas da Universidade Nova de Lisboa.

INE. 2003a. Recenseamento Geral da População e Habitação, 2001 (Resultados Definitivos). Portugal: Instituto Nacional de Estatística.

INE. 2003b. Repartição percentual da população residente segundo concelho e meio de residência ano 2000. Cabo Verde: Instituto Nacional de estatística de Cabo Verde.

INE. 2004. Censos de Población y Viviendas 2001. Resultados definitivos. España: Instituto Nacional de Estadistica.

Larruga JM, Diez F, Pinto FM, Flores C, Gonzalez AM. 2001. Mitochondrial DNA characterisation of European isolates: the Maragatos from Spain. Eur J Hum Genet 9:708–716.

Maca-Meyer N, Arnay M, Rando JC, Flores C, Gonzalez AM, Cabrera VM, Larruga JM. 2004. Ancient mtDNA analysis and the origin of the Guanches. Eur J Hum Genet 12:155–162.

Maca-Meyer N, Gonzalez AM, Pestano J, Flores C, Larruga JM, Cabrera VM. 2003. Mitochondrial DNA transit between West Asia and North Africa inferred from U6 phylogeography. BMC Genet 4:15.

Matos AT. 2005. Nova história da expansão Portuguesa, Vol. III: Tomo 1. Lisboa, Portugal: Editorial Estampa, Lda.

Mendonça L. 1996. História dos Açores—Visão geral (sécs. XV-XIX). PontaDelgada, Azores: Centro de Apoio Tecnológico à Educação.

Mesquita H. 2005. Escravos em Angra no século XVII (uma abordagem a partir dos registos paroquiais) Arquipélago, História, II série vol. IX. 209–230.

Nei M. 1987. Molecular evolutionary genetics. New York: Columbia University Press.

Neto D, Montiel R, Bettencourt C, Santos C, Prata MJ, Lima M. 2007. The African contribution to the present-day population of the Azores Islands (Portugal): analysis of the Y chromosome haplogroup E. Am J Hum Biol 19:854–860.

Olivieri A, Achilli A, Pala M, Battaglia V, Fornarino S, Al-Zahery N, Scozzari R, Cruciani F, Behar DM, Dugoujon JM,

Coudray C, Santachiara-Benerecetti AS, Semino O, Bandelt HJ, Torroni A. 2006. The mtDNA legacy of the Levantine early Upper Palaeolithic in Africa. Science 314:1767–1770.

Perego UA, Achilli A, Angerhofer N, Accetturo M, Pala M, Olivieri A, Kashani BH, Ritchie KH, Scozzari R, Kong QP, Myres NM, Salas A, Semino O, Bandelt HJ, Woodward SR, Torroni A. 2009. Distinctive Paleo-Indian migration routes from Beringia marked by two rare mtDNA haplogroups. Curr Biol 19:1–8.

Pereira L, Prata MJ, Amorim A. 2000. Diversity of mtDNA lineages in Portugal: not a genetic edge of European variation. Ann Hum Genet 64 (Pt 6):491–506.

Pinto F, Gonzalez AM, Hernandez M, Larruga JM, Cabrera VM. 1996. Genetic relationship between the Canary Islanders and their African and Spanish ancestors inferred from mitochondrial DNA sequences. Ann Hum Genet 60 (Pt 4):321–330.

Plaza S, Salas A, Calafell F, Corte-Real F, Bertranpetit J, Carracedo A, Comas D. 2004. Insights into the western Bantu dispersal: mtDNA lineage analysis in Angola. Hum Genet 115:439–447.

Rando JC, Cabrera VM, Larruga JM, Hernandez M, Gonzalez AM, Pinto F, Bandelt HJ. 1999. Phylogeographic patterns of mtDNA reflecting the colonization of the Canary Islands. Ann Hum Genet 63:413–428.

Rando JC, Pinto F, Gonzalez AM, Hernandez M, Larruga JM, Cabrera VM, Bandelt HJ. 1998. Mitochondrial DNA analysis of Northwest African populations reveals genetic exchanges with European Near-Eastern, and sub-Saharan populations. Ann Hum Genet 62:531–550.

Reynolds J, Weir BS, Cockerham CC. 1983. Estimation of the co-ancestry coefficient—basis for a short-term genetic-distance. Genetics 105:767–779.

Rosa A, Brehm A, Kivisild T, Metspalu E, Villems R. 2004. MtDNA profile of West Africa Guineans: towards a better understanding of the Senegambia region. Ann Hum Genet 68:340–352.

Rosa A, Ornelas C, Jobling MA, Brehm A, Villems R. 2007. Y-chromosomal diversity in the population of Guinea-Bissau: a multiethnic perspective. BMC Evol Biol 7:124.

Salas A, Comas D, Lareu MV, Bertranpetit J, Carracedo A. 1998. mtDNA analysis of the Galician population: a genetic edge of European variation. Eur J Hum Genet 6:365–375.

Salas A, Richards M, De la Fe T, Lareu MV, Sobrino B, Sanchez-Diz P, Macaulay V, Carracedo A. 2002. The making of the African mtDNA landscape. Am J Hum Genet 71:1082–1111.

Santos C, Lima M, Montiel R, Angles N, Pires L, Abade A, Aluja MP. 2003. Genetic structure and origin of peopling in the Azores islands (Portugal): the view from mtDNA. Ann Hum Genet 67:433–456.

Santos C, Montiel R, Bettencourt C, Prata MJ, Abade A, Aluja MP, Lima M. 2006. Peopling, demographic history and genetic structure of the Azores Islands: integrating data from mtDNA and Y-chromosome. In: Amorim A, Corte-Real F, Morling N, editors. Progress in forensic genetics II—International Congress Series 1288. Amsterdam, The Netherlands: Elsevier. p 85–87.

Santos C, Sierra B, Alvarez L, Ramos A, Fernandez E, Nogues R, Aluja MP. 2008. Frequency and pattern of heteroplasmy in the control region of human mitochondrial DNA. J Mol Evol 67:191–200.

Slatkin M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462.

Tanaka M, Cabrera VM, Gonzalez AM, Larruga JM, Takeyasu T, Fuku N, Guo LJ, Hirose R, Fujita Y, Kurata M, Shinoda K, Umetsu K, Yamada Y, Oshida Y, Sato Y, Hattori N, Mizuno Y, Arai Y, Hirose N, Ohta S, Ogawa O, Tanaka Y, Kawamori R, Shamoto-Nagai M, Maruyama W, Shimokata H, Suzuki R, Shimodaira H. 2004. Mitochondrial genome variation in eastern Asia and the peopling of Japan. Genome Res A 14:1832–1850.

Torroni A, Achilli A, Macaulay V, Richards M, Bandelt HJ. 2006. Harvesting the fruit of the human mtDNA tree. Trends Genet 22:339–345.

van Oven M, Kayser M. 2009. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat 30:E386–E394.

Vieira A. 2001. As migrações e os descobrimentos Portugueses—Séculos XV e XVI. In: CEHA, editor. Imigração e emigração nas ilhas. Funchal, Portugal: Centro de Estudos de História do Atlântico, Secretaria Regional do Turismo e Cultura. p 27–62.

Zar JH. 1984. Biostatistical analysis. New Jersey: Prentice Hall.

4.3. La colonización aborigen de la isla de La Palma

Para poder estudiar, desde el punto de vista genético, cómo se produjo el proceso de colonización aborigen de las Islas Canarias, es necesario conocer el nivel de estructuración y la diversidad genética existente en la población indígena de las islas, con el fin de determinar el modelo de colonización que se ajusta más a la variabilidad observada.

En este trabajo hemos estudiado la composición genética para el ADNmt de la población aborigen de La Palma, que es, junto a El Hierro, una de las islas más alejadas del continente africano. De los 38 individuos analizados, se obtuvieron resultados para 30 de ellos, lo que se corresponde con un 78,9% de eficiencia.

La mayoría de linajes observados en la población de La Palma (93%) proceden del oeste euroasiático, mientras que el resto (7%) son de adscripción subsahariana. Por otro lado, una gran parte de los haplotipos aborígenes (70%) presentan parejas exactas en el norte de África. Sin embargo, el haplogrupo autóctono canario U6b1, no ha sido aún detectado en el norte de África. Igualmente, el haplotipo H1 más numeroso en La Palma, definido por la transición 16260, es poco abundante en el norte de África. Estos resultados apuntan a que los ancestros directos de los aborígenes canarios no han sido muestreados todavía o que la zona de la que procedían ha sido modificada por migraciones posteriores.

La alta diversidad encontrada en la población indígena de la isla de La Palma se encuentra en el mismo nivel que la diversidad de los aborígenes de Tenerife (92,4 ± 2,8). Estos resultados no coinciden con una colonización desde el continente siguiendo un modelo de salto escalonado con posterior aislamiento. De igual forma, tampoco se ajustan a una colonización marítima independiente para cada isla sin contactos posteriores, ya que existe una gran similitud entre las poblaciones aborígenes de La Palma y Tenerife. De esta forma, nuestros resultados encajan mejor con un modelo de islas con migración frecuente entre ellas.

Los resultados obtenidos en el estudio de la población aborigen de la isla de La Palma han sido publicados en la revista *European Journal of Human Genetics*, con el título “The maternal aborigine colonization of La Palma (Canary Islands).

European Journal of Human Genetics (2009), 1 – 11

www.nature.com/ejhg

ARTICLE

The maternal aborigine colonization of La Palma (Canary Islands)

Rosa Fregel*[,1], Jose Pestano[2], Matilde Arnay[3], Vicente M Cabrera[1], Jose M Larruga[1] and Ana M González[1]

[1]*Departamento de Genética, Facultad de Biología, Universidad de La Laguna, Tenerife, Spain;* [2]*Laboratorio de Genética, Facultad de Medicina, Universidad de Las Palmas de Gran Canaria,Gran Canaria, Spain;* [3]*Departamento de Prehistoria, Universidad de La Laguna, Tenerife, Spain*

Teeth from 38 aboriginal remains of La Palma (Canary Islands) were analyzed for external and endogenous mitochondrial DNA control region sequences and for diagnostic coding positions. Informative sequences were obtained from 30 individuals (78.9%). The majority of lineages (93%) were from West Eurasian origin, being the rest (7%) from sub-Saharan African ascription. The bulk of the aboriginal haplotypes had exact matches in North Africa (70%). However, the indigenous Canarian sub-type U6b1, also detected in La Palma, has not yet been found in North Africa, the cradle of the U6 expansion. The most abundant H1 clade in La Palma, defined by transition 16260, is also very rare in North Africa. This means that the exact region from which the ancestors of the Canarian aborigines came has not yet been sampled or that they have been replaced by later human migrations. The high gene diversity found in La Palma (95.2 ± 2.3), which is one of the farthest islands from the African continent, is of the same level than the previously found in the central island of Tenerife (92.4 ± 2.8). This is against the supposition that the islands were colonized from the continent by island hopping and posterior isolation. On the other hand, the great similarity found between the aboriginal populations of La Palma and Tenerife is against the idea of an island-by-island independent maritime colonization without secondary contacts. Our data better fit to an island model with frequent migrations between islands.
European Journal of Human Genetics advance online publication, 1 April 2009; doi:10.1038/ejhg.2009.46

Keywords: ancient DNA; mtDNA haplogroups; Canarian aborigines; colonization

Introduction

The Canary Islands are a group of seven Atlantic Islands situated about 60 miles off the northwest African coast. Due to their volcanic origin, they were never a part of the African mainland. However, when the Europeans, along the fourteenth and fifteenth centuries, visited and conquered the archipelago they realized that their inhabitants had a primitive Neolithic culture, no knowledge of seafaring techniques, and very little communication between islands.[1] Archeological, anthropological and linguistic studies suggested that the North African Berbers were the most probable ancestors of the Canarian aborigines. This origin was corroborated, at molecular level, when specific North African markers, as mtDNA haplotypes belonging to the U6 subhaplogroup, Y-chromosome haplotypes belonging to the E-M81 subhaplogroup, or the CD4/Alu 110(−) haplotypes were detected in the current Canarian population, attesting the survival of an important aborigine substrate in it.[2–4] Further evidence came from mtDNA analysis carried out directly on historical and aboriginal remains,[5–6] in which U6 lineages were detected

***Correspondence: Dr R Fregel, Department of Genetics, University of La Laguna, Avda. Astrofísico Fco. Sánchez, La Laguna, Santa Cruz de Tenerife 38271, Spain. Tel: +34 922 318 350; Fax: +34 922 318 311; E-mail: rfregel@ull.es**
Received 14 October 2008; revised 17 February 2009; accepted 20 February 2009

at greater frequencies than in the present day Canarian population. However, as no exact matches of the indigenous U6b1 lineages could be found in North Africa,[5] it was not possible to establish a precise origin for the pre-Hispanic colonizers. On the other hand, radiocarbon dating[7] suggested that, most probably, the archipelago was occupied at the beginning of the first millennium BC. However, it is not clear whether the islands were colonized by one or by several migratory waves. Cultural differences between islands and its evolution within islands could be explained by isolation and indigenous progress. Nevertheless, clear-cut differences in pottery horizons strongly suggested that, at least the eastern islands of Gran Canaria, Fuerteventura and Lanzarote, were secondarily influenced by a more advanced culture.[8] Also, for the western island of La Palma, a Saharan cultural influence, dated around the ninth or tenth centuries, has been proposed.[9] In any case, it is difficult to distinguish demic from simple cultural influences based only on archeological remains. Genetic studies on diversity and frequency distribution of polymorphic markers in the current populations of the seven islands have also given inconclusive results. The correlation patterns found for mtDNA, CD4/Alu haplotypes, and AB0 allele frequencies were congruent with only one dominant settlement process,[2,3,10] whereas the distribution and dating of some Y-chromosome North African lineages, present in the Canary Islands, matched with a pattern of settlement of the archipelago in two stages.[4] If the aboriginal colonizers reached the islands by themselves, using primitive boating skills, they most probably landed first on the nearest eastern islands from the mainland and, under demographic pressure, ventured afterwards to the next western island at sight. Under this restrictive stepping stone model of colonization, without important secondary migration between islands, a diminution in genetic diversity from the eastern to the western islands is expected in such a way that the islands more distant from the African shore would have only a subset of the variation held by the eastern islands. However, if the aborigines were brought to the islands in several waves by other people with seafaring experience,[1] different islands could have been colonized independently, so that their respective genetic diversity would not be correlated with the geographical distance of the islands from the Continent. So, three important issues about the aboriginal colonization of the Canary Islands are still ignored: (a) the precise North African point(s) from which the Canarian ancestors originated, (b) whether the indigenous colonizers reached the islands by themselves or were brought to them by seafaring people, and (c) if there was only one main or several settlements. The study of more aboriginal remains could help to shed light on the first issue if new mtDNA lineages with a precise localization in North Africa were found. Furthermore, as there is an aboriginal sample of the central island of Tenerife previously analyzed for mtDNA,[5] the additional analysis of an indigenous sample from one of the two westernmost islands of the Archipelago, El Hierro or La Palma, would make possible their comparison, at the mtDNA gene diversity and structure levels, to test the alternative models of colonization directly on the prehispanic aboriginal population. Due to demographic differences between these two islands at the time of the conquest, with that of El Hierro heavily punished by slavery raids with only around a hundred survivors compared with La Palma ranging from 1200 to over 4000 inhabitants,[11] the latter seemed more adequate for the above commented comparisons. With these objectives in mind, in this study, an aboriginal sample from La Palma island was chosen to be analyzed for the hypervariable region I (HVR I) of mtDNA and for the diagnostic coding region positions that allowed the unequivocal assignation of their lineages to one of the known mtDNA haplogroups, so that its relationship with other populations from the islands and North Africa could be accurately established.

Materials and methods

Sample collection

Prehistoric remains were obtained from five different archeological sites excavated in La Palma Island (Figure 1). In all cases, teeth were chosen as the preferable ancient skeletal DNA source. A total of 38 different individuals were sampled and, in five cases, two independent analyses from the same individual were possible (Supplementary Material 1). One sample was from Barlovento, 21 from Puntallana (El Espigón), 9 from Santa Cruz de La Palma, 6 from Mazo, and 1 from Los Llanos de Aridane (Supplementary Material 1). In all cases well preserved teeth were chosen. Although this material was not directly C-14 dated, it is known that the most ancient settlements in La Palma are not older than the first millennium AD.[12] Ceramic types co-excavated with teeth in El Espigón site confirmed the relatively recent age of these remains, around 600–1200 AD.

External DNA extraction

To assess whether the teeth were externally contaminated the following protocol was used to extract DNA from their external surfaces: Teeth were repeatedly swabbed using a sterilized swab stick soaked in a guanidinium thiocyanate (GuSCN) washing solution (6 M GuSCN, 0.1 M Tris-HCl, pH 6.4). The swab was introduced in a 1.6 ml tube with 300 μl extracting solution (6 M GuSCN, 0.1 M Tris-HCl, pH 6.4, 0.2 M EDTA, 2% Triton X-100) and the stick broken to close the tube. Then the tube was boiled for 10 min and left to room temperature (RT) for other 10 min. After that, its top and bottom were perforated with a sterile syringe needle and fitted in a new 1.6 ml tube to which the solution was transferred by centrifugation. Fifteen microliters of acid-treated size-fractionated silica particles (Sigma), previously autoclaved, were added and the new tube was kept at RT

Figure 1 Geographical position of La Palma within the Canary Islands and locations of the archeological sites sampled.

with occasional vortex during 10 min. Then the silica particles were pelleted by centrifugation, the supernatant discarded and the pellet washed twice with 1:1 washing solution/ethanol, once with acetone and once with 70% ethanol. Finally, the DNA was eluted from the silica pellet resuspending it in 50 μl TE buffer pH 8, incubating for 10 min at 55°C, and transferring supernatant into a new tube after centrifugation to pellet the silica. Five microliters of this solution was used as template for 25 μl volume PCR reactions.

Internal DNA extraction

For internal extraction, teeth were again thoroughly washed, this time, with 15% HCl, rinsed with UV-treated ddH_2O, and then exposed to UV light for 10 min. To reconstruct the tooth after extraction, it was cut through the midline using a dentist electric saw and the internal pulp and dentin drilled out using a dental drill. The powder was collected in 1.5 ml sterile tubes. Then DNA was extracted according to a modified GuSCN-silica-based protocol.[5]

Spectrometric DNA quantification

Ancient DNA (aDNA) was quantified using an ND-1000 (NanoDrop) spectrophotometer following the manufacturer's instructions.

Real-time PCR quantification

Real-time PCR quantification was carried out to assess the number of molecules used as template for PCR amplification.[13] We used iQ SYBR Green Supermix (BioRad) in a iCycler Thermal Cycler (BioRad). Primers and thermal cycling conditions were as previously specified.[5] Tenfold serial dilutions of a purified and quantified standard were included in the experiment to determine the standard curve, to estimate the initial number of DNA molecules in each sample.

Amplification

For mtDNA HVRI sequencing or RFLP analysis on aborigine samples, primers pairs and PCR conditions used were as previously published.[5,14] To improve the reading of each HVRI fragment sequence, the M13 forward primer (Promega) was added, as a tail, at the 5′ end of all the seven forward primers used. In addition, two new RFLP analyses, that characterise subhaplogroups H1[15] and H3,[16] were performed. The first was typed amplifying a fragment of 50 bp, using the forward primer L3005 5′-CTCGATGTTG GATCAGGACA-3′ and a modified reverse primer H3011 5′-GAACCTTTAATAGCGGCTGCACAAT-3′ that creates a *Tsp509*I site when the derived A is present, giving fragments of 24 and 26 bp. The second was checked amplifying a fragment of 53 bp, using the forward primer L6769 5′-CTAGGGTTTATCGTGTGAGC-3′ and a modified reverse primer H6777* 5′-TCTACGTCTATTCCTACTGTA CATAT-3′ that creates an *Nde*I site when the derived C is present, giving fragments of 25 and 28 bp.

For HVRI amplification of the external DNA extracts, primers HVI (L15840) and HVII (H16436) and conditions as detailed elsewhere[17] were used. In case this long-fragment amplification failed, fragments of approximately half size were amplified using primer pairs HVI/H3R and L3F/HVII.[5] Complete mtDNA amplification, in present day samples, was carried out after all the aDNA analyses were

completed. Primer pairs and conditions were as described earlier.[18–19]

Cloning

Amplification products were cloned into pGEM-T vectors (Promega). Several clones were sequenced for each fragment until an unambiguous consensus sequence was obtained. Additional cloning was carried out in those cases in which only one of the three negative PCR controls showed contamination, and this was of a much lower intensity than the sample amplification. Cloned sequences from the sample were used only when: (a) detected variants were different between the contaminated control and the aboriginal sample, and (b) the mutations observed for that segment were phylogenetically congruent with the haplotype obtained with the rest of the fragments.

Sequencing

Ancient DNA PCR fragments were directly sequenced with the M13 forward primer. Clones were sequenced using the M13 reverse primer (Promega). Amplified fragments from external DNA extracts and from modern samples were sequenced with the same forward primer used in the PCR reaction. When necessary the reverse strand was also sequenced. All the sequencing reactions were prepared with the BigDye v3.1 Terminator Cycle Sequencing kit (Applied Biosystems) using 10 μl volume reactions. At La Laguna, sequencing reactions were run on a MegaBACE 1000 sequencing system (Amersham Pharmacia Biotech). At Las Palmas they were run on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). To confirm the accuracy of the fragments amplified for RFLP diagnosis, half of their PCR reactions were directly sequenced at least in two samples. DNA sequences were analyzed using CLUSTAL X 1.83 and confirmed by inspecting the electropherograms manually.

Criteria of authenticity

Standard contamination prevention and monitoring used at La Laguna and at Las Palmas laboratories were as detailed earlier.[5,14] We tried to follow the eight criteria by Pääbo *et al.*,[20] which include those set forth in Cooper and Poinar.[21]

1. Amplification products have been cloned, multiple clones sequenced, and misincorporated bases detected in several instances.
2. One blank extraction and three negative PCR controls per sample and amplification have been always performed.
3. Consistence of the sequences obtained has been confirmed by repeated amplification and by RFLP analysis that, in all cases, matched the haplogroup assignation based on HVSI sequencing.
4. The initial number of amplifiable DNA molecules has been quantified using real-time PCR.
5. To facilitate the aDNA amplification, we analyzed the mtDNA HVSI region using seven overlapping small fragments. Although in some instances, we were able to obtain double fragments, the total HVSI region could not be amplified in any case.
6. Due to the lack of the appropriate technology, biochemical assays of macromolecular preservation could not be performed.
7. Our overlapping approach would detect any amplification of mtDNA sequences integrated into the nuclear genome.
8. Five duplicated samples were assayed in a second independent laboratory.
9. Although there were no associated faunal remains to be analyzed, we had previously extracted mtDNA from lizard remains, obtained from caves with similar environmental conditions as those for the aboriginal graves.[22] Moreover, it has to be taken into account that the estimated age for these animal remains was older than the oldest date for La Palma aboriginal samples.

With the aim to remove and to detect any previous surface modern DNA contamination, two additional criteria have been followed:

We have previously tested that the decontamination step in our extraction protocol was stringent enough to remove chicken DNA contamination put on the surface of teeth.[5]

In addition, in this study, any modern DNA contamination present on the surface of the teeth was retrieved before the extraction protocol for endogenous DNA was carried out. This approach allowed the subsequent comparison of both DNA sources.

Statistical analysis

Sequences were sorted into haplogroups following the most actualized classification,[23] further updated for J1b.[19] Gene diversity was calculated according to Nei.[24] The published samples, used for comparisons, are detailed in Supplementary Material 2. The relationships between populations were estimated using haplogroup and haplotype frequency-based linearized F_{ST}, computed by the Arlequin 2000 program.[25] Both data sets were used to obtain multidimensional scaling plots by means of the SPSS ver 13.0 package (SPSS Inc., Chicago, Illinois). In addition, the absolute number of migrants exchanged between the two aboriginal populations (M) was also estimated using F_{ST} based on haplotype frequencies (Arlequin 2000 program). To diminish the strong influence of the common haplotypes in F_{ST} based distances, an additional measure of haplotypic identity ($I_{XY} = (HT_{XY}^2/(HT_X\ HT_Y))$ was used, where HT_{XY} is the number of shared haplotypes between populations X and Y, and HT_X and HT_Y are the number of different haplotypes in populations

X and *Y*, respectively. Phylogenetic relationships among HVSI and genomic mtDNA sequences were established using the reduced median network algorithm.[26] Coalescence ages, based on HVSI sequences, were estimated using the ρ-statistic[27] and a calibration of 1 transition per 20 180 years.[28]

Results

External extracts for all the 38 samples available for the molecular analysis were obtained. For 21 teeth, no surface sequences could be retrieved. In three cases, the external extract was contaminated by the researcher in charge of the analysis (VMC). For the remaining 18 ones, the external sequences obtained belonged to the two anthropologists who previously performed physical measures on the teeth. In two cases, after cloning, both anthropologist sequences could be identified on the same tooth (Supplementary Material 1). However, the sequence from the archeologist, who participated in all the excavations and in the conservation of this material, was not retrieved in any case. Real-time PCR quantification gave a 3726 ± 2400 mean number of molecules used as initial template, being 931 molecules the minimal estimation. Endogenous informative mtDNA sequences were obtained from a total of 30 individuals, accounting for an efficiency of 78.9%. It seems that poor DNA content and the presence of inhibitors were the main causes for the lack of amplification in the remaining eight samples, as they gave spectrophotometric lower DNA sample concentration (4.7 ± 0.8 ng/μl) and anomalous 260/280 nm absorbance ratios (3.3 ± 1.6) compared with the successful group, 11.5 ± 0.4 and 1.7 ± 0.4, respectively. Inhibition was also a problem for the real-time PCR quantitative molecular estimation as in several cases, samples had to be diluted to get optimal amplification. Cloning was carried out on 15 samples (Supplementary Material 1) and a mean of five clones were sequenced by sample. They were chosen because they had slight PCR contamination in one of the fragments or because, although no contamination was detected, their haplotypes shared some mutations with some of the researchers involved in the analysis. In all cases, the consensus cloned sequences were the same as the corresponding direct sequences. Moreover, the contaminated fragment sequence could be always distinguished from the endogenous haplotype. In addition, the following mutations (16085, 16175, 16176G, 16222, 16235, 16256, 16287, 16298, 16299, 16318T, 16332, 16343, 16354, 16364, and 16388) appeared individually in some of the clones. Because of its sporadic detection and lack of phylogenetic sense within the consensus sequence obtained, its presence was attributed to postmortem DNA damage. Due to its very little phylogenetic information, fragments 1 and 2 were analyzed only when enough DNA extract remained after the other five fragments and the RFLP analysis were carried out. The five samples replicated in Las Palmas resulted identical to those obtained in La Laguna (Supplementary Material 1). In some cases, aboriginal haplotypes shared one or two positions with some of the researchers involved in the molecular analysis. However, in all cases, the haplogroup ascription of these haplotypes was different. For instance, the aborigine sample PA-17 shares transition 16316 with VMC but, whereas the latter belonged to subgroup H1, the former did not (Table 2). In addition, PA-17 lacked 16129 transition present in the VMC haplotype. Furthermore, no external contamination was obtained from this sample (Supplementary Material 1).

Table 1 compares haplogroup frequencies between aboriginal, historical, and modern samples from the Canary Islands and those from Northwest (Morocco, Mauritania, and Sahara) and North Central Africa (Algeria and Tunisia). In general, haplogroup frequencies in aborigines from La Palma and Tenerife are highly coincident ($F_{ST} = 0.0047$, $P = 0.30$), although some haplogroups present in La Palma, such as T1a, W and X were not detected in Tenerife and haplogroup HV0, and subgroup U6a1/a2/a3, present in Tenerife, were not found in La Palma. This could be attributed to small sample size. Curiously, the U6a1/a2/a3 type was also absent in the modern population of La Palma. By far, haplogroup H was the most abundant in the aborigines from La Palma (56.7%) and Tenerife (47.2%). Although there are no significant differences with the historical sample from Tenerife, nor with the modern population of the Archipelago, there was a significant excess of H lineages in the aboriginal sample when compared with North Africa ($P < 0.005$). Macro-haplogroup L was also present in moderate frequencies in the aboriginal populations of La Palma (6.7%) and Tenerife (11.1%). However, as for H, there was only significant differences ($P = 0.003$) with L frequencies (24.8%) in North Africa.

Haplotypic frequencies and RFLP analyses performed in the aboriginal population of La Palma are presented in Table 2. Twenty different haplotypes (67%) were found in the 30 individuals analyzed giving a gene diversity of 95.2 ± 2.3 that was not significantly different from the Tenerife aboriginal sample (92.4 ± 2.8). Although, a slight level of endogamic isolation among aboriginal groups within La Palma and within Tenerife could be deduced from the high frequency of the 16260 haplotype in the Espigón (La Palma) and the CRS type in CP (Tenerife) (Supplementary Material 3), both aboriginal samples presented diversity values not significantly different from their respective modern populations (Table 2). The majority (14) of the aboriginal haplotypes from La Palma (70 %) had exact matches in North Africa (Table 2). Matches for the six remaining ones were of different ascription. The Canarian U6b1 ancestral haplotype (16163 16172 16219 16311) has only exact matches within the Archipelago. Its

Table 1 Haplogroup frequencies in the aboriginal, historical, and modern samples from the Canary Islands, and from Northwest and North central Africa

Haplogroup	*PAA*	*TFA*	*CON*	*LAN*	*FUE*	*GCA*	*TFE*	*GOM*	*PAL*	*HIE*	*NWA*	*NCA*
H/HV*/U*/R*	56.67	47.21	45.88	30.64	38.11	45.00	39.69	21.74	41.19	25.02	27.51	32.83
R0a											1.76	1.40
HV0		2.78	0.82		2.38	2.50	1.72		1.47		8.10	6.19
U1a											0.61	0.82
U2e							1.72		1.47		0.15	
U3			0.82			1.25	0.57		1.47		0.76	2.45
U4						1.25	0.57				0.15	0.47
U5*				4.08							0.69	0.23
U5a				2.04			0.57				0.23	
U5a1*			1.64		2.38		1.15		1.47		0.76	
U5*/U5b			0.82		9.52				1.47	3.12	0.23	0.23
U5b1			1.64	2.04	2.38		3.45	2.17	1.47		2.67	0.35
U6a			1.64	2.04		1.25				3.12	3.97	1.87
U6a1/a2/a3		2.78				1.25	1.72			3.12	3.74	5.37
U6b											0.53	0.47
U6b1	6.67	11.11	9.02	10.20	9.52	8.75	9.22	47.84	14.71	9.39		
U6c				8.16	7.14		1.15	2.17	2.94	6.25	0.61	0.12
U7												0.35
U8b											0.38	1.17
K	3.33	2.78	0.82	6.12		10.00	5.17			9.39	4.89	3.62
T*											1.07	0.70
T1a	3.33				9.52		4.02	8.70	1.47	3.12	1.38	5.14
T2b			1.64			1.25	5.17		11.76		0.46	1.40
T2c	10.01	13.89	1.64	10.20	11.91	5.00	4.60				1.76	0.35
T2e			0.82			2.50				3.12	0.15	0.23
J	3.33	8.33	7.38	12.24		6.25	4.60	4.35	7.35	6.25	2.83	2.92
J1b											0.08	0.23
J1b1a1							1.72				0.15	
J1c7			0.82				1.15				0.08	0.47
J2a				4.08					1.47	3.12	0.38	0.12
J2b							0.57				1.15	0.23
N1a											0.15	0.35
N1b											0.84	0.93
I			0.82				1.15			6.25	0.15	1.17
W	3.33		2.46		2.38				2.94		0.38	0.35
X	6.67		2.46			3.75	2.30	2.17	4.41		1.53	1.17
A			0.82							3.12		
C			0.82				0.57				0.15	
M1					2.38						3.06	4.21
L3*			3.28								0.76	0.35
L3b			0.82				0.57				2.52	1.64
L3b3			0.82			1.25	2.30				0.99	0.93
L3b4			0.82								0.15	0.35
L3d		2.78	0.82								0.99	0.58
L3d3b								6.52		3.12	0.84	0.12
L3d1a1											0.08	
L3e1												0.47
L3e1a			0.82								0.31	
L3e1b											0.08	
L3e2											0.46	0.70
L3e2b											0.31	0.47
L3e4											0.08	
L3e5				2.04						3.12	1.53	2.45
L3f							0.57				0.38	1.17
L3f1b											0.69	1.52
L3h1b												0.35
L3 × 1												0.12
L3 × 2							0.57					
L2/L2c	3.33	5.56	1.64								1.53	0.12
L2a											0.69	1.17
L2a1			0.82	4.08		5.00	2.87		1.47		2.52	2.34
L2a1a4								2.17			0.08	0.59
L2a1beta3						1.25	0.57				1.60	0.70
L2a1d											0.08	0.58
L2a1m											0.15	
L2b			0.82		2.38						0.53	
L2b1											0.69	0.23
L2c1											0.53	
L2c2											0.08	
L2d1											0.53	0.12
L2e		2.78									0.08	0.35
L1b	3.33		0.82			1.25				6.25	0.69	0.23
L1b1			0.82	2.04		1.25		2.17			5.42	2.22
L1c			0.82								0.23	
L1c1a			0.82									
L1c2			1.64									
L1c3a											0.15	
L1c3b1			0.82								0.53	0.35
L0a1b											0.08	0.35
L0a1a											0.15	1.52
N	30	36	122	49	42	80	174	46	68	32	1309	856

Codes are: CON: Conception Church historical sample, FUE: Fuerteventura, GCA: Gran Canaria, GOM: Gomera, HIE: Hierro, LAN: Lanzarote, NCA: North Central Africa, NWA: North West Africa, PAA: Aborigines from La Palma; PAL: La Palma, TFA: Aborigines from Tenerife, TFE: Tenerife.

Table 2 Haplotypes found in the aboriginal sample of La Palma indicating the number of individuals, the RFLP analyzed and the geographical distribution of the lineages

Halplogroup/ motif/site	*N*	*RFLP*	*TFA*	*CON*	*FUE*	*LAN*	*GCA*	*TFE*	*GOM*	*HIE*	*PAL*	*NWA*	*NCA*	*MED*
H*		7025 *AluI*(−) 3010 *Tsp509I*(−)												
CRS	3		√	√	√	√	√	√	√	√	√	√	√	
290	1			√							√	√		
316	1			√										√
H1		7025 *AluI*(−) 3010 *Tsp509I*(+)												
218	1											√		
260	5		√	√	√	√		√		√	√		√	
292	1		√	√										√
311	1		√	√				√	√		√	√	√	
260 278	1													
H3		6776 *NdeI*(+)												
192 260	1										√			
HV/U/R		7025 *AluI*(+)												
189	1		√	√			√	√				√	√	
J		4216 *NlaIII*(+)												
069 126	1		√	√		√	√	√	√	√	√	√	√	
T1a		4216 *NlaIII*(+)												
126 163 186 189 294	1				√		√					√	√	
T2c		4216 *NlaIII*(+)												
126 292 294	3		√	√	√	√	√	√				√	√	
U		12308 *HinfI*(+)												
CRS	1		√							√		√	√	
U6b1		12308 *HinfI*(+)												
163 172 219 311	2		√	√	√	√	√	√	√	√	√			√
K		12308 *HinfI*(+)												
189 224 311	1		√							√		√		
W														
223 292 295	1			√							√			√
X														
189 223 278	2			√				√	√		√	√	√	
L1b		3592 *HpaI*(+)												
126 187 189 223 264 270 278 311	1			√			√			√		√	√	
L2/L2c		3592 *HpaI*(+)												
223 278 (390)	1		√	√								√	√	
N	30		36	122	42	49	80	174	46	32	68	1309	862	—
Gene diversity	95.2 ± 2.3		92.4 ± 2.8	94.8 ± 1.4	96.2 ± 1.3	96.6 ± 1.3	90.9 ± 2.7	96.5 ± 0.8	86.7 ± 3.9	97.8 ± 1.3	94.4 ± 1.7	98.0 ± 0.2	97.2 ± 0.3	—

Codes are as in Table 1. MED: mediterranean basin.

occasional presence in Spain and South America has been attributed to the historical Canarian emigration.[29,30] Of the two haplotypes, which are one-step derived from the most abundant H type of the aboriginal sample (16260), one (16192 16260) belonging to the H3 subgroup has only been found in the modern population of La Palma. The other one (16260 16278) of H1 ascription has never been detected before because, although the same HVRI motif was found in a Yemeni sample,[31] it belonged to H14 subgroup. Finally, the other 3 (15%) haplotypes have only been found in the islands and in the European side of the Mediterranean basin. The aboriginal 16260 H1 type deserved special attention because, within haplogroup H, this transition is very redundant as it has been independently observed in at least 11 different subgroups as deduced from the whole sequencing of six 16260 lineages and the unequivocal subhaplogroup H assignation of eight additional partial sequences (Figure 2). So, RFLP analyses have to be performed to accurately establish exact haplotypic matches. All but one aboriginal 16260 haplotype belonged to the H1 subgroup (Table 2), however, for the modern populations of the Archipelago, albeit H1 is also the most frequent, there are also H3 and H7 representatives (Figure 2). In North Africa, the H1 16260 haplotype has only been detected once in North Central Africa as that found in Northwest Africa, belonged to the H3 subgroup. Furthermore, contrarily to the Canary Islands, 16260 H1-derived haplotypes have not yet been found in North Africa. Due to the relative recent radiocarbon dates estimated for the aboriginal settlement of the Archipelago,[7] it seems improbable that all the derived 16260 types were originated in the islands after their colonization. Their absence or rareness in North Africa, likewise that for the U6b1 motif, has to be explained because the precise site from which the Canarian ancestors originated in North Africa has not yet been sampled or because they have been replaced by later human migrations.[29] In fact, ρ-values for both 16260 H1 and U6b1 authoctonous clusters gave coalescence ages of 6263 ± 2869 and 5911 ± 3536 years ago, respectively. These dates, not

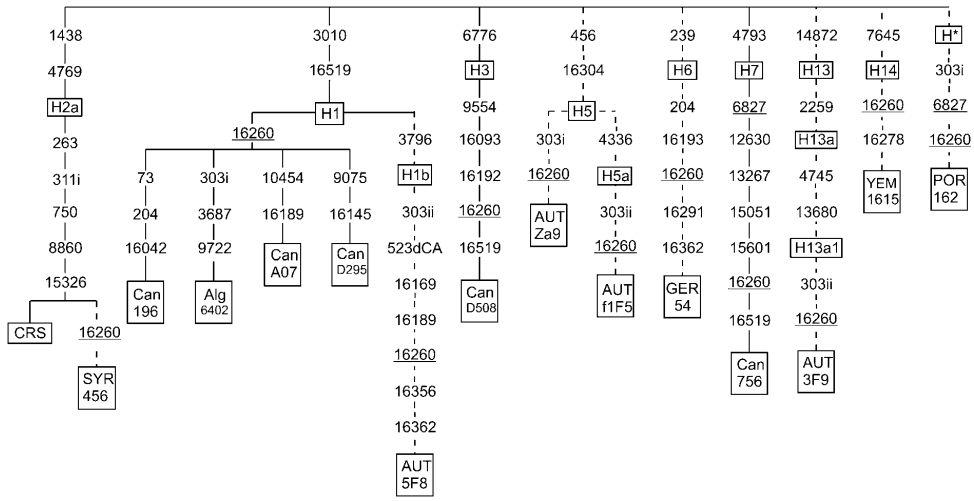


Figure 2 Phylogenetic tree of complete (continuous branches) and incomplete (discontinuous branches) haplogroup H mtDNA sequences carrying the 16260 transition. Numbers along links refer to nucleotide transitions. 'd' Indicate deletions and 'i' insertions. Recurrent mutations are underlined. Sequence references: CRS;[35–36] SYR456 and YEM1615;[31] Can196, CanA07, CanD295, CanD508, Can756, and Alg6402 (this study; GenBank accession nos. J719302-FJ719307); AUT5F8, AUTZa9, AUTf1F5, and AUT3F9;[37] GER54;[38] POR162.[39] Geographic origins: Alg6402: Algerian; AUT5F8, AUTZa9, AUTf1F5, and AUT3F9: Austrians; Can196, CanA07, CanD295, CanD508, and Can756: Canarian; GER54: German; POR162: Portuguese; SYR456: Syrian; YEM1615: Yemeni.

statistically different, clearly precede the age of the indigenous colonization, proposed by radiocarbon dates and archeological criteria at the beginning of the first millennium. Attending to global exact matches between samples, the highest relationship of aborigines from La Palma was with aborigines from Tenerife (0.165) and the most distant populations were those from Northwest (0.018) and North Central (0.016) Africa. Among islands, the aboriginal sample from La Palma had greater affinities with the western islands (0.095 ± 0.020), to which it geographically belongs, than with the eastern islands (0.051 ± 0.003). The high number of migrants exchanged between the two aboriginal population of La Palma and Tenerife ($M = 103$), reinforces the high relationship found at haplogroup and haplotype levels.

The relationships among populations based on haplogroup and haplotype F_{ST} distances are graphically shown in Figure 3. In both cases, La Gomera was the most divergent population mainly due to its comparatively low frequency of haplogroup H (21.7%) and because it presents the highest frequency of the Canarian indigenous U6b1 motif (47.8%). It is also worth mentioning that the aboriginal population from La Palma was always nearer to the aboriginal population from Tenerife than to the modern population of La Palma, whereas the aborigines from Tenerife are closer to their present day descendants in the island. Attending to haplogroup frequencies, Gran Canaria was the nearest island to North Africa. However, when haplotypic frequencies were used, the historical sample from Tenerife was the closest. This apparent discrepancy was due to the fact that exact L haplotypic matches between North Africa and the historical sample from Tenerife [5] were greater than between the former and Gran Canaria.[2]

Discussion

Before any conclusion based on aDNA could be established, the authenticity of the data obtained should be assessed. Although the repetition of the results is not a warranty of lack of modern DNA contamination,[20] the fact that modern contaminating DNA was retrieved from the surface of about 50% of the teeth, and that the endogenous sequences obtained did not match the external ones, seems to be an additional criterion for authenticity. It has been demonstrated that the ancient remains are most susceptible to contamination at the excavation process[32] and subsequent museum preservation.[33] However, from our results, it seems that well preserved teeth are less sensible to these routes of contamination. In our case, the archeologist sequence was not retrieved from the external extracts, possibly due to its elimination by later anthropologists handling. Furthermore, this sequence did not appear as a

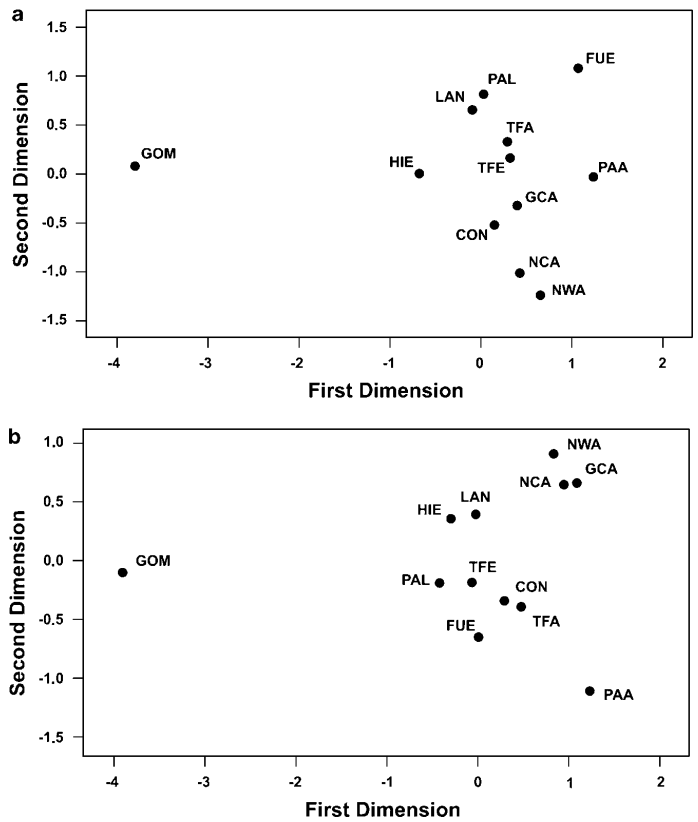


Figure 3 Graphical relationships among the studied populations. MDS plots based on F_{ST} haplotypic (**a**) and haplogroup (**b**) frequency distances. Codes are as in Table 1.

contaminant of the endogenous extracts. In addition, no more than one sequence, with phylogenetic sense, could be constructed from the mutations consistently detected in the different PCR fragments and clones of a same sample. Therefore, we feel confident of the reliability of our results.

On the basis of the probabilistic criteria, eight sequences of putative Northwest African ancestry were proposed as Canarian pre-Hispanic founder haplotypes and 13 additional ones, derived from them, as possible additional founder types.[2] Five of these basic founders were detected in the aboriginal sample of La Palma, where only the U6c1 type was absent.[29] However, previous[5] and the present aDNA study on prehistoric remains have shown that the number of founder lineages was higher than expected. In total, 36 additional lineages have been found. The bulk of them[26] belonged to West Eurasian haplogroups, being the rest[10] of sub-Saharan Africa ascription. As expected from archeological, anthropological and linguistic studies, the majority of the West Eurasian and sub-Saharan African lineages had exact matches in North Africa. Furthermore, there is an indigenous North African haplogroup, U6,[34] that has Canarian aboriginal representatives in its three main branches (U6a, U6b, U6c), which strongly points to this part of Africa as the most probable origin for the pre-Hispanic Canarian colonizers. However, the fact that the specific Canarian subtype U6b1 has not yet been found in North Africa makes the assignation of a precise geographic origin difficult. In addition, it has to be taken into account that there is a significant portion of aboriginal lineages (15%) that have specific matches to the European side of the Mediterranean basin or to the near East and that, until now, have not been found in North Africa. So, a minor demic component from these areas, on the maternal structure of the pre-Hispanic Canarians, cannot be dis-

carded. This possibility introduces the question of the way(s) and time(s) the islands were colonized before the Spanish conquest. The great gene diversity found in the aborigines from La Palma (95.2 ± 2.3), one of the westernmost islands, similar to that of the aborigines from the central Tenerife Island (92.4 ± 2.8), was an unexpected result. It was also surprising that the two aboriginal samples showed diversities in the same range as the current insular population (Table 2). As samples in each island were collected from four different sites, although with a predominant one (Espigón in La Palma 18 of 30 and CP in Tenerife 24 of 36), we wondered whether population structure within islands existed and the sampling process introduced a bias augmenting diversity. To test this possibility, H was calculated only for the most abundant sites. However, their values were even higher than those obtained for the total sample (96.1 ± 3.0 in Espigón and 93.1 ± 3.2 in CP). So we discarded the existence of a strong population structure and considered the high H values real. These results are against the supposition of a settlement process through island hopping and posterior isolation. Therefore, a restrictive stepping stone model would not fit with the maternal genetic pattern found, although it could be accepted supposing frequent secondary migrations between islands. Under a neutral model, the most important evolutionary forces that modulate gene variation are mutation, recombination, migration, and genetic drift. In the case of mtDNA, recombination is absent. On the other hand, the relative recent age of the Canary Island colonization, suggests that mutation could play only a minor role. In fact, coalescence ages of around 6000 years ago found for two founder clades, pointed to an arrival of both, ancestral and derived lineages, from the North African source population. This leaves migration and genetic drift as the two main counteracting factors to explain the high gene diversity present in the islands, as well as, its genetic resemblance. As commented before, the most accepted age for the aboriginal colonization of the island is at the beginning of the first millennium, and the mean age for the analyzed aboriginal remains is around 900 AD. So, assuming a generation time of 25 years, 36 generations passed since the initial colonization of the two islands. During this period, a total of 103 migrants was estimated, which roughly gives an interchange of around three migrants per generation. This value is sufficient to homogenize their genetic pools and would explain the great similarity found between both populations. However, this migratory input could be an overestimation if, as has been suggested, the colonizers of the two islands had the same North African origin.

Under these assumptions, an island-by-island independent maritime colonization, without secondary migrations, is also improbable. Moreover, if the Canary Islands were colonized in several waves, most probably Tenerife and La Palma were affected likewise by these events. At present, our data better fit to an island model with frequent migrations between islands. Future studies on aboriginal remains from other islands and from modern and ancient North African populations would help to elucidate these remaining questions.

Acknowledgements

We gratefully acknowledge P Hernández Carpio and A González and J Espino from SEGAI for technical assistance and to N Maca-Meyer and A Gámez for their helpful comments. The samples were generously donated by J Pais. This research was supported by grants from the Spain Ministry of Science and Technology BFU2006-04490 to JM Larruga. R Fregel was supported by a Gobierno de Canarias predoctoral fellowship.

References

1 Mercer J: The Canary Islanders: their prehistory, conquest, and survival. *London, Rex Collings* 1980.
2 Rando JC, Cabrera VM, Larruga JM *et al*: Phylogeographic patterns of mtDNA reflecting the colonization of the Canary Islands. *Ann Hum Genet* 1999; **63**: 413–428.
3 Flores C, Maca-Meyer N, Pérez JA, Cabrera VM: The peopling of the Canary Islands: a CD4/Alu microsatellite haplotype perspective. *Hum Immunol* 2001; **62**: 949–953.
4 Flores C, Maca-Meyer N, Pérez JA, González AM, Larruga JM, Cabrera VM: A predominant European ancestry of paternal lineages from Canary Islands. *Ann Hum Genet* 2003; **67**: 138–152.
5 Maca-Meyer N, Arnay M, Rando JC *et al*: Ancient mtDNA analisis and the origin of the Guanches. *Eur J Hum Genet* 2004; **12**: 155–162.
6 Maca-Meyer N, Cabrera VM, Arnay M *et al*: Mitochondrial DNA diversity in 17th–18th century remains from Tenerife (Canary Islands). *Am J Phys Anthropol* 2005; **127**: 418–426.
7 Delgado-Darias T, Velasco-Vazquez J, Arnay de la Rosa M, Martin Rodriguez E, Gonzalez-Reimers E: Dental caries among the pre-Hispanic population from Gran Canaria. *Am J Phys Anthropol* 2005; **128**: 560–568.
8 Abercromby J: Prehistoric pottery of the Canary Islands and its makers. *J Royal Anthropol Inst of Great Britain and Ireland* 1914; **44**: 302–323.
9 Navarro Mederos JF: Pasado y presente de la arqueología canaria. Espacio, Tiempo y Forma. *Prehistoria y Arqueología Canaria* 1997; **10**: 447–478.
10 Fregel R, Maca-Meyer N, Cabrera VM, González AM, Larruga JM: Description of a simple multiplex PCR-SSCP method for AB0 genotyping and its application to the peopling of the Canary Islands. *Immunogenet* 2005; **57**: 572–578.
11 Macias-Hernández A: Fuentes y principales problemas metodológicos de la demografía histórica de Canarias. *Anuario de Estudios Atlánticos* 1988; **34**: 51–158.
12 Martín E: La Palma y los auaritas. *Santa Cruz de Tenerife, Centro de la Cultura Popular Canaria* 1992.
13 Alonso A, Martín P, Albarrán C *et al*: Real-time PCR designs to estimate nuclear and mitochondrial DNA copy number in forensic and ancient DNA studies. *For Sci Int* 2003; **139**: 141–149.
14 Casas MJ, Hagelberg E, Fregel R, Larruga JM, González AM: Human mitochondrial DNA diversity in an archaeological site in *al-Andalus*: genetic impact of migrations from North Africa in Medieval Spain. *Am J Phys Anthropol* 2006; **131**: 539–551.
15 Finnilä S, Lehtonen MS, Majamaa K: Phylogenetic network for European mtDNA. *Am J Hum Genet* 2001; **68**: 1475–1484.
16 Herrnstadt C, Elson JL, Fahy E *et al*: Reduced-median-network analysis of complete mitochondrial DNA coding-region sequences for the major African, Asian and European haplogroups. *Am J Hum Genet* 2002; **70**: 1152–1171.

17 González AM, Cabrera VM, Larruga JM *et al*: Mitochondrial DNA variation Mauritania and Mali and their genetic relationship to other Western Africa populations. *Ann Hum Genet* 2006; **70**: 631–657.
18 Maca-Meyer N, González AM, Larruga JM, Flores C, Cabrera VM: Major genomic mitochondrial lineages delineate early human expansions. *BMC Genetics* 2001; **2**: 13–20.
19 Abu-Amero KK, Larruga JM, Cabrera VM, González AM: Mitochondrial DNA structure in the Arabian Peninsula. *BMC Evol Biol* 2008; **8**: 45.
20 Pääbo S, Poinar H, Serre D *et al*: Genetic analyses from ancient DNA. *Annu Rev Genet* 2004; **38**: 645–679.
21 Cooper A, Poinar HN: Ancient DNA: do it right or not at all. *Science* 2000; **18**: 289–1139.
22 Maca-Meyer N, Carranza S, Rando JC, Arnold EN, Cabrera VM: Status and relationships of the extinct giant Canary Island lizard *Gallotia goliath* (Reptilia: Lacertidae), assessed using ancient mtDNA from its mummified remains. *Biol J Linn Soc* 2003; **80**: 659–670.
23 van Oven M, Kayser M: Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Hum Mutat* 2008; online.
24 Nei M: *Molecular evolutionary genetics*. New York: Columbia University Press, 1987.
25 Schneider S, Roessli D, Excoffier L: *Arlequin ver. 2.0: A software for population genetic data analysis*. *Genetics and Biometry Laboratory*. Switzerland: University of Geneva, 2000.
26 Bandelt H-J, Forster P, Röhl A: Median-ing networks for inferring intraspecific phylogenies. *Mol Biol Evol* 1999; **16**: 37–48.
27 Saillard J, Forster P, Lynnerup N, Bandelt HJ, Norby S: mtDNA variation among Greenland Eskimos. The edge of the Beringian expansion. *Am J Hum Genet* 2000; **67**: 718–726.
28 Forster P, Harding R, Torroni A, Bandelt H-J: Origin and evolution of Native American mtDNA variation. A reappraisal. *Am J Hum Genet* 1996; **59**: 935–945.
29 Maca-Meyer N, González AM, Pestano J, Flores C, Larruga JM, Cabrera VM: Mitochondrial DNA transit between West Asia and North Africa inferred from U6 phylogeography. *BMC Genetics* 2003; **4**: 15.
30 Plaza S, Calafell F, Helal A *et al*: Joining the pillars of Hercules. mtDNA sequences show multidirectional gene flow in the western Mediterranean. *Ann Hum Genet* 2003; **67**: 312–328.
31 Roostalu U, Kutuev I, Loogvali E-L *et al*: Origin and expansion of haplogroup H, the dominant human mitochondrial DNA lineage in West Eurasia: the Near East and Caucasian perspective. *Mol Biol Evol* 2007; **24**: 436–448.
32 Sampietro ML, Gilbert MTP, Lao O *et al*: Tracking down human contamination in ancient human teeth. *Mol Biol Evol* 2006; **23**: 1801–1807.
33 Pruvost M, Schwarz R, Correia VB *et al*: Freshly excavated fossil bones are best for amplification of ancient DNA. *Proc Nat Acad Sci USA* 2007; **104**: 739–744.
34 Rando JC, Pinto F, González AM *et al*: Mitochondrial DNA análisis of northwest African populations reveals genetic exchanges with European, Near Eastern and sub-Saharan populations. *Ann Hum Genet* 1998; **62**: 531–550.
35 Anderson S, Bankier AT, Barrell BG *et al*: Sequence and organization of the human mitochondrial genome. *Nature* 1981; **290**: 457–465.
36 Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N: Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat Genet* 1999; **23**: 147.
37 Brandstaetter A, Zimmermann B, Wagner J *et al*: Timing and deciphering mitochondrial DNA macrohaplogroup R0 variability in Central Europe and Middle East. *BMC Evol Biol* 2008; **8**: 191.
38 Poetsch M, Witting H, Krause D, Lignitz E: Mitochondrial diversity of a northeast German population. *For Sci Int* 2003; **137**: 125–132.
39 Pereira L, Richards M, Goios A *et al*: Evaluating the forensic informativeness of mtDNA haplogroup H sub-typing on a Eurasian scale. *Foren Sci Int* 2006; **159**: 43–50.

Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)

4.4. Determinación del sexo en aborígenes de las Islas Canarias, basado en parámetros mandibulares y contrastados mediante el análisis del gen de la amelogenina

Como paso previo al análisis del cromosoma Y en la población aborigen de las Islas Canarias, se realizó un estudio del gen de la amelogenina para determinar qué muestras pertenecían a varones y establecer además cuales eran susceptibles de ser amplificadas para un locus autosómico.

En el caso de las muestras aborígenes de Gran Canaria, dado que el tamaño muestral fue mayor y que los dientes se obtuvieron directamente de la mandíbula, los resultados se utilizaron para contrastarlos con el sexaje basado en parámetros mandibulares e inspección visual.

La determinación del sexo mediante amplificación del gen de la amelogenina fue posible en 56 de las 76 mandíbulas estudiadas (73,68% de eficiencia). De los individuos analizados, 31 resultaron ser varones (55,36%) y 25 hembras (44,64%).

Una vez sexados los individuos, se contrastaron con los resultados del sexaje mediante inspección visual y medidas osteométricas, que habían sido obtenidas de forma independiente. En general se encontró que el sexaje basado en la inspección visual o en medidas mandibulares en la población prehispánica de Gran Canaria sólo permite una asignación correcta en aproximadamente el 70% de los casos, comparado con el sexaje molecular.

Este trabajo, titulado “Canary islands aborigin sex determination based on mandible parameters contrasted by amelogenin analysis”, se publicó en la revista *Journal of Archaeological Science*.

ELSEVIER

Journal of Archaeological Science 34 (2007) 1515–1522

Journal of Archaeological SCIENCE

http://www.elsevier.com/locate/jas

Canary islands aborigin sex determination based on mandible parameters contrasted by amelogenin analysis☆

M. Arnay-de-la-Rosa [a], E. González-Reimers [b,*], R. Fregel [c], J. Velasco-Vázquez [d], T. Delgado-Darias [e], Ana M. González [c], J.M. Larruga [c]

[a] Dpto. de Prehistoria, Antropología e Historia Antigua, Universidad de La Laguna, 38071 Tenerife, Canary Islands, Spain
[b] Dpto. de Medicina Interna, Hospital Universitario de Canarias, Ofra s/n 38230 Tenerife, Canary Islands, Spain
[c] Dpto. de Genética, Universidad de La Laguna, Tenerife, Canary Islands, Spain
[d] Unidad de Patrimonio, Cabildo de Gran Canaria, Las Palmas, Gran Canaria, Canary Islands, Spain
[e] Museo Canario, Las Palmas, Gran Canaria, Canary Islands, Spain

Received 21 August 2006; accepted 13 November 2006

Abstract

Sex determination using mandible parameters is population dependent. In order to assess which measurements better characterize sex in prehispanic individuals from the Canary Islands, we blindly contrasted the results obtained by visual inspection and osteometric measurements with those obtained by molecular sexing using amelogenin ancient DNA analysis on teeth from the same material. Unambiguous sex classification was achieved by amplification of sex specific amelogenin alleles in 56 out of 76 mandibles (73.78% of the cases). Visual inspection led to a correct diagnosis in 66.04% of cases, with a greater proportion of errors for female (54.17%) than male (17.24%) mandibles. Osteometric measurements were able to assign sex correctly in 72.2% in the best of cases (mandibular height), a proportion similar to that obtained using a discriminant function (71.2%). By logistic regression analysis, ramus breadth, index ramus breadth/ramus height and mandibular length were the parameters independently related with a mistaken diagnosis of female sex, whereas bigonial width, ramus height and mandibular length were the parameters more closely and independently related to a mistaken diagnosis of male sex. In conclusion, diagnosis based on visual examination of the mandible or on its metric measurement only serves to roughly estimate sex with an accuracy of around 70% or less, at least among the prehispanic population from Gran Canaria. Amplification of amelogenin alleles leads to unambiguous identification of male and female alleles in 73.68% of cases, at least among the prehispanic population from Gran Canaria.

Keywords: Sexing mandibles; Amelogenin alleles; Ancient skeletal remains; Ancient teeth DNA extraction

1. Introduction

Sex determination from skeletal remains is an important aspect of the osteologic analysis of a given population. Sexual dimorphism is especially marked in some bones, which are, therefore, suitable for sexing skeletons with high accuracy. Indeed, accurate sex estimation rate with only the pelvis reaches 95% (Ubelaker, 1989). The skull also offers a marked sexual dimorphism, which also allows accurate sexing of skeletons, although sexing from cranial bones is not as precise as from the pelvis (Brothwell, 1972; Ferembach et al., 1979; Mays, 1998). In addition, discriminant functions combining several measurements on long bones may help (Dibennardo and Taylor, 1982, 1983; Dittrick and Suchey, 1986; Iscan and Ding, 1995; Iscan and Miller-Shaivitz, 1984; Iscan et al., 1994; Ubelaker, 1989), but there is considerable variation of these functions among different populations, so the standards used for one population should not be used for

☆ Grant sponsorship: This study was partly supported by grants BMC2001-3511 from Ministerio de Ciencia y Tecnología and COF2002-015 from Gobierno de Canarias to Vicente M Cabrera.
* Corresponding author. Tel.: +34 922 678 600; fax: +34 922 319 279.
E-mail address: egonrey@ull.es (E. González-Reimers).

0305-4403/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jas.2006.11.008

another (Stein and Iscan, 1997). However, in many instances, just a few bones often constitute the sole remains of a dead individual, especially when we are dealing with ancient populations. One of these bones is the mandible. Several measurements and non-metric traits (Duric et al., 2005; Loth and Henneberg, 1996; Sutter, 2003) have been used to discriminate sex, although there is some controversy among different researchers regarding their validity (Balci et al., 2005; Koski, 1996).

The discovery that the human enamel protein gene amelogenin is expressed from both the X and the Y chromosomes and that there are sequence differences between both alleles (Salido et al., 1992) has provided a useful tool for sex determination in ancient remains (Faerman et al., 1995). Cortical and cranial bone, as well as teeth, may provide sufficiently preserved DNA to perform molecular genetic analyses and identify sex-specific DNA sequences of X and Y alleles of the amelogenin gene. Based on these facts we have analyzed in the present study: 1) the accuracy of several mandibular measurements for the diagnosis of sex; 2) the accuracy of visual inspection of the mandible by an expert anthropologist based on the general aspect of the mandible and on the evaluation of non-metric traits; and 3) the determination of the X and Y-specific amelogenin alleles, obtained from the same mandibles and used as molecular controls for the metric traits. The osteological study was performed on mandibles of prehispanic individuals from different archaeological sites from Gran Canaria, and the molecular study was performed on teeth from the same material.

2. Materials and methods

2.1. Sample

We analyzed seventy-six complete or nearly complete mandibles housed in the Museo Canario of the city of Las Palmas, belonging to prehispanic individuals from diverse archeological sites of the island Gran Canaria. Most of the cases come from Guayadeque, a ravine with several huge collective burial caves, located in the eastern part of the island. Antiquity of the samples, based on radiocarbon dating performed on skeletal material from the same burials, ranges from 1213 ± 60 to 1410 ± 60 BP.

2.2. Methods

2.2.1. Anthropometrics

We recorded chin height, height of the mandibular body, breadth of the mandibular bone, bigonial width (a), bicondylar breadth (b), minimum ramus breadth (c), maximum ramus height (d), mandibular length (e) and mandibular angle, following Buikstra and Ubelaker (1994), using a sliding caliper and a mandibulometer. We also calculated the indexes $a \times 100/b$, $c \times 100/d$, $d \times 100/e$.

Non-parametric traits, such as general robustness, aspect of the gonion and gnathion, or robustness of the mandibular ramus were considered in the diagnosis of sex by an experienced anthropologist.

2.2.2. Genetic sex determination

This was performed using dentin and pulp, usually from the second premolar and third molar, following the protocol outlined below.

2.2.2.1. DNA extraction. The steps followed for teeth decontamination and posterior DNA extraction were as previously published (Loth and Henneberg, 1996). Briefly, the procedure is as follows:

Prior to extraction, the surface of the tooth was thoroughly washed with 15% HCl, rinsed with ultraviolet (UV)-treated ddH2O and dried under UV lamp for 5 min on each side. Each tooth was then placed between two sterilized metal plates and crushed with a hammer, and the pieces were introduced into sterile 15 ml tubes (Costar). DNA was extracted according to a modified silica-based protocol (Höss and Pääbo, 1993). Briefly, 1–2 ml of a commercial guanidine thiocyanate solution (DNAzol R; Chomcynski et al., 1997) were added to each tube and incubated, at room temperature, for 3–4 days. After this incubation, the supernatant was passed through commercial silica columns (QIAquic R, Qiagen; Yang et al., 1998), according to the manufacturer's recommendation.

2.2.2.2. Amplifications. The X and Y amelogenin alleles were amplified using primer Amel-A (CCCTGGGCTCTGTAAAG AATAGTG) from Sullivan et al. (1993) and primer Amel-C (AATRYGGACCACTTGAGAAAC) described by Maca-Meyer et al. (2005). These primers amplify a small region in intron 1 of the amelogenin gene that encompasses a deletion polymorphism giving a product of 66 bp for the X allele and a product of 72 bp for the Y allele, so both products should be present in males, but only one in females. The polymerase chain reaction (PCR) was carried out in 10 μl reaction, containing 1 μl of 10X Tris–HCl buffer, 200 μM of each dNTP, 1 pmol of each primer, 5 mM $MgCl_2$, 15 ng of bovine serum albumin (BSA), 1 unit of Taq polymerase (Ecogen) and 3 μl of DNA extract. If no amplification product was obtained, DNA was increased to 6 μl in subsequent PCRs. To overcome PCR inhibition, detectable by the lack of primer-dimers in the reaction, DNA was reduced to 1 μl and/or the Taq and BSA amounts were doubled. Reactions were submitted to 40 amplification cycles with denaturation at 94 °C for 10 s, annealing at 45 °C for 10 s and extension at 72 °C for 10 s. PCR products were completely loaded in 10% Acrylamide:Bisacrylamide (19:1) gels, stained with ethidium bromide and visualized under UV.

2.2.2.3. RFLP tests. The specificity of the amplified products was tested by PCR re-amplification of each band and restriction fragment length polymorphism (RFLP) analysis. Bands were tooth-picked from the gel, dissolved in 10 μl sterile ddH_2O and 2 μl used in a 20 μl re-amplification PCR carried out with the same primers and conditions as those used in the first amplification. After checking, 10 μl of positive reactions were RFLP analyzed using the restriction enzyme *Hin*fI that give bands of 30 and 36 bp for the X allele and of 30 and

42 bp for the Y allele. In addition, the Y allele is specifically cut by *Mbo*II giving bands of 28 and 44 bp.

2.2.2.4. Cloning and sequencing. PCR products were ligated into pGEM-T vectors (Promega). Colonies were plated on selective Amp/IPTG/X-gal plates, and white colonies were selected.

PCR fragments were directly sequenced using the same primers as for amplification and clones were sequenced using M13 universal primers. Sequencing reactions were prepared in 10 μl volumes using the BigDye 3.1 Terminator Cycle Sequencing kit (Applied Biosystems) and the products were ethanol precipitated and run on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

2.2.2.5. Contamination prevention and authentication. All the procedures followed to prevent contamination from modern sources and to monitor contamination were as previously reported (Maca-Meyer et al., 2004). The authenticity of ancient DNA (aDNA) was assessed performing the following criteria: a) one negative extract control and three negative PCR controls were included in each extraction and amplification to detect possible contamination of extraction and/or PCR reagents, in order to be sure that the products obtained were amplified from the aDNA extracts; and b) previous to our amelogenin study all the teeth had been successfully analyzed for mitochondrial DNA (mtDNA) using our seven fragment amplification strategy (Maca-Meyer et al., 2004). Although in several cases we were able to amplify double fragments we could not amplify the total HVSI mtDNA region in one step in any sample. These results were taking as an additional proof that we were analyzing aDNA (Pääbo et al., 2004); c) amplified products were cloned and several clones sequenced; d) quantification of the number of amplifiable DNA molecules was carried out performing two amplifications and sequencing several clones from each of them, as recommended by others (Pääbo et al., 2004); e) reproduction of novel and unexpected result in a second laboratory is commonly requested (Pääbo et al., 2004). Regrettably, for sex, modern contamination is the same in all laboratories. For this reason and as contamination in our aDNA laboratory was sporadic and could be overcome in a short time period, whenever possible, two independent tooth extraction from the same individual were performed in our aDNA laboratory, but separated in time by a six month period and carried out by different individuals (RF for the first and AMG for the second). In addition, to confirm the amelogenin authenticity of the amplified products the following tests were carried out: a) the correct electrophoretic migration of the X and Y amelogenin alleles was assessed by running authenticated controls in parallel. These controls were amplified and sequenced from modern extracts using the same primers and conditions as those established for aDNA. Amplifications were performed in an independent laboratory by one of us (JML) who had not been in contact with the ancient material; b) faint bands, or bands with slightly different migration than controls, were re-amplified and RFLP checked using *Hin*fI. Occasionally, only the Y allele band was amplified. In these cases the specificity of the product was checked by *Mbo*II digestion; c) initial successful male and female reactions were cloned and 3 clones (females) or 7 clones (males) were sequenced to authenticate the amplified products as real X and Y amelogenin alleles. This cloning strategy was also applied in the cases of individual duplicates; d) at least two additional amplifications were carried out for male extracts to confirm the presence of its sex specific band, and 4–5 additional amplifications were performed when only the female band was amplified the first time in order to diminish the possibility that the extract was from a male but only the X band, common to both sexes, was amplified.

2.3. Statistics

We assessed the proportion of individuals for whom sex was undoubtedly diagnosed by osteological traits using genetic results as controls.

We calculated differences in the metric parameters between men and women before they were diagnosed by genetic analysis. For parameters presenting differences between men and women, we performed stepwise discriminant function analysis, and also depicted the corresponding ROC (receiver operating characteristic) curves and calculated the area under the curve (AUC).

We also calculated differences in the metric parameters between mandibles classified as male and female by visual evaluation. Comparing sex assessed by genetics (as control) and visual evaluation, we calculated the proportion of true positive estimations of sex by visual examination of the mandible, and the overall accuracy. We also studied possible differences in the metric parameters between mandibles correctly classified or incorrectly classified, and then performed another logistic regression analysis to discern which parameters contribute to misdiagnosis, both for men and women.

3. Results

For amelogenin amplification we usually doubled DNA sample amounts used for mtDNA amplifications. This worked for roughly 60% of the samples sexed. For the rest we had to double again the quantity of template DNA. Definitive DNA results were obtained for 56 of the 76 individuals analyzed (73.68%). Twelve individuals were discarded because two or four additional duplicates were not possible for males or females, respectively (15.8%). Five individuals were discarded because no amplification was obtained in any case (6.6%), and three more were excluded due to contamination in the extraction blanks (3.9%). Six additional teeth belonging to the sexed individuals were replicated. Five of them generated repetitive results, whereas no amplification was obtained for the other one.

PCR contamination was sporadic and could be overcome by leaving the lab inoperative for at least three days and, after that, repeating the amplifications using new aliquots of PCR solutions and primers.

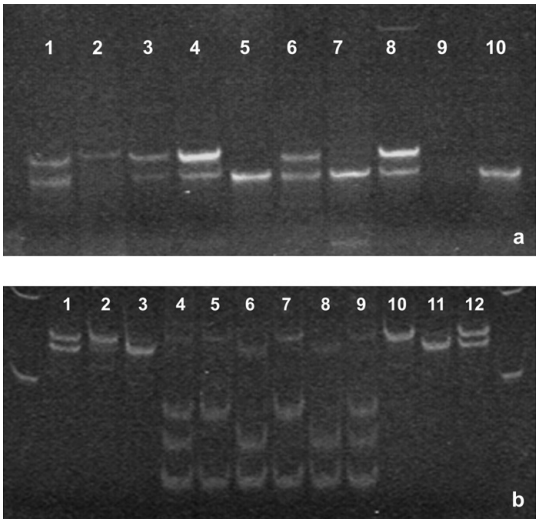


Fig. 1. Polyacrylamide gels showing the band patterns for sex determination. (a) shows migration of amplified fossils, compared with authenticated male and female controls (samples 1 and 10, respectively): samples 2, 3, 4, 6 and 8 correspond to male individuals; sample 5 and 7 to females and 9 was a PCR negative control. (b) shows the *Hin*fI enzymatic assay: samples 1 and 12 correspond to undigested male controls, samples 2 and 10 correspond to undigested male reamplified bands, samples 3 and 11 correspond to female reamplified bands, samples 4 and 9 correspond to *Hin*fI digested male controls, samples 5 and 7 correspond to experimental *Hin*fI digested male bands and 6 and 8 to females. Flanking lanes correspond to bands of 50 bp and 100 bp from a 50 bp ladder.

The authenticity of the results was confirmed by all the designed tests. The electrophoretic migration of the amelogenin bands in ancient bone was identical to the authenticated controls (Fig. 1a). In addition, the amplified fragments produced the expected band patterns in the restriction assays (Fig. 1b), and the sequences obtained by cloning from male and female samples were the same as those previously published (Nakahori et al., 1991), except for one female tooth that showed a transition from T to C at position 4375 in the three clones sequenced. Occasional bands with abnormal migration were picked from the gel, reamplified and sequenced in 17 instances. None of them had a sequence related to the amelogenin gene.

Comparative statistics of the means and standard deviations of the anthropometric parameters for men and women are given in Table 1. As shown, only bigonial width, maximum ramus height, mandibular angle, and the c × 100/d index clearly serve to discriminate between men and women, although when ROC curves are plotted we can see that sensitivity and specificity are not optimal (Figs. 2–5). Indeed, the area under the curve is, in the best of cases (maximum ramus height) 0.722 (confidence interval (CI) = 0.582–0.862). When the anthropometric parameters are step-wisely introduced in a discriminant function analysis, only maximum ramus height and mandibular angle enter as independent factors, leading to a formula ($y = 0.194 \times$ maximum ramus height $- 0.110 \times$ mandibular angle $+ 1.66$) which shows significantly different values for females (-121.19 ± 6.24) and for males (-116.72 ± 5.42, $t = 2.73$, $p = 0.009$), but which does not improve the overall accuracy of the diagnosis of sex provided by the individual anthropometric parameters (AUC = 0.712, CI = 0.565–0.859, Fig. 6). When a logistic regression analysis was performed, mandibular ramus height entered the regression analysis in the first place, but with a low beta value (-0.24, $p = 0.009$). Despite a lack of differences between men and women in the univariate analysis, the index bigonial width/bicondylar breadth entered the logistic regression analysis in second place (beta $= -0.156$, $p = 0.061$).

An expert anthropologist, by visual inspection, was able to make an unambiguous diagnosis of sex in 68 cases (89.47%). This method led to a correct classification of male sex in 82.76%, but only in 45.83% of female cases, with an overall

Table 1
Differences in several parameters between men and women (sex assessed by genetics)

	Men		Women		*t* value
	n	X ± SD	*n*	X ± SD	
Chin Height (mm)	28	31.09 ± 2.91	23	30.07 ± 2.71	1.29
Height (mm) of the mandibular body	31	30.46 ± 2.19	25	29.66 ± 2.37	1.31
Breadth (mm) of the mandibular body	31	11.61 ± 1.77	25	11.10 ± 1.94	1.02
Bigonial width (mm)	29	97.45 ± 6.78	24	93.01 ± 5.54	2.57**
Bicondylar breadth (mm)	27	119.14 ± 4.03	24	116.65 ± 6.61	1.65
Minimum ramus breadth (mm)	30	32.64 ± 2.67	25	31.85 ± 2.70	1.09
Maximum ramus breadth (mm)	30	44.88 ± 3.59	24	44.80 ± 3.36	0.09
Maximum ramus height (mm)	30	61.63 ± 5.22	24	58.20 ± 4.07	2.65**
Mandibular length (mm)	31	83.19 ± 7.11	25	80.26 ± 6.86	1.55
Mandibular angle (°)	29	118.47 ± 4.86	22	121.82 ± 5.54	2.30*
Bigonial breadth × 100/bicondylar breadth	26	81.98 ± 4.86	23	79.69 ± 4.02	1.78
Minimum ramus breadth × 100/Maximum ramus height	30	53.32 ± 6.32	24	54.72 ± 3.59	1.41
Maximum ramus breadth × 100/Maximum ramus height	30	73.27 ± 7.97	24	77.17 ± 5.74	2.01*
Mandibular length × 100/Bicondylar breadth	27	69.71 ± 5.89	24	68.78 ± 5.40	0.59

* = $p < 0.05$; ** = $p < 0.01$.

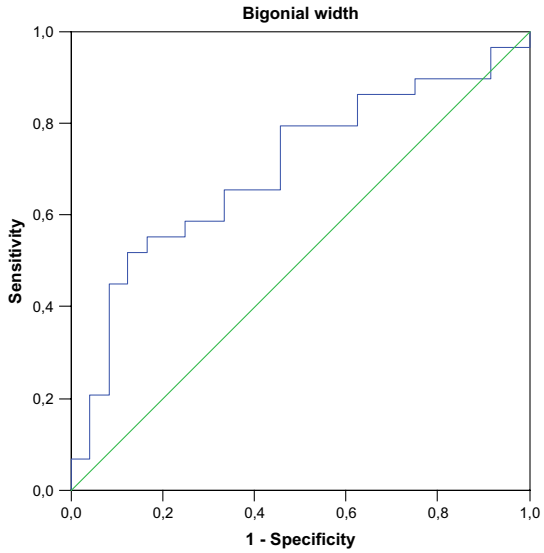


Fig. 2. Receiver operating characteristic curve of bigonial width in the diagnosis of male sex. (AUC = 0.698; confidence interval = 0.555–0.842.)

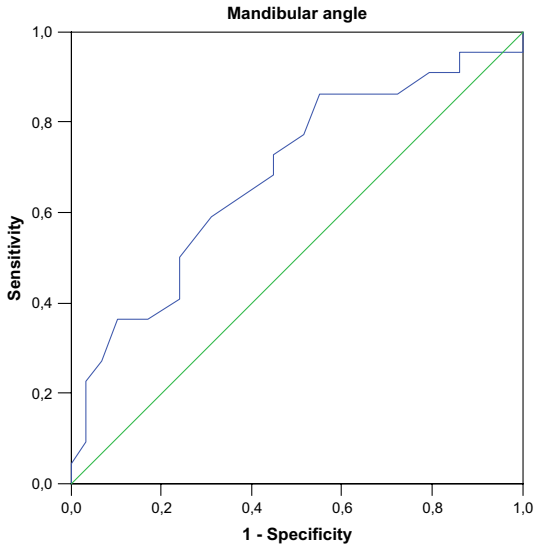


Fig. 4. Receiver operating characteristic curve of mandibular angle in the diagnosis of female sex. (AUC = 0.683; confidence interval = 0.533–0.833.)

accuracy of 66.04% (Table 2). As shown, this figure is slightly less than the accuracy observed with metric parameters: AUC is 0.640 (CI = 0.486–0.794, Fig. 7).

When anthropometric measures were compared between individuals classified as males and females by visual assessment, differences were more marked than when these parameters were compared for genetically-assessed sex (Table 3).

Incorrectly classified women showed a greater chin height, height of the mandibular body, bicondylar breadth, minimum ramus breadth and maximum ramus height than those who

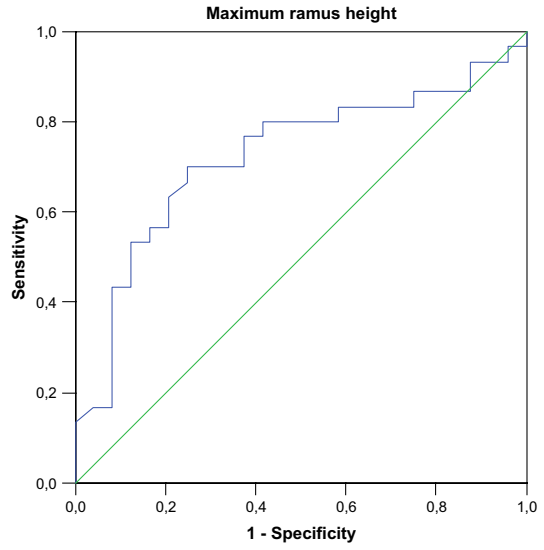


Fig. 3. Receiver operating characteristic curve of maximum ramus height in the diagnosis of male sex. (AUC = 0.722; confidence interval = 0.582–0.862.)

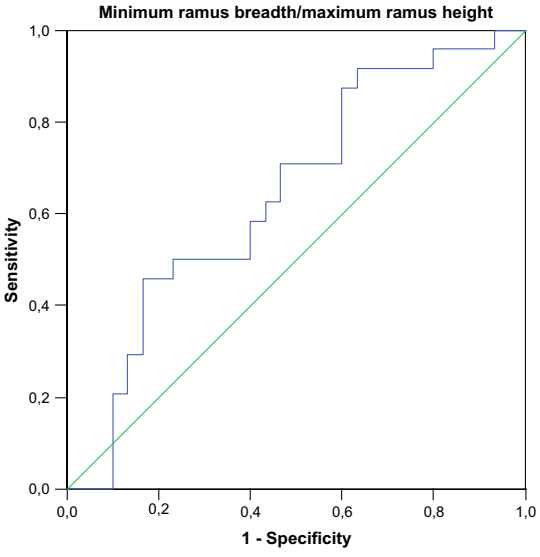


Fig. 5. Receiver operating characteristic curve of the index minimum ramus breadth/maximum ramus height in the diagnosis of female sex. (AUC = 0.642; confidence interval = 0.493–0.791.)

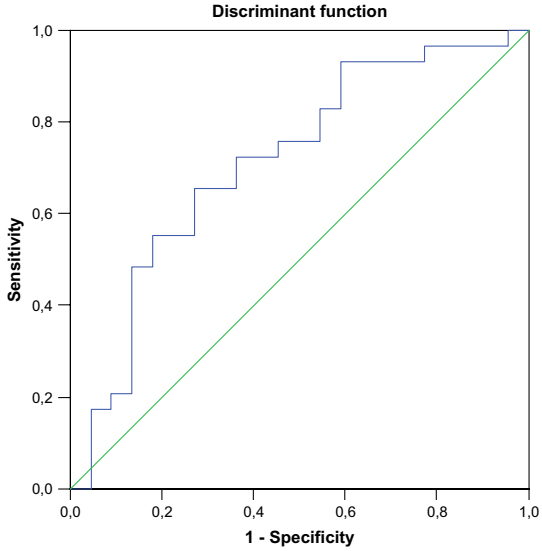


Fig. 6. Receiver operating characteristic curve of the discriminat function obtained combining maximum ramus breadth and mandibular angle in the diagnosis of male sex. (AUC = 0.712; confidence interval = 0.565–0.859.)

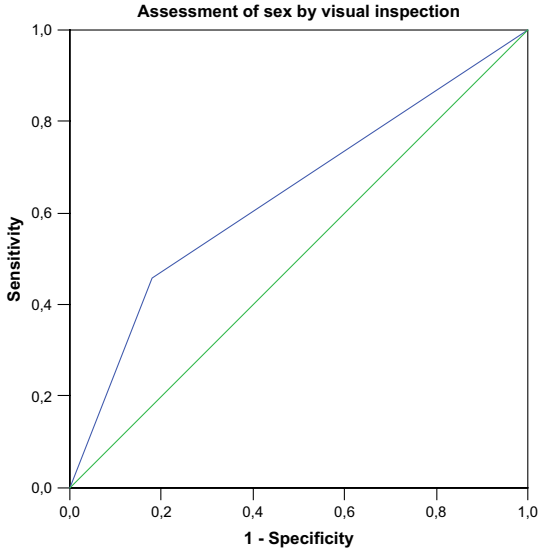


Fig. 7. Receiver operating characteristic curve of visual inspection of the mandible in the diagnosis of sex. (AUC = 0.640; confidence interval = 0.486–0.794.)

were correctly classified (Table 4). On the contrary, incorrectly classified males showed narrower bigonial width and the bigonial-bicondylear index, and shorter mandibular length than correctly classified ones (Table 5). Logistic regression analysis showed that minimum ramus breadth, the index minimum ramus breadth * 100/maximum ramus height and mandibular length, in this order, are the factors which independently lead to misdiagnosis of female sex when assessed by visual inspection. For the males, bigonial width, maximum ramus height and mandibular length, in this order, are the factors which led to misdiagnosis.

4. Discussion

Sequence differences between the X and Y alleles of the amelogenin gene offer an exceptional tool for the assessment of sex. However, genetic assessment of sex, when applied to prehistoric population groups (Stone et al., 1996) is strikingly dependent on burial conditions and preservation of the skeletal remains. The fact that different amelogenin patterns are obtained for males and females minimizes the rate of false negatives, which was too high when amplification of chromosome Y specific sequences were used (Hummel and Herrmann, 1991). Based on the amelogenin gene test we were able to perform unambiguous sex identification in 76.25% of cases. Our results, however, are in the range of others reported. Faerman et al. (1995), were able to identify sex correctly in 81.82% of cases, using teeth and bone powder from skeletons of variable antiquity (200–8000 years), and Stone et al. (1996) reached 95% in 20 skeletons dated from 1300 A.D. Although the precise antiquity of our samples is not known, radiocarbon dating of other specimens from the same burial site yield an antiquity ranging from 1000–1500 BP. We have used teeth without fractures, which provide sufficient DNA and minimizes the risk of non-preservation and contamination.

Table 2
Correctly and incorrectly sexed individuals by visual inspection of the mandible

		Visually assessed	
		Male	Female
Genetically assessed sex	Male	24	5
	Female	13	11

Using genetically assessed sex as control, we have tested the ability of several classical mandible measurements in the sex diagnosis, as well as non-metric assessment of sex. We found that accuracy ranges from around 70–75% with the metric analysis, and 66% (46–83%,) with the non-metric analysis. Sexual differences in the mandible of modern human beings have at least two components: differences related to musculoskeletal development and differences related to a different growth trajectory in males and females (Rosas et al., 2002). Sexing based on mandibular features is not 100% accurate. For instance, sexing based on the presence of ramus flexure is 85.8% accurate, a figure which drops to 60% for a correct classification of a female sex (Balci et al., 2005). Other authors have observed accuracies of 79.1% (Hill, 2000), while others have reported an accuracy of only 66% (Kemkes-Grottenthaler et al., 2002), and only 32% for

Table 3
Differences in several parameters between men and women (sex assessed by visual inspection)

	n	X ± SD	*n*	X ± +/SD	*t* value
Chin Height (mm)	47	31.37 ± 2.61	16	28.69 ± 2.25	3.66***
Height (mm) of the mandibular body	49	31.02 ± 2.14	19	28.35 ± 1.66	4.90***
Breadth (mm) of the mandibular body	49	11.74 ± 1.87	19	10.57 ± 1.61	2.40*
Bigonial width (mm)	46	97.70 ± 6.11	17	89.70 ± 5.43	4.74***
Bicondilar breadth (mm)	44	119.24 ± 4.98	15	115.56 ± 3.81	2.61**
Minimum ramus breadth (mm)	48	32.87 ± 2.66	19	30.62 ± 2.10	3.29***
Maximum ramus breadth (mm)	47	45.16 ± 3.53	17	44.52 ± 2.88	0.68
Maximum ramus height (mm)	48	62.56 ± 4.99	17	55.10 ± 3.54	5.66
Mandibular length (mm)	48	83.60 ± 6.59	18	76.39 ± 5.42	4.14
Mandibular angle (°)	44	119.88 ± 5.69	17	121.94 ± 5.62	1.28
Bigonial breadth × 100/bicondylar breadth	43	82.03 ± 4.61	14	78.51 ± 4.33	2.52*
Minimum ramus breadth × 100/Maximum ramus height	48	52.75 ± 4.77	17	55.69 ± 5.61	2.01*
Maximum ramus breadth × 100/Maximum ramus height	47	72.32 ± 6.89	17	81.00 ± 6.29	4.55***
Mandibular length × 100/Bicondylar breadth	44	70.11 ± 5.73	15	66.12 ± 3.81	2.51*

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 4
Differences among female individuals correctly and incorrectly classified by visual inspection

	Incorrectly classified		Correctly classified		*t* value
	n	X ± SD	*n*	X ± SD	
Chin Height (mm)	12	31.73 ± 2.64	10	28.23 ± 1.32	3.79***
Height (mm) of the mandibular body	13	31.03 ± 2.09	11	28.44 ± 1.53	3.40**
Breadth (mm) of the mandibular body	13	11.78 ± 2.09	11	10.50 ± 1.03	1.69
Bigonial width (mm)	13	94.44 ± 6.49	10	91.62 ± 3.85	1.22
Bicondylar breadth (mm)	13	119.44 ± 7.36	10	113.87 ± 3.38	2.21*
Minimum ramus breadth (mm)	13	33.52 ± 2.24	11	29.81 ± 1.81	3.71**
Maximum ramus breadth (mm)	13	45.47 ± 3.95	10	43.82 ± 2.49	1.15
Maximum ramus height (mm)	13	60.63 ± 3.25	10	55.00 ± 2.88	4.32***
Mandibular length (mm)	13	83.32 ± 5.87	11	75.99 ± 5.82	3.06**
Mandibular angle (°)	11	121.05 ± 6.24	10	121.85 ± 4.50	0.34
Bigonial breadth * 100/bicondylar breadth	13	79.14 ± 4.36	9	80.27 ± 3.81	0.62
Minimum ramus breadth * 100/Maximum ramus height	13	55.38 ± 3.97	10	53.79 ± 3.20	1.03
Maximum ramus breadth * 100/Maximum ramus height	13	75.05 ± 6.06	10	79.76 ± 4.58	2.05
Mandibular length * 100/Bicondylar breadth	13	69.86 ± 4.71	10	66.15 ± 4.33	1.94

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Table 5
Differences among male individuals correctly and incorrectly classified by visual inspection

	Correctly classified		Incorrectly classified		*t* value
	n	X ± SD	*n*	X ± SD	
Chin Height (mm)	22	31.81 ± 2.58	3	31.81 ± 2.58	0.33
Height (mm) of the mandibular body	23	30.89 ± 2.07	5	29.17 ± 1.29	1.77
Breadth (mm) of the mandibular body	23	11.68 ± 2.01	5	11.08 ± 0.74	0.66
Bigonial width (mm)	22	100.02 ± 5.38	5	90.14 ± 3.98	3.85***
Bicondylar breadth (mm)	21	119.62 ± 4.05	4	119.57 ± 1.53	0.03
Minimum ramus breadth (mm)	23	32.75 ± 2.85	5	32.69 ± 2.01	0.04
Maximum ramus breadth (mm)	23	45.01 ± 3.80	5	45.26 ± 3.28	0.13
Maximum ramus height (mm)	23	63.33 ± 4.18	5	55.98 ± 5.24	3.42**
Mandibular length (mm)	23	84.64 ± 7.19	5	79.86 ± 1.48	1.46
Mandibular angle (°)	22	117.98 ± 4.46	5	120.10 ± 6.27	0.89
Bigonial width * 100/bicondylar breadth	20	83.50 ± 4.38	4	76.77 ± 1.84	2.97**
Minimum ramus breadth * 100/Maximum ramus height	23	51.85 ± 4.97	5	59.02 ± 8.89	2.52*
Maximum ramus breadth * 100/Maximum ramus height	23	71.30 ± 6.82	5	81.38 ± 9.39	2.81**
Mandibular length * 100/Bicondylar breadth	21	70.72 ± 6.22	4	67.15 ± 1.91	1.12

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

females. On the other hand, Haun (2000) correctly classified 96.3% of the males and 62.5% of females. Based on visual evaluation of the robustness of mandibles, Duric et al. (2005) correctly classified only 70.93% in a modern Balkan population.

We have tried to analyze which factors lead to misdiagnosis of sex by visual inspection. It can be seen that these factors are different for men and women. Indeed, female mandibles which were incorrectly classified as male ones showed features related to robustness, such as breadth of the ramus in relation to height and to mandibular length. On the contrary, more "gracile" male mandibles, with a narrower bigonial width, lead to misclassification by visual inspection.

Several conclusions can be extracted from this study. At first, extraction of DNA from teeth in sufficient amounts to diagnose sex unambiguously was achieved in nearly 75% of cases. Secondly, assessment of sex either by metric analysis or by visual inspection of the mandibles yields an accuracy of 65–70%. Visual inspection is especially misleading in the case of female individuals who show some features of robustness such as a wider ramus breadth, or a reduced index ramus breadth/ramus height. In contrast, misclassification of male individuals depends on bigonial width and mandibular length.

References

Balci, Y., Yavuz, M.F., Cagdir, S., 2005. Predictive accuracy of sexing the mandible by ramus flexure. Homo 55, 229–237.

Brothwell, D.R., 1972. Digging Up Bones. Trustees of the British Museum, London.

Buikstra, J.E., Ubelaker, D.H., 1994. Standards for data collection from Human Skeletal remains. Arkansas Archaeol. Surv. Res. Series 44, 78.

Chomcynski, P., Mackey, K., Drews, R., Wilfinger, W., 1997. DNAzol R: a reagent for the rapid isolation of genomic DNA. Biotechniques 22, 550–553.

Dibennardo, R., Taylor, J.V., 1982. Classification and misclassification in sexing the black femur by discriminant function analysis. Am. J. Phys. Anthropol. 58, 145–151.

Dibennardo, R., Taylor, J.V., 1983. Multiple discriminant function analysis of sex and race in the postcranial skeleton. Am. J. Phys. Anthropol. 61, 305–314.

Dittrick, J., Suchey, J.M., 1986. Sex determination of prehistoric central California skeletal remains using discriminant analysis of the femur and humerus. Am. J. Phys. Anthropol. 70, 3–9.

Duric, M., Rakocevic, Z., Donic, D., 2005. The reliability of sex determination of skeletons from forensic context in the Balkans. Forensic Sci. Int. 147, 159–164.

Faerman, M., Filon, D., Kahila, G., Greenblatt, C.L., Smith, P., Oppenheim, A., 1995. Sex identification of archaeological human remains based on amplification of the X and Y amelogenin alleles. Gene 167, 327–332.

Ferembach, D., Schwidetzky, I., Stloukal, M., 1979. Recommendations pour determiner l'age et le sexe sur le squelette. Bull. Mem. Soc. Anthropol. (Paris) 6 (série XIII), 7–45.

Haun, S.J., 2000. A study of the predictive accuracy of mandibular ramus flexure as a single morphological indicator of sex in an archaeologicals sample. Am. J. Phys. Anthropol. 111, 429–432.

Hill, C.A., 2000. Technical note: evaluating mandibular ramus flexure as a morphological indicator of sex. Am. J. Phys. Anthropol. 111, 573–577.

Höss, M., Pääbo, S., 1993. DNA extraction from Pleistocene bones by a silica-based purification method. Nucleic Acid Res. 21, 3913–3914.

Hummel, S., Herrmann, B., 1991. Y-chromosome-specific DNA amplified in ancient human bone. Naturwissenschaften 78, 266–267.

Iscan, M.Y., Ding, S., 1995. Sexual dimorphism in the Chinese femur. Forensic Sci. Int. 74, 79–87.

Iscan, M.Y., Miller-Shaivitz, P., 1984. Discriminant function sexing of the tibia. J. Forensic Sci. 29, 1087–1093.

Iscan, M.Y., Yoshino, M., Kato, S., 1994. Sex determination from the tibia: standards for contemporary Japan. J. Forensic Sci. 39, 785–792.

Kemkes-Grottenthaler, A., Löbig, F., Stock, F., 2002. Mandibular ramus flexure and gonial eversion as morphologic indicators of sex. Homo 53, 97–111.

Koski, K., 1996. Mandibular ramus flexure-Indicator of sexual dimorphism? Am. J. Phys. Anthropol. 101, 545–546.

Loth, S.R., Henneberg, M., 1996. Mandibular ramus flexure: a new morphologic indicator of sexual dimorphism in the human skeleton. Am. J. Phys. Anthropol. 99, 473–485.

Maca-Meyer, N., Arnay de la Rosa, M., Rando, J.C., Flores, C., Fregel, R., González, A.M., Cabrera, V.M., Larruga, J.M., 2004. Ancient mtDNA analysis and the origin of the Guanches. Eur. J. Hum. Genet. 12, 155–162.

Maca-Meyer, N., Cabrera, V.M., Arnay de la Rosa, M., Flores, C., Fregel, R., González, A.M., Larruga, J.M., 2005. Mitochondrial DNA diversity in 17th–18th Century remains from Tenerife (Canary Islands). Am. J. Phys. Anthropol. 127, 418–426.

Mays, S., 1998. The Archaeology of Human Bones. Routledge, London-New York.

Nakahori, Y., Takenaka, O., Nakagome, Y., 1991. A human X-Y homologous region encodes "amelogenin". Genomics 9, 264–269.

Pääbo, S., Poinar, H., Serre, D., Jaenicke-Després, V., Hebler, J., Rohland, N., Kuch, M., Krause, J., Vigilant, L., Hofreiter, M., 2004. Genetic analyses from ancient DNA. Annu. Rev. Genet. 38, 645–679.

Rosas, A., Bastir, M., Martínez-Maza, C., Bermúdez de Castro, J.M., 2002. Sexual dimorphism in the Atapuerca-SH hominids: the evidence from the mandibles. J. Hum. Evol. 42, 451–474.

Salido, E.C., Yen, P.H., Koprivnikar, K., Yu, L.C., Shapiro, L.J., 1992. The human enamel protein gene amelogenin is expressed from both the X and the Y chromosomes. Am. J. Human Genet. 50, 303–316.

Stein, M., Iscan, M.Y., 1997. Sex determination from the femur and tibia in South African whites. Forensic Sci. Int. 90, 111–119.

Stone, A.C., Milner, G.R., Pääbo, S., Stoneking, M., 1996. Sex determination of ancient human skeletons using DNA. Am. J. Phys. Anthropol. 99, 231–238.

Sullivan, K.M., Mannuci, A., Kimpton, C.P., Grill, P., 1993. A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. Biotechniques 15, 636–641.

Sutter, R.C., 2003. Non-metric subadult skeletal sexing traits: I. A blind test of the accuracy of eight previously proposed methods using prehistoric known-sex mummies from northern Chile. J. Forensic Sci. 48, 927–935.

Ubelaker, D.H., 1989. Human Skeletal Remains. Taraxacum, Washington.

Yang, D.Y., Eng, B., Waye, J.S., Dudar, J.C., Saunders, S.R., 1998. Improved DNA extraction from ancient bones using silica-based spin columns. Am. J. Phys. Anthropol. 105, 539–543.

4.5. Historia demográfica de los linajes paternos en las Islas Canarias

El estudio de marcadores uniparentales, ADNmt y cromosoma Y, en la población actual de las Islas Canarias detectó una marcada asimetría sexual, de tal forma que los linajes maternos habían sobrevivido en mayor número, comparado con los paternos. Sin embargo, en estos trabajos, se utilizó como parental para estimar la aportación aborigen una muestra de la población norteafricana actual. Dado que no podemos asumir que los primeros pobladores de las Islas Canarias fueran idénticos a los del norte de África actual, es imprescindible analizar directamente la composición genética indígena para obtener una estima más exacta. En el caso del ADNmt, estudios previos en los aborígenes han confirmado su procedencia y su grado de pervivencia en la población actual. Con la misma intención, en el presente trabajo, se ha estudiado la población indígena y una población histórica (S.XVII-XVIII) de las Islas Canarias para determinar la evolución temporal de los linajes paternos.

La detección del haplogrupo E-M81, autóctono de poblaciones bereberes, y los haplogrupos E-M78 y J-M267, que también aparecen con frecuencia en estas poblaciones, confirma el origen norteafricano de los aborígenes canarios y los resultados obtenidos con el ADNmt.

A diferencia de los linajes maternos, que se han mantenido a lo largo del tiempo con un leve descenso, los paternos han ido disminuyendo de forma constante desde la conquista hasta la actualidad, siendo sustituidos progresivamente por linajes de origen europeo.

Con respecto a la aportación subsahariana, asociada al tráfico de esclavos, ésta ha sido detectada tanto desde el punto de vista de los linajes maternos, como de los paternos. Sin embargo, en ambos casos, su frecuencia fue mayor durante los siglos XVII-XVIII que en la actualidad.

Este estudio del cromosoma Y en poblaciones aborígenes e históricas de las Islas Canarias fue publicado en la revista *BMC Evolutionary Biology* con el título “Demographic history of Canary Islands male gene-pool: replacement of native lineages by European”.

BMC Evolutionary Biology

BioMed Central

Research article

Open Access

Demographic history of Canary Islands male gene-pool: replacement of native lineages by European

Rosa Fregel*[1], Verónica Gomes[2,3], Leonor Gusmão[2], Ana M González[1], Vicente M Cabrera[1], António Amorim[2] and Jose M Larruga[1]

Address: [1]Department of Genetics, University of La Laguna, Avda. Astrofísico Fco. Sánchez, La Laguna, 38271 Santa Cruz de Tenerife, Spain, [2]Institute of Pathology and Molecular Immunology of the University of Porto (IPATIMUP), University of Porto, Porto, Portugal and [3]Institute of Legal Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain

Email: Rosa Fregel* - rfregel@gmail.com; Verónica Gomes - vgomes@ipatimup.pt; Leonor Gusmão - lgusmao@ipatimup.pt; Ana M González - amglez@ull.es; Vicente M Cabrera - vcabrera@ull.es; António Amorim - aamorim@ipatimup.pt; Jose M Larruga - jlarruga@ull.es

* Corresponding author

Published: 3 August 2009

BMC Evolutionary Biology 2009, **9**:181 doi:10.1186/1471-2148-9-181

Received: 16 January 2009
Accepted: 3 August 2009

This article is available from: http://www.biomedcentral.com/1471-2148/9/181

Abstract

Background: The origin and prevalence of the prehispanic settlers of the Canary Islands has attracted great multidisciplinary interest. However, direct ancient DNA genetic studies on indigenous and historical 17th–18th century remains, using mitochondrial DNA as a female marker, have only recently been possible. In the present work, the analysis of Y-chromosome polymorphisms in the same samples, has shed light on the way the European colonization affected male and female Canary Island indigenous genetic pools, from the conquest to present-day times.

Results: Autochthonous (E-M81) and prominent (E-M78 and J-M267) Berber Y-chromosome lineages were detected in the indigenous remains, confirming a North West African origin for their ancestors which confirms previous mitochondrial DNA results. However, in contrast with their female lineages, which have survived in the present-day population since the conquest with only a moderate decline, the male indigenous lineages have dropped constantly being substituted by European lineages. Male and female sub-Saharan African genetic inputs were also detected in the Canary population, but their frequencies were higher during the 17th–18th centuries than today.

Conclusion: The European colonization of the Canary Islands introduced a strong sex-biased change in the indigenous population in such a way that indigenous female lineages survived in the extant population in a significantly higher proportion than their male counterparts.

Background

The Canary Islands are a volcanic archipelago consisting of seven main islands situated in the Atlantic Ocean, facing the western Saharan coast of Africa. Fuerteventura and Lanzarote are the easternmost islands, the former being only a hundred km from the continent.

The Islands were already known to Mediterranean Classical cultures, but the Archipelago was rediscovered and visited by Genovese, Majorcan, Portuguese and French sailors during the 13th and 14th centuries. Under the auspices of the Castilian crown, Europeans conquered the Canary Islands during the 15th century, beginning with

Lanzarote in 1402 and finishing with Tenerife in 1496. The conquest was rather violent because the Guanches often fought fiercely against the invaders. Even islands such as Lanzarote or Gomera, which pacifically received the first Norman and Castilian expeditions, were the scene of violent revolts because the natives were enslaved in large numbers to defray the cost of the military expeditions. In retaliation, the rebels, mainly men, were killed and massively deported by the conquerors [1].

There are several questions about the past and present of the Guanches that have attracted the curiosity of scientists since the 19th century. They refer to the time(s) and way(s) they arrived on the islands, their geographic origin, and whether their descendants persist in the present-day population [2]. The oldest human settlement seems to be no earlier than the first millennium B.C., according to absolute C^{14} dating [3]. Coalescence age estimates obtained from mitochondrial DNA (mtDNA) [4] and Y-chromosome [5] putative founder lineages concord with archeological results.

As the islands were never connected with the African Continent, they had to be reached by sea. Their inhabitants did not supposedly have seafaring skills and communication among islands was thus absent at the time of the Spanish conquest. This poses the unresolved dilemma of whether the first settlers reached the islands by themselves and after that forgot their sailing skills or if they were transported to the islands by another maritime people [6].

From the beginning of the conquest, Guanche dialects and customs were found to be related to those of their N African Berber neighbors. Since then, anthropological, archeological and linguistic studies have provided further support to the N African origin of the indigenous population [7]. Furthermore, the different human types discovered and the heterogeneity of their cultural remains again points to the possibility of successive arrivals of N African settlers [8-13].

In spite of the aggressive conquest and subsequent massive European immigration and North and sub-Saharan African slave importation to the islands, historians estimated that approximately two-thirds of the Canary population were Africans and aborigines at the end of the 16th century [14]. Moreover, osteological studies comparing aboriginal remains and modern rural populations, support the persistence of indigenous traits in the current population [10,15].

From the genetic perspective, strong evidence in support of a N African origin of the indigenous ancestors and their present-day persistence was only obtained when uniparental genetic markers were analyzed. Mitochondrial DNA (mtDNA) lineages, belonging to the U6 haplogroup [16], and Y-chromosome haplotypes of the E1b1b1b haplogroup, characterized by the M81 marker [17], both with a clear Berber origin, were detected in the Canary islanders at a significantly higher presence than in Iberians, their main colonizers [4,5]. In addition, admixture analysis taking the Iberians, Northwest and sub-Saharan West African populations as parental sources of the present-day Canary population, showed that the indigenous contribution was estimated to be 33% of maternal lineages [4] and only 7% for paternal lineages [5]. This strong sexual asymmetry was explained by a sociological bias favoring matings between Iberian males and indigenous females, and the greater indigenous male mortality during the Conquest [2]. Accordingly, intermediate admixture estimates were obtained when autosomal markers were used [18,19]. It is also worth mentioning that the detection of significant correlations between relative frequencies and/or diversity values for mtDNA, CD4/Alu haplotypes and ABO gene data, and geographical distances of the islands from Africa were explained assuming only one main colonization event [4,18,19]. On the contrary, using Y-chromosome markers, two opposite correlations were found [5], which was explained by at least two independent waves of colonists from NW Africa, still detectable today. These genetic results, although congruent with previous anthropological, archeological and linguistic data, have not been free of criticism. It is well known that admixture values strongly depend on the appropriate choice of the parental populations. To extrapolate the unknown indigenous population from a NW African sample pool seems unsuitable, because although the mtDNA haplogrup U6 present in the Canary Islanders and in North Africa originates in the latter [20], the most abundant Canary sublineage, U6b1, is absent in NW Africa, and the most abundant U6a sublineages on that continent are very scarce in the archipelago [4,16,21] pointing to different N African sources. Moreover, the unquestionably N African lineages present in the present-day Canary population may not be wholly due to the indigenous heritage but to Iberian colonizers, since these lineages, albeit in low frequencies, are also present in Spain and Portugal [22,23]. Another possibility is that those U6 lineages present in the islands may derive from slaves brought from the NW African coast after the conquest. However, all these concerns vanished when mtDNA information was obtained directly from indigenous remains [24], and exhumed 17th–18th century remains from Tenerife [25]. The presence of U6b1 lineages and other presumed founder lineages were detected in both samples, confirming their prehispanic origin. In addition, the direct incorporation of the indigenous sample as a parental source of the admixed Canary Islands populations provided greater indigenous female component estimates (42–73%) than those based on the present-day NW African maternal gene pool (33–43%).

Although most of the populational molecular genetic studies carried out on skeletal remains have used mtDNA, mainly because of its copy number per cell, sex-typing based on the XY amelogenin test has also been frequently and successfully used since the beginning of the ancient DNA (aDNA) typing era [26-28]. Recent achievements in Neanderthal whole nuclear genome [29,30] and gene specific [31,32] studies prompted us to undertake a Y-chromosome SNP analysis in the indigenous population of the islands, which is crucial to determine the relative survival of the prehispanic male genetic pool in the present-day population. The goal was to directly type North-African geographically structured Y-chromosome binary markers in samples from indigenous and 17^{th}–18^{th} century remains that were already successfully analyzed for mtDNA [24,25] and proven to be males by an amelogenin-based sexing test [33]. The statistical null hypotheses of these analyses would be that male haplogroup frequencies in the indigenous and historical samples should not be significantly different from those found in the modern Canary population.

Results and discussion

Sample typing and methods

First of all, contamination was not detected in extraction or PCR negative controls, in any case of Y-chromosome analysis, although sporadic contamination was observed when the previous mtDNA analysis was performed. PCR efficiency with the samples selected for the present Y-chromosome analysis was 58% in the indigenous material and 63% in La Concepción historical material. However, taking into account previous mtDNA and amelogenin analysis, only 30 (10%) of the total indigenous and 42 (21%) of the historical samples produced Y-chromosome positive results. From the 30 successfully amplified indigenous samples, 24 were from Gran Canaria, 3 from Fuerteventura, 2 from Tenerife and 2 from Gomera.

For the direct Y-chromosome markers amplification, a mean of 366 ± 254 initial molecules was quantified by real-time PCR. However, the limited amount of DNA substrate left after the mtDNA and amelogenin analysis, and the frequent PCR inhibition problems due to the relatively large amount of extract necessary to directly amplify each marker, required the inclusion of a prior preamplification step. At first, we unsuccessfully tried whole genome amplification. Secondly, we turned to a specific multiplex approach using the whole sixteen primer-pair set in one reaction but, although some specific products were obtained, the relative abundance of unspecific amplifications made this method difficult to apply. Only when the sixteen markers were subdivided into three different multiplex assays (Additional file 1), clean specific products were obtained in subsequent nested PCR reamplifications. Cloning and sequencing confirmed the PCR amplification specificity for all the markers used.

Although a hierarchical approach was followed in the RFLP analysis in La Laguna (Additional file 2), all the samples were first typed for the phylogenetically basal M89 marker, the three most frequent North African markers (M78, M81, M267) and M269, the most abundant European marker. The same five markers were also replicated in the Porto lab using a first multiplex SNaPshot analysis (Additional file 3). Samples derived for M78, M81, M267 and M269 were not included in further analyses; those derived for M89 were subsequently analyzed for the M9, M45, M170, M172, M173 and M201 multiplex set; and ancestral ones for the M2, M33, M34, M60 and M96 multiplex set.

Authenticity of ancient DNA results

We are confident in the authenticity of our results for several reasons. First, only those samples that showed a relatively high initial copy number in the real-time PCR quantification assay were successfully analyzed. Second, we never detected contamination in any of the negative controls performed in extraction and amplification. Third, all the markers analyzed in the same individual always gave genealogically congruent results for their respective ancestral or derived status. Fourth, replication of all the samples in two independent laboratories produced identical results. Fifth, haplogroup types and frequencies obtained for the indigenous and historical samples were very different, but in accordance with the predictions based on historical and archeological records. Sixth, haplogroups crucial to the correct interpretation of the results, such as E-M81, were not detected in the panel of male researchers that handled the remains from each excavation (Additional file 4).

Y-SNP haplogroups in indigenous and historical Canary Island populations

Y-SNP haplogroups in indigenous and historical Canary Island populations are shown in Table 1. The autochthonous N African E-M81 haplogroup was the most abundant type in the indigenous sample (26.7%). It is also the most common in NW Africa (64%) with its highest frequency in the Western Sahara (76%) [17,34]. The E-M81 marker is rare outside N Africa and its presence in the Iberian Peninsula has mainly been considered a result of Moorish influence [5,17]. In the historical sample, the E-M81 frequency was 11.9%, more similar to that found in the current Canary Islands (8.3%) than to the indigenous sample (26.7%). Taking into account the low frequency of this haplogroup in sub-Saharan Africa, its presence in the historical sample could be better explained by indigenous persistence than by later trade in sub-Saharan slaves. However, it is also to be expected that some E-M81 line-

Table 1: Y-chromosome haplogroup frequencies in the studied populations

HG	MARKER	ABO	CON	HIE[1]	PAL[1]	GOM[1]	TFE[1]	GCA[1]	LAN[1]	FUE[1]	CAN[1]	NWA[2,3]	SAH[2,3]	NCA[4]	IBE[5]
ADC	-														0.15
B	M60														
E*	M96						0.56				0.15	0.45			0.76
E1a*	M33	3.33	2.38						1.03	2.67	0.46	1.82	8.99	0.99	0.46
E1b1a*	M2		4.76	4.25	2.35			1.28	1.03		0.92	4.55	11.24	0.99	0.31
E1b1b1*	M35											4.09		2.97	
E1b1b1a*	M78	23.33	11.9	6.38	2.35	4.35	3.37	3.85	3.09	2.67	3.53	6.82		5.94	2.44
E1b1b1b*	M81	26.67	11.9	2.13	5.88	4.35	10.68	11.54	6.19	13.33	8.28	65.00	59.55	39.1	5.19
E1b1b1c1*	M34			2.13	3.53	2.17	3.93	2.56			2.30			2.97	1.68
F*	M89											0.91		6.44	0.31
G*	M201		2.38	4.25	2.35	5.44	3.93	3.85	5.16	2.67	3.99	0.91			4.27
I*	M170	6.67		2.13	9.41	20.65	7.30	6.41	13.40	5.33	9.66	0.45			9.77
J1*	M267	16.67	11.90	4.25	2.35	7.61	1.12	1.28	3.09	8.00	3.53	5.00	20.22	29.2	2.14
J2*	M172		2.38	14.89	14.12	10.87	7.30	7.69	12.37	10.67	10.43	4.09		3.47	7.02
K*	M9	10.00			4.71	1.09	6.18	5.13	1.03	1.33	3.37	1.82		0.99	3.21
P*	M45	3.33												0.50	0.46
R1a	M17		9.52	2.13	2.35	2.17	2.25	1.28	4.12	5.33	2.76			0.50	1.83
R1b1b2	M269	10.00	42.88	57.46	50.60	41.30	53.38	55.13	49.49	48.00	50.62	4.09		5.94	60.00
Sample		30	42	47	85	92	178	78	97	75	652	221	89	202	655

[1]Flores et al. 2001; [2]Bosch et al. 2001; [3]Flores 2001; [4]Arredi et al. 2004; [5]Flores et al. 2004
Comparison of Y-chromosome haplogroup frequencies (%) among the indigenous (ABO), historical (CON), and extant samples from the seven Canary Islands (Lanzarote: LAN; Fuerteventura: FUE; Gran Canaria: GCA; Tenerife: TFE; Gomera: GOM; La Palma: PAL; Hierro: HIE), total Canaries sample (CAN) and current Northwest African (NWA), North Central African (NCA), Saharan (SAH) and Iberian Peninsula (IBE) populations.

ages reached the islands due to the minor NW African slave-trade. The notable E-M81 frequency decrease in the historical sample, relative to the indigenous one, is in agreement with a strong European replacement of the indigenous males at the beginning of the conquest [5].

Congruently, the European R-M269 haplogroup was already the most frequent in the historical sample (42.9%). R-M269 reaches 60% in the Iberian peninsula [35] but is found at a low frequency in NW Africa (4–6%), and seems to have been introduced there from Europe in historical times [17]. Its frequency in the extant Canary population (53.2%) is similar to that found in the Iberian Peninsula, pointing to a mainly European origin for the present-day male pool in the Canaries [5]. The fact that a similar frequency has been found in the historical sample, again points to a strong European replacement of the male indigenous pool since the early conquest period. Surprisingly, R-M269 was also found in the indigenous sample in a moderate frequency (10%). Its presence in the indigenous people could be explained in two ways: (a) R-M269 was introduced into NW Africa in prehistoric not historical times, or (b) the presence of this marker in the aborigines was due to a prehispanic European gene flow

BMC Evolutionary Biology 2009, **9**:181 http://www.biomedcentral.com/1471-2148/9/181

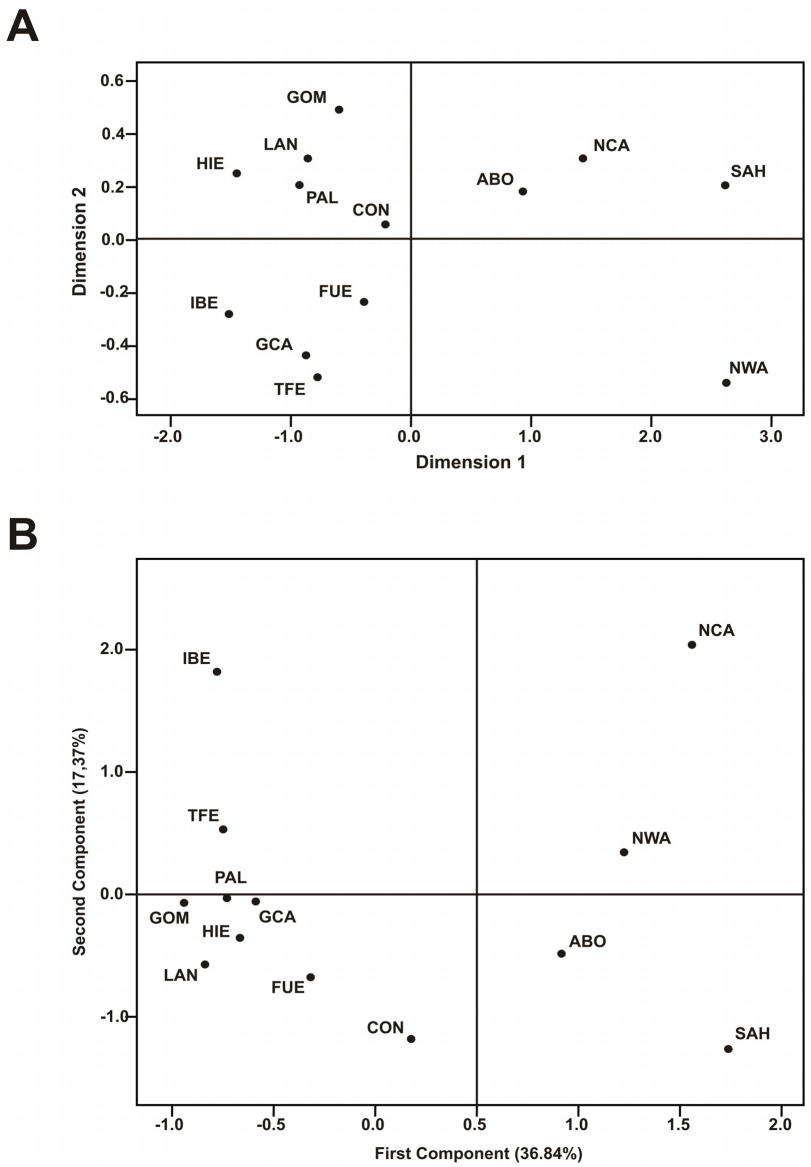


Figure 1
MDS and PCA graphical representations. A) MDS plot based on pairwise Fst genetic distances. B) PCA results based on haplogroup frequencies. Population codes are as in Table 1.

Table 2: F_{ST} distances between populations based on Y-chromosome haplogroup frequencies

	ABO	CON	FUE	LAN	GCA	TFE	GOM	HIE	PAL	NWA	SAH	NCA	IBE
ABO	-												
CON	**0.083****	-											
FUE	**0.135****	0.000	-										
LAN	**0.177****	**0.021***	0.000	-									
GCA	**0.188****	0.018	0.000	0.001	-								
TFE	**0.187****	**0.022***	0.002	0.003	0.000	-							
GOM	**0.133****	**0.030***	0.014	0.000	**0.024***	**0.023****	-						
HIE	**0.233****	0.022	0.005	0.002	0.000	0.005	**0.029***	-					
PAL	**0.181****	**0.023***	0.000	0.000	0.000	0.000	0.008	0.000	-				
NWA	**0.160****	**0.363****	**0.383****	**0.470****	**0.463****	**0.443****	**0.441****	**0.588****	**0.480****	-			
SAH	**0.140****	**0.329****	**0.364****	**0.457****	**0.468****	**0.449****	**0.409****	**0.579****	**0.469****	**0.032****	-		
NCA	**0.040***	**0.158****	**0.196****	**0.264****	**0.273****	**0.273****	**0.223****	**0.319****	**0.269****	**0.096****	**0.050****	-	
IBE	**0.285****	**0.046****	**0.017***	0.008	0.000	0.004	**0.035****	0.003	0.006	**0.565****	**0.583****	**0.381****	-

*p < 0.05; **p < 0.01; ***p < 0.001
Codes as in Table 1.

into the indigenous population. As NW African R-M269 chromosomes showed close STR-similarity to the Iberian ones [17], pointing to recent contacts between both regions, the second option appears more plausible.

A sub-Saharan component is detected in both indigenous (3.3%) and historical (7.1%) samples. E-M33 was the only sub-Saharan marker found in aborigines. In Africa, its highest frequencies have been detected in Southern (51%) and Central areas (57%) [17,36]. However, as its frequencies in North-Central Moroccan Berbers (3.2%) and in Saharan people (3.5%) [34] are similar to that found in the indigenous sample, its prehispanic presence in the islands could be due to the same NW African colonization that brought E-M81. E-M33 was also detected in the historical population (2.4%) which, together with E-M81, could indicate a moderate indigenous Y-lineage persistence in the 17th–18th centuries. Although its presence could also be the result of the later sub-Saharan slave trade, its limited frequency in the Gulf of Guinea [17], the main source of slaves, makes this second option less probable. The E-M2 branch is another sub-Saharan haplogroup [37,38] present in the historical sample (4.76%). It reaches its highest frequency in Mali and has been proposed as a marker of the Bantu expansion [38]. So, its presence in the 17th–18th century population could indicate direct influence due to slavery. In fact, it is well documented that, for instance, in Gran Canaria more than 10,000 slaves were introduced during the 16th century. The majority of these slaves came from regions of sub-Saharan Africa [39] where E-M2 is the most abundant Y-chromosome haplogroup [17,36]. E-M2 is also present in

NW African populations [17,34] so, although this marker was not detected in our small indigenous sample, a prehispanic NW African origin cannot be ruled out.

Some additional haplogroups detected in indigenous and/or historical Canaries samples (M78, M172, M173, M201 and M267), appear in the Iberian Peninsula as well as in NW Africa [17,34-36,40-47]. Nevertheless, M78 and M267, which are more abundant in the latter (Table 1), have a higher frequency in the indigenous sample (23.3% and 16.7%, respectively) than in the 17th–18th centuries population (11.9% in both cases), which is again in accordance with a NW African origin for the prehispanic colonizers of the islands.

Due to the low variance of J-M267 in N Africa compared to that in the Middle East, its presence in the former has been related to the Arab expansion in the 7th century A.D. [36]. However, if the arrival of the indigenous people in the islands was around 1,000 years B.C. [48], the presence of J-M267 in NW Africa could be previous to the Arab expansion. Alternatively, this marker might have reached the islands with a second wave of colonists.

Similarly to E-M81, the frequencies of E-M78 and J-M267 decrease in the historical and present-day Canary populations, again highlighting the strong demic impact of the European colonists before the 17th–18th centuries. On the other hand, haplogroups with a comparatively higher European presence such as M172, M201 and M173 (comprising SRY1532 and M17) were only detected in the historical sample, therefore, they most probably reached the islands after the European conquest.

The presence of the I-M170 haplogroup in the indigenous sample (6.7%) deserves special attention. This haplogroup is the only major clade of the Y-chromosome phylogeny that is widespread over Europe and almost absent elsewhere, suggesting that it originated there [49]. It is especially abundant in the eastern Mediterranean area, with its highest frequencies in the Balkans [50]. Therefore, the presence of this European Y-chromosome lineage in the indigenous pool is compatible with a direct Mediterranean input, or to a more ancient demic influx from Europe to N Africa than has yet been proposed [17].

Genetic distances and AMOVA

In order to detect genetic differences between populations, pairwise F_{ST} comparisons (Table 2) were carried out. It was found that the indigenous Canary Island population has its highest affinities with N Central Africa ($p = 0.01$) and with the historical population ($p = 0.002$), compared to the rest of the samples ($p < 0.0001$). In turn, the historical sample was more closely related to the present-day Canary populations (from $p = 0.43$ to $p = 0.02$) than to the Iberian Peninsula ($p = 0.003$), being highly divergent from Africa ($p < 0.0001$). These relative relationships are graphically represented in the bidimensional plot of the multidimensional scaling (MDS) analysis performed with the F_{ST} distance matrix (Figure 1a). The indigenous sample is halfway between N Central Africa and the 17th–18th century sample; the latter standing closer to the present-day Canary populations and to the Iberian Peninsula. Results from the principal component analysis (PCA) are highly congruent with the MDS plot (Figure 1b). The only discrepancy is that, in this case, the indigenous sample is closer to NW Africa than to N Central Africa. The first principal component (accounting for 37% of the whole variance) clearly separates Canarian aborigines and Africans from the present-day Canary and Iberian samples, leaving the historical sample in an intermediate position. Haplogroups E-M81 and J-M267 on the one hand, and R-M269, G-M201 and J-M172 on the other, are mainly responsible for these positive and negative displacements. Additionally, in the second component (17% of the whole variance) the sub-Saharan haplogroup E-M96 is the main source of the positive displacements of the Iberian peninsula and N Central Africa from the present-day Canary Islanders and the group composed by NW African, indigenous and Saharan populations, respectively. On the negative side, the sub-Saharan E-M2 and E-M33 haplogroups clearly make the Eastern islands of Fuerteventura and Lanzarote closer to

Table 3: Admixture estimations

		Iberian peninsula	Aborigines	Sub-Saharan Africa
Canarian males				
	17th–18th centuries	63.2 ± 14.5	31.1 ± 14.0	5.8 ± 4.5
	Present day	83.0 ± 4.7	16.1 ± 4.6	0.9 ± 0.7
Canarian females				
	17th–18th centuries	47.9 ± 23.3	39.9 ± 22.9	12.2 ± 6.5
	Present day	55.4 ± 15.6	41.8 ± 15.8	2.8 ± 3.7

Relative indigenous, Iberian and West sub-Saharan African contributions to the 17th–18th century and present-day Canary Islands populations, were estimated based on Y-chromosome and mtDNA haplogroup frequencies in the three paternal populations.

the historical sample, and the aborigines to the Sahara (Figure 1b).

AMOVA analyses were performed to assess the relative amount of variance attributed to differences among and within natural geographic areas. When the indigenous and historical samples were included within the Canarian group, 80% of the total variance was observed within populations (F_{ST}), 3% among populations within groups (F_{SC}) and 17% among groups (F_{CT}). However, when the indigenous sample was removed from the analysis or grouped with N Africa, F_{CT} increased to 18%, whereas F_{SC} decreases to 2.4%. When, in addition to the aborigines, the historical sample was also removed, the variance partition values did not change. These results indicate, once more, that the indigenous sample is comparatively more similar to the N African than to the present-day Canary population, while the C 17th–18th historical sample shows more affinities with the modern Canary Island population.

Male vs. female contributions

To explain the current demographic composition of the Canaries, in previous genetic approaches their present-day inhabitants were considered as a hybrid population with a NW African substrate, contributed most probably by Berber indigenous founders, a substantial European input and, to a lesser degree, a sub-Saharan African component, introduced after the conquest as slaves. Consequently, admixture estimates were calculated using present-day Iberian, NW African and sub-Saharan W African populations as parental sources. Results based on uniparental markers have provided contradictory evidence pointing to a considerable sexually asymmetric contribution, with a predominant (92%) male European contribution [5] and a high (33–43%) NW African female component [4,51]. More recently, the maternal indigenous substrate has been directly estimated from ancient remains [24] showing a higher indigenous contribution (42–73%) than that estimated when the present-day NW African mtDNA gene pool was used. Admixture proportions based on mtDNA were also calculated for the 17th–18th century population of Tenerife [25] and a higher sub-Saharan African influence was found (14%) than in the present-day population (5%).

The Y-chromosome data obtained in the present study, from the same indigenous and historical populations, allowed a similar direct analysis of the male gene-pool (Table 3). Iberian males appear as the main contributors to the extant Canary population (83.0% ± 4.7%) but to a lesser extent than the indirect estimation (92%). Accordingly, the indigenous component (16.1% ± 4.6%) is also higher than before (7%), whereas the sub-Saharan input was similar (1%). When these indigenous and sub-Saharan male contributions were compared to their respective female contributions, a significant sex bias favoring indigenous ($p < 0.01$) and sub-Saharan ($p < 0.05$) female persistence was observed. As for the 17th–18th century historical population, although the main contribution was already Iberian (63.2 ± 14.5%), at that time the indigenous (31.1 ± 14.0) and sub-Saharan (5.8 ± 4.4) influences were greater than today but, in this case, male and female contribution comparisons did not reach significant differences. Nevertheless, these results have to be taken with caution because the historical sample has been taken from a single burial in Tenerife, whereas the indigenous sample is made up of several archeological sites from different islands.

Conclusion

The presence of autochthonous North African E-M81 lineages, and also other relatively abundant markers (E-M78 and J-M267) from the same region in the indigenous population, strongly points to that area as the most probable origin of the Guanche ancestors. This is in accordance with previous genetic studies performed on the same material at mtDNA level [24], and in support of the cultural connections found between the Berbers and the indigenous islanders people [9,15,52]. In addition to this mainly NW African colonization, the detection in the indigenous sample of markers like I-M170 and R-M269 of clear European ascription might suggest that other secondary waves also reached the Archipelago, most likely from the Mediterranean basin. This would again be in agreement with the multiple settlement theory proposed to explain the physical and cultural diversity found between and within the different islands [3,52]. However, as these markers are also present in N Africa, albeit in low frequencies, it could be that they arrived in the islands during the same African wave(s) that brought E-M81 and reached relatively high frequencies there due to founder and genetic-drift effects. If so, the presence of these markers in N Africa may be older than previously proposed [17].

Compared to the original natives, the 17th–18th century historical sample mainly differs by harboring lower frequencies of NW African haplogroups ($p < 0.05$), such as E-M81 (11.9% vs 26.7%), E-M78 (11.9% vs 23.3%) and J-M267 (11.9% vs 16.7%), and higher frequencies for European haplogroups ($p < 0.001$) like R-M269 (42.9% vs 10.0%) or R-M173, (9.5% vs 0.0%). A notable exception was I-M170 because it was not detected in the historical sample, despite being moderately frequent in the aborigines (6.7%).

Different founder effects on different islands could be a plausible explanation, since all the natives carrying I-M170 were from Gran Canaria, whereas the historical

sample was taken from Tenerife. Another difference between these two samples is the higher, albeit not significant, frequency of sub-Saharan lineages (7.1% vs 3.3%) in this historical population. However, these differences were not detected at mtDNA level [24,25], as the NW African haplogroup U6 (10.2% vs 10.0%) and the most abundant and widespread European haplogroup H (46.9% vs 52.1%) showed similar frequencies in both samples. The sharp and swift change observed for the indigenous male and female genetic pools can be satisfactorily explained if it is accepted that indigenous females were reproductively more successful after the conquest than males, who were displaced by male European colonizers. Although sampling bias and drift effects could also explain these differences, the genetic data corroborate historical chronicles that narrated frequent mass killings and deportations of mainly males during the conquest [1,9]. Even after that first violent period, the better social and economical position held by the Europeans continued to favor their mating with indigenous females.

The asymmetric sexual evolution of the mixed population is also corroborated when quantitative admixture estimates are independently applied to their female and male genetic pools at different times (Table 3). The Iberian contribution to the male genetic pool increases from 63% in the 17th–18th centuries to 83% in the present-day population, which is accompanied by a parallel dropping of the male indigenous (31% vs 17%) and sub-Saharan (6% vs 1%) contributions. However, relative proportions in the female pool are strikingly constant for Iberians (48% vs 55%) and aborigines (40% vs 42%), from the 17th–18th centuries to the present [53], and only the sub-Saharan female contribution shows an important decrease (12% vs 3%).

These results indicate that indigenous males were negatively discriminated, not only at the beginning of the conquest but also afterwards. In the case of the sub-Saharan lineages, it seems that their mating disadvantage affected both sexes, although more so in males.

It has been stated that the Canary Islands served as a laboratory for the later conquest and settlement of the American Continent by the Spaniards [54,55]. In fact, recent genetic studies on Iberoamerican populations [56-58] have also detected considerable sexual asymmetry, showing that the European male contribution to their present-day genetic pools is significantly greater than the female, as happens in the Canary Islands. Ironically, autochthonous male M81 and female U6 lineages from the Canaries have also been detected in Iberoamerica [57], demonstrating that Canary Islanders with indigenous ancestors actively participated in the American colonization.

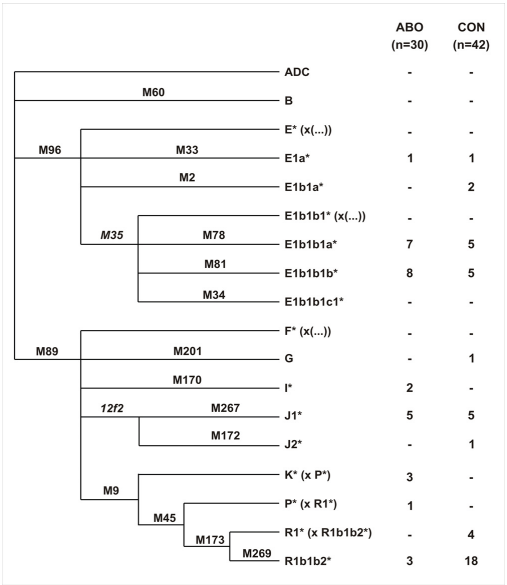


Figure 2
Y-chromosome tree of haplogroups and absolute frequencies for each population. Y-chromosome tree, taken from Karafet et al. (2008), representing the genealogical relationships of the haplogroups characterized in this study, using diagnostic SNPs and their absolute frequencies in the indigenous (ABO) and historical (CON) samples from the Canaries.

Methods

Samples

Samples used in this study were excavated by different authorized archeological teams. The material ceded to perform molecular analyses consisted, in all cases, of teeth without fractures. Whenever possible, teeth were directly taken from their mandible alveolus. A total of 643 teeth corresponding to 493 different individuals were analyzed. This material belonged to different indigenous burials sampled from six of the seven islands: Fuerteventura (13 teeth from 10 individuals), Gran Canaria (230 teeth from 115 individuals), Tenerife (45 teeth from 39 individuals), Gomera (62 teeth from 52 individuals), Hierro (44 teeth from 44 individuals) and La Palma (43 teeth from 38 individuals). Calibrated radiocarbon dating was performed in the Beta Analytic Radiocarbon Dating Laboratory (Miami). At least two samples for site were analyzed. Aboriginal remains were clearly pre-conquest for all the analyzed islands: Tenerife (2210 ± 60 to 1720 ± 60 BP), Gomera (1743 ± 40 to 1493 ± 40 BP), Hierro (1740 ± 50 to 970 ± 50 BP) and Gran Canaria (1410 ± 60 to 750 ± 60

BP) [33]. Although the Fuerteventura and La Palma [59] materials were not directly C-14 dated, ceramic types co-excavated with the remains indicate that they were also prehispanic and not older than 1000 years BP. Historical remains, from 17^{th}–18^{th} century, exhumed from La Concepción Church in Tenerife (206 teeth from 195 individuals), were also analyzed. In order to avoid sampling repetitions, individuals from different graves were preferably chosen, and only one type of tooth was taken when more than one individual was sampled in the same grave [25].

Ancient DNA laboratory

To ensure the reliability of the results, strict measures were taken to avoid contamination, as recommended for aDNA work [60,61]. Analyses were performed in three independent aDNA-dedicated laboratories. In the first, the excavated material was decontaminated and processed to obtain powdered samples. In the second, DNA extraction and pre-PCR procedures were carried out. PCR amplifications were performed in a third area. Finally, post-PCR analyses were done in another physically isolated laboratory.

In each aDNA dedicated area, all personnel were required to wear lab-coats, face-shields, hats and multiple pairs of gloves. The equipment and work areas were constantly irradiated with UV lamps and frequently cleaned with bleach. All sample manipulations were performed in laminar flow cabinets, with dedicated pipettes and sterile filter tips (Tip One, Star Lab). Solutions were commercially acquired whenever possible; otherwise, they were autoclaved and UV-treated. All metallic material was sterilized in an oven at 200°C for at least 4 h.

Ancient DNA extraction

Initial decontamination steps were carried out on all samples prior to extraction. Teeth were thoroughly washed with 15% HCl, rinsed with UV-treated ddH_2O and exposed to UV light for 10 min. In order to reconstruct teeth after extractions, they were transversely cut through the mid-line, using a dental electric saw, and the internal pulp and dentine drilled out using a dental drill. The powder was collected in 1.5 ml sterile tubes and DNA extracted according to a modified GuSCN-silica based protocol [24,25,62].

Previous mtDNA and amelogenin analysis

As it there are estimated to be about 3,000 mtDNA molecules per cell [63], previous to the Y-chromosome study all the teeth were analyzed for mtDNA [24]. Those individuals that could not be amplified for mtDNA (35%) were not included in subsequent analysis. The successfully amplified mtDNA samples were sexed using an amelogenin test as previously published [25,33]. For those samples carrying the Y-chromosome specific band, two additional amelogenin typings were performed to confirm the result. When only the female band was amplified, 4 to 5 additional repetitions were carried out, in order to avoid false results due to allelic dropout during the first few PCR cycles [33]. In the indigenous sample, only 49% of the individuals were unequivocally sexed and 17% proved to be male, so 89 teeth from 52 individuals (14.6%) were analyzed for Y-chromosome binary markers. For the historical sample, 56% of the individuals gave results for the amelogenin locus and 34% resulted male, so 67 individuals were included in the Y-chromosome analysis.

Y-SNP selection

Sixteen biallelic markers (M2, M9, M33, M34, M45, M60, M78, M81, M89, M96, M170, M172, M173, M201, M267, M269; see Figure 2), that characterize the most prevalent lineages in NW Africa, Sub-Saharan Africa and Europe, were chosen from the literature [5,17,34-36,38,40]. The Y-SNP haplogroup nomenclature and tree topology, represented in Figure 2, were established following the nomenclature of Karafet et al. 2008 [64].

To amplify the Y-SNPs, primers were designed to define fragments with less than 100 base pairs (bp), as recommended for aDNA studies, using Primer3 software http://primer3.sourceforge.net[65]. Different sets of primers were used for: a) direct SNP amplification, b) primer-extension preamplificaction (PEP), c) nested-PCR reamplification and d) SNaPshot multiplex SNP typing. Primer sequences are shown in Additional files 1, 2 &3.

Real-time PCR quantification

To assess the number of molecules used as template for PCR amplification [66], we used iQ™ SYBR® Green Supermix (BioRad) in an iCycler Thermal Cycler (BioRad). Primers and thermal cycling conditions were as described for Y-SNP amplifications. Tenfold serial dilutions of a purified and quantified standard were included in the experiments to determine the standard curve in order to estimate the initial number of DNA molecules in each sample.

Primer-Extension Preamplification (PEP)

Primer-extension preamplification of the whole genome [67] was carried out using DOP PCR Master Mix Kit (Roche) and following the manufacturer's protocol.

Multiplex preamplification

Multiplex amplification was performed in two different ways. In the first approach, the thirty-two primers of the sixteen markers, detailed in Additional file 1, were used in a PCR multiplex. Subsequently, the sixteen markers were amplified in three different multiplex assays (Additional

file 1). PCR was performed in 10 μl volume, containing 1 μl of 10× Tris-HCl buffer, 200 μM of each dNTP, 5 mM of $MgCl_2$, 1.5 ng of bovine serum albumin (BSA), 1 unit of Taq polymerase (Ecogen), the optimal concentration for each pair of primers (Additional file 1) and 5 μl of DNA extract. When no amplification product was obtained, the DNA extract volume was increased to 7 μl in subsequent PCRs. To overcome PCR inhibition, detectable by the lack of primer-dimers, DNA was reduced to 3 μl and/or the Taq and BSA amounts were doubled. Reactions were submitted to 40 amplification cycles with denaturation at 94°C for 10 s, annealing at 55°C for 10 s and extension at 72°C for 10 s. Extraction and PCR controls were included to detect modern DNA contamination. Ancient female DNA was used as an additional negative control.

Nested-PCR reamplification

Each marker was reamplified using a nested-PCR approach. In these amplifications, one of the previous PCR primers was used together with a newly designed nested-primer (Additional file 2). The PCR was run in 40 μl, containing 4 μl of 10× Tris-HCl buffer, 200 μM of each dNTP, 40 pmoles of each primer, 5 mM of $MgCl_2$, 3 units of Taq polymerase (Ecogen) and 8 μl of 1:200 diluted multiplex PCR product. Reactions were submitted to 40 amplification cycles with denaturation at 94°C for 10 s, annealing at 55°C for 10 s and extension at 72°C for 10 s. A 5 μl aliquot of the PCR product was loaded in 10% acrylamide:bis-acrylamide (19:1) gels, stained with ethidium bromide and visualized under UV to assess the amplification yield.

RFLP analysis

0.5–1 unit of the appropriate restriction enzyme (Additional file 2) was used to directly digest 10 μl of the nested-PCR product under the manufacturers' recommendations. RFLP patterns were resolved on 8% acrylamide:bis-acrylamide (19:1) in 1× TBE buffer and stained with ethidium bromide (1 μg/ml) for 15 min.

Multiplex SNaPshot analysis

Products of each nested PCR were pooled in 0.5-ml sterile eppendorf tubes at comparatively optimal amounts, and ethanol precipitated in order to purify and concentrate the samples in a 10 μl volume. In order to remove any primers and dNTPs left by the previous ethanol precipitation, 1 μl of the concentrated PCR products was treated with 0.5 μl of Exo-SAP-it (USB) and incubated at 37°C for 15 min, followed by heating at 85°C for 15 min to inactivate the enzyme.

The multiplex minisequencing reactions were carried out in a 5 μl final volume containing 1 μl of SNaPshot™ Multiplex Ready Mix (Applied Biosystem) and 1.5 μl of the previously treated PCR products. Concentrations of primers in the reaction mix are specified in Additional file 3. Reactions were submitted to 25 cycles of denaturation at 96°C for 10 s, annealing at 50°C for 5 s, and extension at 60°C for 30 s. Final extension products were treated with 1 μl of SAP (USB) and incubated at 37°C for 1 h, followed by enzyme inactivation by heating at 85°C for 15 min.

For capillary electrophoresis, 0.5 μl purified extension products were mixed with 9 μl Hi-Di™ formamide (Applied Biosystems, (AB)) and 0.5 μl of internal size standard GeneScan-120 LIZ™ (AB). Samples were run on an ABI PRISM 3130 Genetic Analyzer (AB) using POP-7® (AB). Results were analyzed using GeneMapper 4.0 software (AB).

Cloning and sequencing

To check the specificity of the primers, PCR products of each marker were ligated into pGEM-T vectors (Promega). Colonies were plated on selective Amp/IPTG/X-gal plates, and white colonies were selected. Clones were directly sequenced using M13 universal primers. Sequencing reactions were prepared in 10 μl volumes using the BigDye 3.1 Terminator Cycle Sequencing kit (AB) and the products were run on an ABI PRISM 310 Genetic Analyzer (AB).

Contamination prevention and authentication

To avoid modern contaminant DNA during the mtDNA and amelogenin analyses, all the previously reported procedures were followed [24,25,33,62]. In addition, for the Y-chromosome analysis, aDNA was exclusively manipulated by female researchers and all sample analyses were duplicated, in La Laguna, using RFLP assays and in Porto, using SNaPshot analysis.

RFLP analysis on modern populations

In order to make comparisons among populations feasible, after concluding the analysis of all the extant aDNA samples from the Canary Islands [5], samples from the Iberian peninsula [35] and North Africa [68] were additionally typed for M269 marker as previously described [69].

Statistical analyses

The indigenous and historical samples were compared between each other and with each present-day island population, with West Saharan (including Mauritanian samples), NW African (comprising Arabs and Berbers from Morocco) and North Central African populations (including Algerian and Tunisian samples), and with an overall sample from the Iberian Peninsula as detailed in Additional file 5. To make comparisons possible, frequencies were calculated for haplogroups at the same level of SNP resolution as the indigenous and historical samples. Analysis of molecular variance (AMOVA) and pairwise F_{ST} genetic distances based on haplogroup frequencies [70]

were performed using ARLEQUIN 2000 package [71]. Principal component (PC) and multidimensional scaling (MDS) analyses were carried out using the SPSS statistical package 11.5 (SPSS, Inc). Admixture analysis, using Y-chromosome SNPs (k = 18) as alleles of a single locus, was performed using ADMIX 2.0 program [72]. Admixture coefficients and their standard deviations were obtain from 3000 bootstrap replicates. Contingency and Fisher exact tests were used to assess the significance of haplogroup frequency differences. To test the significance of admixture proportions between male and female lineages, we used a significance test of independent proportions [73].

Authors' contributions

The experiments were designed by all the authors. RF and AMG carried out the RFLP analyses, VG and LG the SNaPshot analyses, while VMC and JML analyzed extant samples for M269. All the authors participated in data analysis, discussion of results and drafting the manuscript. All authors read and approved the final manuscript.

Additional material

Additional file 1

***PCR conditions**. Primer sequences, optimal concentrations and product lenghs for the Multiplex PCR assays*
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2148-9-181-S1.xls]

Additional file 2

***PCR and RFLP conditions**. Primer sequences for reamplification PCRs and RFLP assay patterns*
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2148-9-181-S2.xls]

Additional file 3

***SNaPshot conditions**. Single Base Extension (SBE) primer sequences and optimal concentrations for SNaPshot analysis*
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2148-9-181-S3.xls]

Additional file 4

***Y-chromosome haplogroup data**. Y-chromosome haplogroups for male researchers involved in the study*
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2148-9-181-S4.xls]

Additional file 5

***Sample size and references for populations used in this study**. Populations used in analysis*
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2148-9-181-S5.xls]

Acknowledgements

We are grateful to P. Hernández Carpio and A. González and J. Espino from the Servicio de Secuenciación y Genotipado de ADN (SEGAI) for technical assistance. Samples were ceeded by Dirección General de Patrimonio Histórico del Gobierno de Canarias and/or Unidad de Patrimonio del Cabildo de La Palma, El Hierro and La Gomera, with the consent of Museo Canario (Gran Canaria and Lanzarote samples), Unidad de Patrimonio de El Hierro (Hierro samples), Museo Arqueológico de La Palma (La Palma samples), Museo Arqueológico de La Gomera (Gomera samples) and Instituto de Canarias Cabrera Pinto (Tenerife samples). We would like to specially acknowledge the help of M. Arnay, A. Gámez, J.C. Hernández, J. Pais, J. Velasco and T. Delgado. This research was supported by grants from the Spanish Ministry of Science and Technology BFU2006-04490 to J.M. Larruga. R. Fregel is supported by a predoctoral fellowship from the Gobierno de Canarias, and V. Gomes by Fundação para a Ciência e a Tecnologia through grant SFRH/BD/36045/2007.

References

1. Viera y, Clavijo J: **Historia de la Islas Canarias.** vol. Tomo I. Madrid: Editorial Cupsa; 1772.
2. Flores C, Larruga JM, González AM, Hernández H, Pinto FM, Cabrera VM: **The origin of the Canary Island aborigines and their contribution to the modern population: A molecular genetics perspective.** *Current Anthropology* 2001, **42(5):**749-754.
3. Onrubia-Pintado J: **Les cultures préhistoriques des Îles Canaries, état de la question.** *L'Anthropologie* 1987, **91:**653-678.
4. Rando JC, Cabrera VM, Larruga JM, Hernandez M, Gonzalez AM, Pinto F, Bandelt HJ: **Phylogeographic patterns of mtDNA reflecting the colonization of the Canary Islands.** *Ann Hum Genet* 1999, **63(Pt 5):**413-428.
5. Flores C, Maca-Meyer N, Perez JA, Gonzalez AM, Larruga JM, Cabrera VM: **A predominant European ancestry of paternal lineages from Canary Islanders.** *Ann Hum Genet* 2003, **67(Pt 2):**138-152.
6. Mercer J: **The Canary Islanders: their prehistory, conquest, and survival.** London: Rex Collings; 1980.
7. Navarro JF: **Arqueología de las Islas Canarias.** *Espacio, tiempo y forma Serie I, Prehistoria y Arqueología* 1997, **10:**447-478.
8. Verneau R: **Rapport d'une mission scientifique dans l'Archipel Canarien.** *Archives des Missions Sc et Litté 3er sér* 1887, **XVIII:**567-817.
9. Hooton EA: **Preliminary remarks on the Archaeology and Physical Anthropology of Tenerife.** *Am Anthropol New Series* 1916, **18:**358-365.
10. Fusté M: **Algunas observaciones acerca de la antropología de las poblaciones prehistórica y actual de Gran Canaria.** Las Palmas: Ediciones del Museo Canario; 1958.
11. Schwidetzky I, ed: **La población prehispánica de las Islas Canarias.** *Cabildo Insular de Tenerife; Tenerife* 1963.
12. López de Ipiña S, Bermúdez de Castro JM: **Análisis de la variabilidad dental en las poblaciones prehistóricas de Canarias.** *Zainak* 1987, **4:**133-168.
13. Arco MC, Navarro JF: **Historia popular de Canarias. Vol. 1. Los aborígenes.** S/C de Tenerife: Centro de la Cultura Popular Canaria; 1987.
14. Wölfel DJ: **Sind die Ureinwohner der Kanaren ausgestorben?** *Zeitschrift für Ethnologie* 1930, **62:**282-302.
15. Schwidetzky I: **Investigaciones antropológicas en las Islas Canarias: Estudio comparativo entre la población actual y la**

prehispánica. Tenerife: Publicaciones del Museo Arqueológico de Tenerife; 1975.

16. Rando JC, Pinto F, Gonzalez AM, Hernandez M, Larruga JM, Cabrera VM, Bandelt HJ: **Mitochondrial DNA analysis of northwest African populations reveals genetic exchanges with European, near-eastern, and sub-Saharan populations.** *Ann Hum Genet* 1998, **62(Pt 6):**531-550.
17. Bosch E, Calafell F, Comas D, Oefner PJ, Underhill PA, Bertranpetit J: **High-resolution analysis of human Y-chromosome variation shows a sharp discontinuity and limited gene flow between northwestern Africa and the Iberian Peninsula.** *Am J Hum Genet* 2001, **68(4):**1019-1029.
18. Flores C, Maca-Meyer N, Perez JA, Cabrera VM: **The peopling of the Canary Islands: a CD4/Alu microsatellite haplotype perspective.** *Hum Immunol* 2001, **62(9):**949-953.
19. Fregel R, Maca-Meyer N, Cabrera VM, Gonzalez AM, Larruga JM: **Description of a simple multiplex PCR-SSCP method for AB0 genotyping and its application to the peopling of the Canary Islands.** *Immunogenetics* 2005, **57(8):**572-578.
20. Maca-Meyer N, Gonzalez AM, Pestano J, Flores C, Larruga JM, Cabrera VM: **Mitochondrial DNA transit between West Asia and North Africa inferred from U6 phylogeography.** *BMC Genet* 2003, **4:**15.
21. Brakez Z, Bosch E, Izaabel H, Akhayat O, Comas D, Bertranpetit J, Calafell F: **Human mitochondrial DNA sequence variation in the Moroccan population of the Souss area.** *Ann Hum Biol* 2001, **28(3):**295-307.
22. Pereira L, Prata MJ, Amorim A: **Diversity of mtDNA lineages in Portugal: not a genetic edge of European variation.** *Ann Hum Genet* 2000, **64(Pt 6):**491-506.
23. Gonzalez AM, Brehm A, Perez JA, Maca-Meyer N, Flores C, Cabrera VM: **Mitochondrial DNA affinities at the Atlantic fringe of Europe.** *Am J Phys Anthropol* 2003, **120(4):**391-404.
24. Maca-Meyer N, Arnay M, Rando JC, Flores C, Gonzalez AM, Cabrera VM, Larruga JM: **Ancient mtDNA analysis and the origin of the Guanches.** *Eur J Hum Genet* 2004, **12(2):**155-162.
25. Maca-Meyer N, Cabrera VM, Arnay M, Flores C, Fregel R, Gonzalez AM, Larruga JM: **Mitochondrial DNA diversity in 17th–18th century remains from Tenerife (Canary Islands).** *Am J Phys Anthropol* 2005, **127(4):**418-426.
26. Faerman M, Filon D, Kahila G, Greenblatt CL, Smith P, Oppenheim A: **Sex identification of archaeological human remains based on amplification of the X and Y amelogenin alleles.** *Gene* 1995, **167(1–2):**327-332.
27. Stone AC, Milner GR, Paabo S, Stoneking M: **Sex determination of ancient human skeletons using DNA.** *Am J Phys Anthropol* 1996, **99(2):**231-238.
28. Meyer E, Wiese M, Bruchhaus H, Claussen M, Klein A: **Extraction and amplification of authentic DNA from ancient human remains.** *Forensic Sci Int* 2000, **113(1–3):**87-90.
29. Green RE, Krause J, Ptak SE, Briggs AW, Ronan MT, Simons JF, Du L, Egholm M, Rothberg JM, Paunovic M, *et al.*: **Analysis of one million base pairs of Neanderthal DNA.** *Nature* 2006, **444(7117):**330-336.
30. Noonan JP, Coop G, Kudaravalli S, Smith D, Krause J, Alessi J, Chen F, Platt D, Paabo S, Pritchard JK, *et al.*: **Sequencing and analysis of Neanderthal genomic DNA.** *Science* 2006, **314(5802):**1113-1118.
31. Krause J, Lalueza-Fox C, Orlando L, Enard W, Green RE, Burbano HA, Hublin JJ, Hanni C, Fortea J, de la Rasilla M, *et al.*: **The derived FOXP2 variant of modern humans was shared with Neandertals.** *Curr Biol* 2007, **17(21):**1908-1912.
32. Lalueza-Fox C, Rompler H, Caramelli D, Staubert C, Catalano G, Hughes D, Rohland N, Pilli E, Longo L, Condemi S, *et al.*: **A melanocortin 1 receptor allele suggests varying pigmentation among Neanderthals.** *Science* 2007, **318(5855):**1453-1455.
33. Arnay M, González-Reimers E, Fregel R, Velasco-Vázquez J, Delgado-Darias T, González AM, Larruga JM: **Canary islands aborigin sex determination based on mandible parameters contrasted by amelogenin analysis.** *J Archaeol Sci* 2007, **34(9):**1515-1522.
34. Arredi B, Poloni ES, Paracchini S, Zerjal T, Fathallah DM, Makrelouf M, Pascali VL, Novelletto A, Tyler-Smith C: **A predominantly neolithic origin for Y-chromosomal DNA variation in North Africa.** *Am J Hum Genet* 2004, **75(2):**338-345.
35. Flores C, Maca-Meyer N, Gonzalez AM, Oefner PJ, Shen P, Perez JA, Rojas A, Larruga JM, Underhill PA: **Reduced genetic structure of the Iberian peninsula revealed by Y-chromosome analysis: implications for population demography.** *Eur J Hum Genet* 2004, **12(10):**855-863.
36. Semino O, Magri C, Benuzzi G, Lin AA, Al-Zahery N, Battaglia V, Maccioni L, Triantaphyllidis C, Shen P, Oefner PJ, *et al.*: **Origin, diffusion, and differentiation of Y-chromosome haplogroups E and J: inferences on the neolithization of Europe and later migratory events in the Mediterranean area.** *Am J Hum Genet* 2004, **74(5):**1023-1034.
37. Cruciani F, Santolamazza P, Shen P, Macaulay V, Moral P, Olckers A, Modiano D, Holmes S, Destro-Bisol G, Coia V, *et al.*: **A back migration from Asia to sub-Saharan Africa is supported by high-resolution analysis of human Y-chromosome haplotypes.** *Am J Hum Genet* 2002, **70(5):**1197-1214.
38. Underhill PA, Shen P, Lin AA, Jin L, Passarino G, Yang WH, Kauffman E, Bonne-Tamir B, Bertranpetit J, Francalacci P, *et al.*: **Y chromosome sequence variation and the history of human populations.** *Nat Genet* 2000, **26(3):**358-361.
39. Lobo-Cabrera M: **La esclavitud en las Canarias orientales en el siglo XVI.** Las Palmas de Gran Canaria: Excmo. Cabildo Insular de Gran Canaria; 1982.
40. Semino O, Passarino G, Oefner PJ, Lin AA, Arbuzova S, Beckman LE, De Benedictis G, Francalacci P, Kouvatsi A, Limborska S, *et al.*: **The genetic legacy of Paleolithic Homo sapiens sapiens in extant Europeans: a Y chromosome perspective.** *Science* 2000, **290(5494):**1155-1159.
41. Jobling MA, Tyler-Smith C: **The human Y chromosome: an evolutionary marker comes of age.** *Nat Rev Genet* 2003, **4(8):**598-612.
42. Behar DM, Garrigan D, Kaplan ME, Mobasher Z, Rosengarten D, Karafet TM, Quintana-Murci L, Ostrer H, Skorecki K, Hammer MF: **Contrasting patterns of Y chromosome variation in Ashkenazi Jewish and host non-Jewish European populations.** *Hum Genet* 2004, **114(4):**354-365.
43. Cinnioglu C, King R, Kivisild T, Kalfoglu E, Atasoy S, Cavalleri GL, Lillie AS, Roseman CC, Lin AA, Prince K, *et al.*: **Excavating Y-chromosome haplotype strata in Anatolia.** *Hum Genet* 2004, **114(2):**127-148.
44. Cruciani F, La Fratta R, Santolamazza P, Sellitto D, Pascone R, Moral P, Watson E, Guida V, Colomb EB, Zaharova B, *et al.*: **Phylogeographic analysis of haplogroup E3b (E-M215) y chromosomes reveals multiple migratory events within and out of Africa.** *Am J Hum Genet* 2004, **74(5):**1014-1022.
45. Nasidze I, Quinque D, Ozturk M, Bendukidze N, Stoneking M: **MtDNA and Y-chromosome variation in Kurdish groups.** *Ann Hum Genet* 2005, **69(Pt 4):**401-412.
46. Regueiro M, Cadenas AM, Gayden T, Underhill PA, Herrera RJ: **Iran: tricontinental nexus for Y-chromosome driven migration.** *Hum Hered* 2006, **61(3):**132-143.
47. Sengupta S, Zhivotovsky LA, King R, Mehdi SQ, Edmonds CA, Chow CE, Lin AA, Mitra M, Sil SK, Ramesh A, *et al.*: **Polarity and temporality of high-resolution y-chromosome distributions in India identify both indigenous and exogenous expansions and reveal minor genetic influence of Central Asian pastoralists.** *Am J Hum Genet* 2006, **78(2):**202-221.
48. Martínez VMF: **Arqueología prehistórica de África.** Madrid: Editorial Síntesis; 1996.
49. Rootsi S, Magri C, Kivisild T, Benuzzi G, Help H, Bermisheva M, Kutuev I, Barac L, Pericic M, Balanovsky O, *et al.*: **Phylogeography of Y-chromosome haplogroup I reveals distinct domains of prehistoric gene flow in europe.** *Am J Hum Genet* 2004, **75(1):**128-137.
50. Fechner A, Quinque D, Rychkov S, Morozowa I, Naumova O, Schneider Y, Willuweit S, Zhukova O, Roewer L, Stoneking M, *et al.*: **Boundaries and clines in the West Eurasian Y-chromosome landscape: insights from the European part of Russia.** *Am J Phys Anthropol* 2008, **137(1):**41-47.
51. Pinto F, Gonzalez AM, Hernandez M, Larruga JM, Cabrera VM: **Genetic relationship between the Canary Islanders and their African and Spanish ancestors inferred from mitochondrial DNA sequences.** *Ann Hum Genet* 1996, **60(Pt 4):**321-330.
52. González R, Tejera A: **Los aborígenes canarios: Gran Canaria y Tenerife.** Oviedo: Ediciones Itsmo; 1990.
53. Maca-Meyer N: **Composición genética de poblaciones históricas y prehistóricas humanas de las Islas Canarias.** La Laguna: Universidad de La Laguna; 2002.

54. Albulafia D: **The discovery of mankind: Atlantic encounters in the age of Columbus.** New Heaven: Yale University Press; 2008.
55. Elliot J: **Empires of the Atlantic World: Britain and Spain in America 1492–1830.** New Heaven: Yale University Press; 2006.
56. Bonilla C, Bertoni B, Gonzalez S, Cardoso H, Brum-Zorrilla N, Sans M: **Substantial Native American female contribution to the population of Tacuarembo, Uruguay, reveals past episodes of sex-biased gene flow.** *Am J Hum Biol* 2004, **16(3):**289-297.
57. Mendizabal I, Sandoval K, Berniell-Lee G, Calafell F, Salas A, Martinez-Fuentes A, Comas D: **Genetic origin, admixture, and asymmetry in maternal and paternal human lineages in Cuba.** *BMC Evol Biol* 2008, **8:**213.
58. Salas A, Jaime JC, Alvarez-Iglesias V, Carracedo A: **Gender bias in the multiethnic genetic composition of central Argentina.** *J Hum Genet* 2008, **53(7):**662-674.
59. Martín E: **La Palma y los auaritas.** Santa Cruz de Tenerife: Centro de la Cultura Popular Canaria; 1992.
60. Cooper A, Poinar HN: **Ancient DNA: do it right or not at all.** *Science* 2000, **289(5482):**1139.
61. Paabo S, Poinar H, Serre D, Jaenicke-Despres V, Hebler J, Rohland N, Kuch M, Krause J, Vigilant L, Hofreiter M: **Genetic analyses from ancient DNA.** *Annu Rev Genet* 2004, **38:**645-679.
62. Casas MJ, Hagelberg E, Fregel R, Larruga JM, Gonzalez AM: **Human mitochondrial DNA diversity in an archaeological site in al-Andalus: genetic impact of migrations from North Africa in medieval Spain.** *Am J Phys Anthropol* 2006, **131(4):**539-551.
63. Iborra FJ, Kimura H, Cook PR: **The functional organization of mitochondrial genomes in human cells.** *BMC Biol* 2004, **2:**9.
64. Karafet TM, Mendez FL, Meilerman MB, Underhill PA, Zegura SL, Hammer MF: **New binary polymorphisms reshape and increase resolution of the human Y chromosomal haplogroup tree.** *Genome Res* 2008, **18(5):**830-838.
65. Rozen S, Skaletsky H: **Primer3 on the WWW for general users and for biologist programmers.** *Methods Mol Biol* 2000, **132:**365-386.
66. Alonso A, Martin P, Albarran C, Garcia P, Garcia O, de Simon LF, Garcia-Hirschfeld J, Sancho M, de La Rua C, Fernandez-Piqueras J: **Real-time PCR designs to estimate nuclear and mitochondrial DNA copy number in forensic and ancient DNA studies.** *Forensic Sci Int* 2004, **139(2–3):**141-149.
67. Zhang L, Cui X, Schmitt K, Hubert R, Navidi W, Arnheim N: **Whole genome amplification from a single cell: implications for genetic analysis.** *Proc Natl Acad Sci USA* 1992, **89(13):**5847-5851.
68. Flores C: **Composición genética y posible origen paterno de las poblaciones humanas canarias, deducidos de su polimorfismo en el cromosoma Y.** In *PhD thesis* La Laguna: Universidad de La Laguna; 2001.
69. Alonso S, Flores C, Cabrera V, Alonso A, Martin P, Albarran C, Izagirre N, de la Rua C, Garcia O: **The place of the Basques in the European Y-chromosome diversity landscape.** *Eur J Hum Genet* 2005, **13(12):**1293-1302.
70. Slatkin M: **A measure of population subdivision based on microsatellite allele frequencies.** *Genetics* 1995, **139(1):**457-462.
71. Schneider S, Roessli D, Excoffier L: **Arlequin ver. 2000: A software for population genetics data analysis.** Switzerland: Genetics and Biometry Laboratory, University of Geneva; 2000.
72. Dupanloup I, Bertorelle G: **Inferring admixture proportions from molecular data: extension to any number of parental populations.** *Mol Biol Evol* 2001, **18(4):**672-675.
73. Zar JH: **Biostatistical Analysis.** 2nd edition. New Jersey: Prentice Hall .

4.6. Estudio del grupo sanguíneo AB0 mediante PCR-SSCP multiplex y su aplicación al estudio del poblamiento de las Islas Canarias.

En este trabajo presentamos un método simple y económico para el genotipado del locus ABO, basado en amplificación por PCR multiplex y análisis mediante polimorfismos conformacionales de cadena sencilla. Además, hemos aplicado este método al estudio del poblamiento de las Islas Canarias, analizando un total de 2.200 cromosomas tanto en el Archipiélago Canario, como en sus poblaciones parentales (Península Ibérica y noroeste de África).

En las poblaciones parentales se observa que el alelo *A101* es más abundante en la Península Ibérica que el noroeste africano, mientras que, el alelo *B101* es más frecuente en esta área que en la Península. Las Islas Canarias tienen unas frecuencias similares a las peninsulares para el alelo *A101*, excepto en el caso de Gran Canaria y La Gomera, que son más parecidas a las del noroeste africano. Respecto al alelo *B101*, su frecuencia en Canarias cubre los valores medios entre la de la Península Ibérica y la del norte de África. Por otro lado, la proporción entre la frecuencia de los alelos *O01* (*O101* en la nomenclatura de Yamamoto (2000)) y *O02* (*O201*) también es diferente en las dos poblaciones parentales: en el noroeste africano el valor de la relación se aproxima a 1, mientras que en la Península Ibérica es próximo a 2. En Canarias, esta proporción tiene un valor medio de 1,66, oscilando entre 2,18 (Tenerife) y 1,14 (El Hierro). Para los alelos *O12* (*O210*) y *O03* (*O303*), considerados alelos poco frecuentes previamente detectados en vascos y bereberes, se confirma su presencia tanto en la población canaria como en otras regiones de la Península Ibérica.

Respecto al proceso de colonización de las Islas Canarias, el alelo *B101* muestra una correlación negativa ($R=-0.822$, $p=0.023$) entre la distancia geográfica a la costa africana y su frecuencia alélica insular. Este resultado puede explicarse suponiendo una colonización aborigen que llegó en primer lugar a las islas orientales, más cercanas al continente africano, y se desplazó de este a oeste siguiendo un modelo de salto escalonado.

Finalmente, la estima de mezcla en la población de las Islas Canarias, usando como parentales a la Península Ibérica y el noroeste africano, señala una contribución peninsular mayoritaria (82.0 ± 0.5%), cuyo valor es similar a los obtenidos con otros marcadores autosómicos.

Sin embargo, en los cálculos realizados isla a isla observamos que existen diferencias dentro del archipiélago. Un caso llamativo es el de La Gomera, isla occidental en la que la contribución del norte de África (61.7 ± 4.3%) es mayoritaria. Este resultado podría explicarse por una mayor pervivencia aborigen, o bien, por efectos de deriva debido al aislamiento.

Además, se observa que las islas del oeste (El Hierro, La Palma y Tenerife) poseen un componente africano insignificante, de manera que sus frecuencias alélicas se pueden explicar con la única participación de la Península Ibérica. En cambio, las islas del este, Gran Canaria (38.7 ± 3.0%), Fuerteventura (24.3 ± 2.4%) y Lanzarote (28.3 ± 1.9%) aún muestran una notable contribución norteafricana. Esta diferencia parece reflejar un mayor impacto de la colonización castellana en las islas del oeste del Archipiélago Canario.

Este trabajo, titulado “Description of a simple multiplex PCR-SSCP method for AB0 genotyping and its aplication to the peopling of the Canary Islands” fue publicado en la revista *Immunogenetics*.

Immunogenetics
DOI 10.1007/s00251-005-0029-6

ORIGINAL PAPER

Rosa Fregel · Nicole Maca-Meyer · Vicente Martínez Cabrera Ana María González · José María Larruga

Description of a simple multiplex PCR-SSCP method for AB0 genotyping and its application to the peopling of the Canary Islands

Received: 25 May 2005 / Accepted: 17 July 2005

Abstract A simple and affordable multiplex polymerase chain reaction–single-strand conformation polymorphism method is proposed for the molecular study of AB0 polymorphisms. Application of this method to the peopling of the Canary Islands, analyzing a total of 2,200 chromosomes, detected that in addition to Berbers and Basques, the rare alleles *0210* and *O303* are also present in the Iberian Peninsula and in the Canary Islands. Allele *B101*, with the highest frequency in Northwest (NW) Africa, shows a negative correlation (R=−0.822, p=0.023) between geographic distances from this continent and insular frequencies, congruent with a main aborigine colonization from East to West still detectable today. Similar to previous autosomal studies, admixture estimations point to a major Iberian contribution (82±0.5%) to the Canary Islands, although, in some islands as La Gomera, the NW African component raised to 62±4.3%.

Keywords PCR-SSCP · Multiplex · AB0 · Human populations · Canary Islands

Introduction

Since its discovery in 1900 (Landsteiner 1901), the AB0 system has played a central role in clinical, forensic, and anthropological studies. In fact, it was the first human gene to be described (Hirszfeld and Hirszfeld 1919). The elucidation of the molecular basis of this locus (Yamamoto et al. 1990) prompted numerous analyses at the DNA level and the discovery of new alleles, some of them with relatively high frequencies in some ethnic groups (Olsson et al. 1997; Roubinet et al. 2001). However, population studies that take into account this new molecular diversity are still relatively scarce (Villa et al. 1996; Ohashi et al. 2004). Perhaps, one of the causes of this delay is the lack of a cheap and simple molecular method to detect these new AB0 polymorphisms in laboratories with limited recourses. Although several methods for molecular AB0 genotyping have been reported (Watanabe et al. 1997; Yip 2000; Doi et al. 2004), they need complex primer mixtures and/or elaborate detection techniques, like silver staining or fluorescence-based single-base primer extensions. Here, we propose a simple and inexpensive polymerase chain reaction–single-strand conformation polymorphism (PCR-SSCP) method based on a post-PCR addition of primers (Almeida et al. 1998) that only needs conventional ethidium bromide (EtBr) staining for its detection. The method allows a multiplex PCR amplification and SSCP analysis in a single-tube procedure and has the potential to identify new AB0 alleles.

We further applied this method to study the genetic composition of the present Canary Islands inhabitants from an AB0 molecular perspective. The peopling of the Canary Islands is of anthropological and genetic interest, since their present-day inhabitants are a complex mixture of people with different ethnic origins, including their primitive inhabitants, today commonly known as Guanches, with a most probable Berber ancestry, Spaniards and other Europeans that, since the end of the fifteenth century conquered and colonized the archipelago, and North and sub-Saharan (SAH) Africans that arrived at the Canary Islands as victims of the Atlantic European slave trade during the sixteenth to eighteenth centuries. Previous serological blood group and enzymatic polymorphism analysis found that the archipelago's variation for these systems were within the European range, with only a minor African contribution (Morilla et al. 1988; Afonso et al. 1989; Pinto et al.

R. Fregel · N. Maca-Meyer · V. M. Cabrera · A. M. González · J. M. Larruga (✉)
Departamento de Genética,
Facultad de Biología,
Universidad de La Laguna,
Avda. Astrofísico Fco. Sánchez, s/n,
38271 La Laguna, Tenerife, Spain
e-mail: jlarruga@ull.es
Tel.: +34-922-318351
Fax: +34-922-318311

N. Maca-Meyer
Unidad de Investigación,
Hospital Universitario NS de Candelaria,
Carretera del Rosario s/n,
38010 Santa Cruz de Tenerife, Spain

1994, 1996). Recent Y-chromosome studies, based on binary and microsatellite markers (Flores et al. 2003), corroborated a main European male origin, with less than 10% of African admixture. In contrast, mtDNA data, based on present-day Canarian samples (Rando et al. 1999), and in historical (Maca-Meyer et al. 2005) and aboriginal (Maca-Meyer et al. 2004a) remains, detected a notable maternal African component (33% for North Africans and 5% for sub-SAH Africans) in the archipelago that, in some islands as La Gomera (GOM), can be higher (Rando et al. 1999; Flores et al. 2001a). These contradictory results point to an important sexual asymmetric contribution, with a predominant male European component and a comparatively high female aboriginal component. Finally, a recent molecular study using *Alu* insertion polymorphisms (Maca-Meyer et al. 2004b) detected congruent intermediate values (23–38%) for the Northwest (NW) African contribution that have to be confirmed with new independent autosomal markers.

Materials and methods

Samples

A total of 508 blood samples, comprising all the seven Canary Islands, were obtained from unrelated healthy blood donors with at least two generations of Canarian ancestors: 130 from Tenerife (TFE), 65 from La Palma (PAL), 66 from GOM, 41 from El Hierro (HIE), 72 from Gran Canaria (GCA), 62 from Fuerteventura (FUE), and 72 from Lanzarote (LAN). Two hundred seven blood samples from NW Africa [42 Berbers (BERs), 69 Moroccans (MORs), 30 Mauritanian (MAU) and 66 SAHs] and 385 from the Iberian peninsula [165 Basques (BASs), 61 from the South (PES), 65 from the North (PEN), 58 from the East (PEE), and 36 of the central area (PEC)] were also analyzed. All DNA samples were extracted from whole blood as in Rudbeck and Dissing (1998).

PCR amplification and SSCP analysis

As there is no consensus terminology for all the identified AB0 alleles, in this paper, we follow the nomenclature proposed by Yamamoto (2000). To distinguish the seven most common alleles (*A101*, *A201*, *O101*, *O201*, *O210*, *O303*, and *B101*), three fragments were amplified. To detect 261 and 297 mutations, primers ABOex6U and ABOex6L (Hummel et al. 2002) were used. For detection of 595, 646, 657, and 681 mutations, we designed primers 11L* (5′atcagtgacttctgcgaccgg3′) and 12R* (5′ggtgcagggtgccgaccag3′). To detect the single C deletion between nucleotides 1059 and 1061 (1060delC), which characterized *A201* allele, we designed primers ABOex7M (5′accaggcggtccggaac3′) and ABOex7P (5′gcagccctcccagagcc3′). First, three independent amplifications, with the same PCR conditions, were carried out in a PTC-100 thermal cycler (MJ Research) in 15-µl reactions containing the following components: 100 ng of template DNA, 16.6 mM $(NH_4)SO_4$, 67 mM Tris–HCl pH 8.8, 2.5 mM $MgCl_2$, 0.2 mM deoxynucleoside 5c-triphosphates (dNTPs), 1.5 pmol of both primers, 1.5 µg of bovine serum albumin (BSA), and 0.5 U Eco*taq* DNA polymerase (Ecogen). The PCR conditions consisted of a first step of 94°C for 2 min, 35 cycles of 15 s at 94°C, 15 s at 55°C, and 15 s at 72°C, and a final extension step of 5 min at 72°C. Following Almeida et al. (1998), 7 µl of each PCR product was mixed with 7 µl of formamide, 10 pmol of both primers, and 3 µl of denaturing loading buffer (Promega). This mixture was heated at 100°C for 5 min, quickly introduced into liquid nitrogen, and then plugged into ice. Similar results were obtained when the tubes were directly plugged into an ice–ethanol mixture. Samples were kept at −20°C until used. Products were resolved on 15% polyacrylamide gel electrophoresis (PAGE) (39:1 acrylamide is to bisacrylamide) for 11L*/12R* and ABOex7M/ABOex7P, and 18% PAGE (39:1 acrylamide is to bisacrylamide) for ABOex6U/ABOex6L, using glycerol (0.5%) and standard 1× Tris–HCl Boric acid ethylenediaminetetraacetic acid (TBE) (0.089 M, 0.089 M, and 10 mM, respectively, pH 8.2). To minimize DNA renaturation during loading, gels and buffers were kept at 4°C before use. For the same reason, gels were run at maximum voltage for the first 3 min and then kept at 20 mA during 2.5 h for 11L*/12R* and ABOex7M/ABOex7P, and 4 h for ABOex6U/ABOex6L. Gels were EtBr-stained for 15 min. Strands with different mobilities were pricked from the gel with a sterile toothpick and transferred to tubes with 10 µl sterile water. Two microliters of this solution were reamplified by PCR, and ammonium acetate was precipitated and sequenced using the dideoxy method (BigDye v3.1 Terminator Cycle sequencing kit). Products were again ammonium-acetate-precipitated and run on an ABI PRISM 310 Genetic Analyzer (Applied Biosystem).

Multiplex PCR-SSCP optimization

With the aim of simplifying the AB0 genotyping by SSCP-PCR, we implemented a multiplex assay. The multiplex amplification was carried out in 50-µl reactions that contained the following components: 100 ng of template DNA, 16.6 mM $(NH_4)SO_4$, 67 mM Tris–HCl pH 8.8, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 5 pmol of each of the six primers, 2.5 µg of BSA, and 1.5 U Eco*taq* DNA polymerase. The PCR conditions consisted of a first step of 94°C for 2 min, 35 cycles of 15 s at 94°C, 15 s at 55°C, and 15 s at 72°C, and a final extension step of 5 min at 72°C. Multiplex product (10 µl) was mixed with 10 µl of formamide, 20 pmol of each of the six primers, and 6 µl of denaturing loading buffer. Samples were run on PAGE 25% (49:1 acrylamide is to bisacrylamide) for 3.5 h at the same conditions as in separate amplifications.

Primer design

Primers 11L*, 12R*, ABOex7M, and ABOex7P were designed using the software Oligo.exe structure ver. 3.4 and Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) program.

Statistical analysis

Arlequin ver. 2000 (Schneider et al. 2000) was used to calculate allele frequencies and to test for Hardy–Weinberg equilibrium by means of an exact test (Guo and Thompson 1992). The genetic differentiation was examined by two complementary methods: (a) hierarchical analysis of molecular variance (AMOVA), determined by Arlequin ver. 2000, and (b) principal component analysis (PCA), as performed using an SPSS ver. 11 package (SPSS, Inc.). Three frequency-based admixture estimators were used to calculate the genetic contribution from Iberians and NW Africans to the Canarian population: m_L (Long 1991), m_R (Roberts and Hiorns 1965), and m_C (Chakraborty et al. 1992). The ADMIX.PAS program (kindly provided by Dr. J. Long) was used to implement m_L, a weighted least-squares estimator that takes into account sampling error and drift. The m_R and m_C estimators were performed using means of the ADMIX 1.0 program (Bertorelle and Excoffier 1998). The m_R, the first proposed frequency-based estimator, is a least-squares estimator that neglects stochastic effects apart from the sampling of the hybrid population. The m_C is a closed form of m_L.

Results

SSCP AB0 genotyping

The SSCP method, utilized here for the AB0 genotyping, avoids the use of radioactive-, fluorescence-, or silver-stain-based techniques for product detection. The addition of primers just before the DNA denaturation step avoids reannealing of the complementary strands and produces double-stranded segments that increases band separation and favors the EtBr incorporation, and therefore allows a simple and fast detection. The patterns of the AB0 genotypes observed by this PCR-SSCP analysis are shown in Fig. 1. The correspondence of each SSCP banding pattern to AB0 genotypes was determined by direct sequencing of each band. Figure 1a and c shows results of independent amplifications, and Fig. 1d presents a multiplex PCR-SSCP routine gel. Although the three instances in which bands with rare migrations appeared resulted in PCR artifacts, we think the technique has enough discrimination power to identify at least a high proportion of putative new alleles. Furthermore, the addition of primers is a versatile strategy with many possible variations. To facilitate the strand separations, only one of the primers can be added (Almeida et al. 1998), one or both of the primers can be 5′-

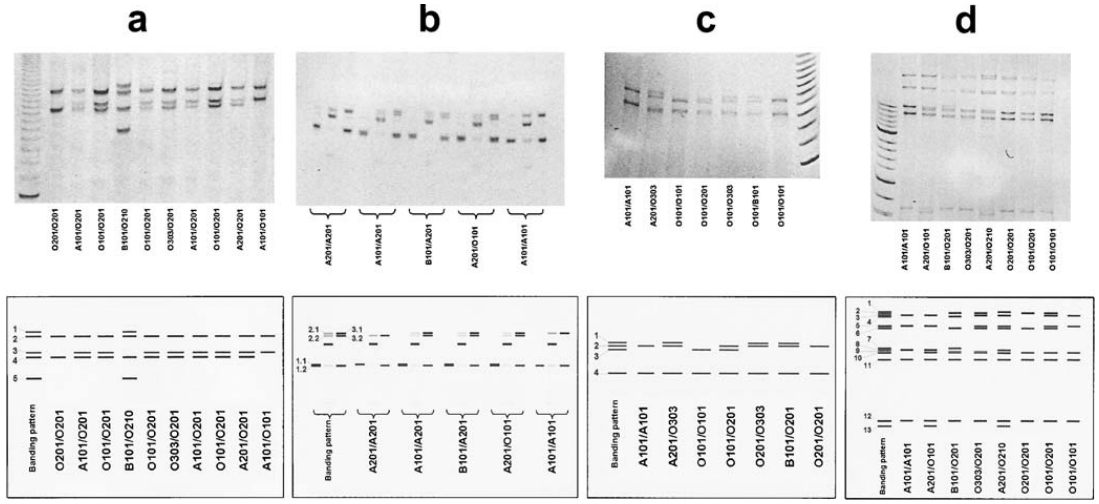


Fig. 1 Gels and schematic drawings showing the SSCP results for the determination of the ABO genotypes. **a** SSCP banding patterns for 11L*/12R* fragment: alleles *A101*, *A201*, *O101*, and *O303* show *bands 2* and *3*, allele *O201* (*bands 2* and *4*), allele *O210* (*bands 1* and *4*), and allele *B101* (*bands 2* and *5*). **b** SSCP banding patterns for ABOex7M/ABOex7P fragment with forward, reverse, and both primers for each sample: allele *A201* shows *bands 1.1*, *2.2*, and *2.3* for each assay, respectively, while the others show *bands 1.2*, *2.1*, and *3.1* in all cases. **c** SSCP banding patterns for ABOex6U/ABOex6L fragment: alleles *A101*, *A201*, *O201*, and *O210* show *bands 2* and *4*, alleles *B101* and *O303* (*bands 1* and *4*) and allele *O101* (*bands 3* and *4*). **d** SSCP banding patterns for multiplex: *A* alleles are characterized by *band 8*, whereas *A101* and *A201* alleles are distinguished by *bands 12* and *13*, respectively. *O101* allele is similar to *A101*, but *band 10* replaces *band 8*. *O201* and *O210* alleles differentiate from *O101* by *band 6*, being further characterized by *bands 2* and *1*, respectively. *B101* and *O303* both show *band 9*; in addition, allele *B101* is further characterized by *bands 4* and *7*, while *bands 3* and *5*, identical to the *A* alleles, identified *O303*, a recombinant allele

Table 1 AB0 frequencies in the Canary Island, Iberian Peninsula, and NW Africa

Allele	TFE	GCA	PAL	HIE	GOM	FUE	LAN	PES	PEC	PEN	PEE	BAS	BER	MOR	MAU	SAH
A101	0.242	0.153	0.200	0.317	0.152	0.266	0.222	0.287	0.306	0.262	0.267	0.194	0.179	0.145	0.117	0.152
A201	0.065	0.056	0.046	0.061	0.061	0.032	0.069	0.057	0.056	0.031	0.069	0.024	0.048	0.072	0.067	0.076
B101	0.073	0.083	0.031	0.024	0.068	0.089	0.069	0.082	0.097	0.062	0.095	0.027	0.107	0.145	0.133	0.152
O101	0.404	0.444	0.485	0.293	0.386	0.315	0.361	0.377	0.361	0.369	0.371	0.445	0.310	0.362	0.333	0.311
O201	0.185	0.222	0.223	0.256	0.311	0.258	0.250	0.189	0.167	0.262	0.190	0.248	0.298	0.232	0.300	0.288
O210	0.019	0.042	0.015	0.024	0.000	0.040	0.021	0.008	0.000	0.000	0.000	0.052	0.036	0.022	0.033	0.008
O303	0.012	0.000	0.000	0.024	0.023	0.000	0.007	0.000	0.014	0.015	0.009	0.009	0.024	0.022	0.017	0.015

tailed, or internal primers might be used instead of, or in addition to, the PCR primers. As an example, Fig. 1b shows the effect of adding only one of the PCR primers or both of them to the ABOex7M/ABOex7P fragment.

The peopling of the Canary Islands

A total of 2,200 chromosomes have been AB0-genotyped to study the peopling of the Canary Islands. All the samples analyzed were in Hardy–Weinberg equilibrium. Allelic frequencies for all the populations sampled are shown in Table 1. Allele *A101* has lower frequencies in NW Africa (0.148±0.022) than in the Iberian Peninsula (0.263±0.038), whereas in the Canaries, it ranges from 0.317 in HIE to 0.152 in GOM. In contrast, allele *B101* has higher frequencies in NW Africa (0.134±0.017) compared to Iberian samples (0.073±0.026). The low frequency of this allele in BASs (0.027) is outstanding. Again, the dispersion range in Canary Islands (from 0.089 in FUE to 0.024 in HIE) covers the mean values of both continental areas. The sum of the *O101* and *O201* alleles conform the highest frequency contribution in all samples. However, the ratio between both alleles shows different geographical distributions: while in NW Africa, it approximates to 1 (1.20±0.21), in the Iberian Peninsula, it nearly reaches 2 (1.88±0.28). In the Canary Islands, the mean value is 1.66±0.46, ranging from 2.18 in TFE to 1.14 in HIE. As a mean, the *O210* allele is more frequent in Africa (0.025±0.011) than in the Iberian Peninsula (0.002±0.003), excepting BASs that show a frequency (0.052) that nearly doubles the NW African value. For this allele, Canary Islands (0.023± 0.013) approximates to Africa. On the contrary, frequencies for *O303* relate Canary Islands (0.009±0.009) more to the Iberian Peninsula (0.009±0.005) than to NW Africa (0.020± 0.004). Contingence tests among the Canarian populations show that they constitute a heterogeneous group (p=0.044). On the contrary, the NW African populations are highly homogeneous for the AB0 system (p=0.96). In the Iberian Peninsula, the BAS sample is highly discrepant from the rest (p=0.001) as when they are excluded; the Iberian populations are very similar between them (p=0.91). AMOVA analysis for the three areas showed that the bulk of the variance is within populations (99.24%). Residual values point to a significant difference only among groups (p=0.010). Pairwise comparisons between populations through F_{ST}-based genetic distances (Table 2) showed that among islands, the small island of HIE is the

Table 2 FST genetic distances between populations

	TFE	GCA	PAL	HIE	GOM	FUE	LAN	PES	PEC	PEN	PEE	BAS	BER	MOR	MAU	SAH
TFE	–															
GCA	0.003	–														
PAL	0.003	0.000	–													
HIE	0.009	0.028**	0.027*	–												
GOM	0.011*	0.002	0.008	0.018*	–											
FUE	0.005	0.014*	0.019*	0.000	0.009	–										
LAN	0.000	0.002	0.006	0.001	0.000	0.000	–									
PES	0.000	0.009	0.008	0.001	0.015*	0.000	0.000	–								
PEC	0.000	0.014	0.014	0.000	0.021*	0.000	0.000	0.000	–							
PEN	0.001	0.008	0.007	0.000	0.003	0.000	0.000	0.000	0.000	–						
PEE	0.000	0.007	0.008	0.002	0.012	0.000	0.000	0.000	0.000	0.000	–					
BAS	0.006*	0.000	0.000	0.021*	0.005	0.013*	0.004	0.011*	0.016*	0.005	0.011*	–				
BER	0.011	0.008	0.021*	0.007	0.000	0.000	0.000	0.010	0.012	0.001	0.006	0.013*	–			
MOR	0.007	0.001	0.015*	0.023*	0.002	0.008	0.001	0.010	0.011	0.008	0.005	0.013*	0.000	–		
MAU	0.015*	0.004	0.021*	0.022*	0.000	0.006	0.001	0.019*	0.022	0.009	0.013	0.015	0.000	0.000	–	
SAH	0.017**	0.012*	0.029**	0.020*	0.001	0.006	0.003	0.017*	0.018*	0.010	0.012	0.024**	0.000	0.000	0.000	–

*p<0.05; **p<0.01

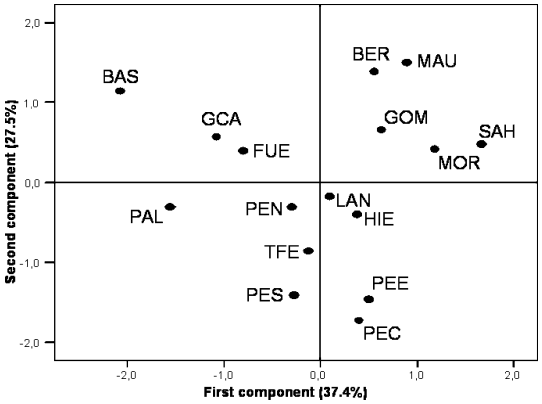


Fig. 2 Plot of the first two principal components of the seven AB0 alleles in Canary Islands and continental populations

most discrepant, and LAN is the most similar to all the others. In the Iberian Peninsula, again, only BASs have significant differences with the rest, excepting the PEN area in which they are geographically included. Congruent with the contingence test results, distances between NW African populations are not significant. Distances between NW African and Iberian populations showed that only the SAH and MAU samples, geographically more distant, are significantly different from some Iberian regions (Table 2). Again, BASs are also peculiar, as they appear highly differentiated from NW Africa. A graphical picture of these relationships can be observed by means of a PCA plot (Fig. 2). The first component, accounting for 37.4% of the variation, clearly points to the BASs as clear outliers. One of the Canary Islands, GOM, groups with NW African populations, whereas TFE clusters with the Iberian populations. Frequencies in alleles *O210* and *O303* are mainly responsible for the positive grouping, and the *A* alleles are mainly responsible for the negative group. The second component, that accounts for 27.5% of the variation, further separates NW Africa from the Iberian Peninsula, while the Canary Islands occupy a central position between both continental areas. In this case, *B101* and *A201* pull samples up, and *O101* and *O210* pull samples down the *Y* axis. Admixture estimates have been obtained using the total Canary Islands sample and each of the islands as the admixed population (Table 3). The three statistics gave rather congruent estimations. For the total Canarian sample, the Iberian contribution is 82.0±0.5%; however, in the western islands (HIE, PAL, and TFE) it seems that the colonization impact was very important, as the NW African component is almost negligible. On the other hand, the eastern islands of GCA (38.7±3.0%), FUE (24.3±2.4%), and LAN (28.3±1.9%) still show a noticeable NW African contribution. An exceptional case is GOM, for which the NW African contribution (61.7±4.3%) is higher than that of the Iberian Peninsula.

Discussion

Basque and BER samples have already been studied for molecular polymorphism of *O* alleles (Roubinet et al. 2001). Alleles *O210* and *O303* were considered rare alleles shared by BERs and BASs, but not encountered in sub-SAH or Amerindian samples. Here, both alleles have also been found in other Iberian samples and in the Canary Islands. As in Roubinet et al. (2001), the *O210* allele is more abundant in BAS (0.052) than in BER (0.025±0.011), whereas the contrary happens with *O303* that is more frequent in BER (0.020±0.003) than in BASs (0.009); curiously, in Iberia, this allele is more abundant in the PEN (0.015) than in the PES (0.000). In an mtDNA study on the colonization of the Canary Islands (Rando et al. 1999), it was suggested that the distribution and variation across the islands of U6 lineages, a specific mtDNA clade of NW African ancestry, was due to one dominant initial settlement process that affected all the islands from east to west. In a more recent study on CD4/Alu haplotypes (Flores et al. 2001b), a significant negative correlation between geographic distances to Africa and insular heterozygosity values was also found, which, in a similar vein, was attributed to a still detectable East to West aborigine colonization. Two AB0 alleles are significantly more abundant in NW Africa (*B101* and *0210*) than in the Iberian Peninsula. Pearson correlations between the islands' distance to Africa and insular frequencies for both alleles also showed negative values (*B101*, R=−0.822; *0210*, R=−0.589), but only the *B101* reached significance (p=0.023; two-tailed test). This result can be explained supposing an aboriginal col-

Table 3 Admixture estimates (±SD) for the Canarian population and for each island

	m_L		m_R		m_C	
	Iberian Peninsula	NW Africa	Iberian Peninsula	NW Africa	Iberian Peninsula	NW Africa
Canary Island	0.816±0.073	0.184±0.073	0.805±0.128	0.195±0.128	0.831±0.113	0.169±0.113
El Hierro	1.104±0.334	0.104±0.334	1.116±0.437	0.116±0.437	1.257±0.390	0.257±0.390
La Palma	1.243±0.181	0.243±0.181	1.082±0.280	0.082±0.280	1.213±0.267	0.213±0.267
La Gomera	0.385±0.288	0.615±0.288	0.294±0.265	0.706±0.265	0.474±0.295	0.526±0.295
Tenerife	0.846±0.156	0.154±0.156	1.056±0.230	0.056±0.230	0.892±0.181	0.108±0.181
Gran Canaria	0.656±0.269	0.344±0.269	0.642±0.267	0.358±0.267	0.539±0.253	0.461±0.253
Fuerteventura	0.805±0.253	0.195±0.253	0.711±0.346	0.289±0.346	0.753±0.282	0.247±0.282
Lanzarote	0.705±0.131	0.295±0.131	0.682±0.229	0.318±0.229	0.763±0.212	0.237±0.212

onization beginning in the nearest island to Africa, going westwards following a step-stone model (Flores et al. 2001b), but can also be explained by a stronger Iberian gene flow on the western islands.

AB0 admixture estimates, obtained using Iberian and NW Africans as the main contributors to the present-day Canarian population, showed an Iberian contribution around 82%, which is not significantly different of the 70% obtained from *Alu* polymorphisms (Maca-Meyer et al. 2004b). Data based on autosomal classical markers (Flores et al. 2001a) gave similar results (65–75%). Furthermore, using the published CD4/*Alu* microsatellite haplotypes (Flores et al. 2001b), we estimated the Iberian contribution to the archipelago as 89±9%. However, quite different percentages were obtained with unisexual markers. As a mean from different studies (Pinto et al. 1996; Rando et al. 1999; Flores et al. 2001a), the female mtDNA NW African contribution was estimated around 40%, ranging from 33 to 60%. On the contrary, the African Y-chromosome contribution was less than 10% (Flores et al. 2003). This sexual disequilibrium was attributed to the asymmetrical migration of the Iberian conquerors to the Canary Islands that, in the early occupation times, was mainly a male migration and to the selective male aboriginal extermination during the violent conquest of the Archipelago (Flores et al. 2001b). Congruent with this asymmetry, autosomal markers show contribution values which are intermediate between the sexual markers. However, these mean values greatly differ between islands. The case of GOM deserves special mention as its inhabitants have, by far, the highest percentage (36%) of the NW African mtDNA U6 cluster (Rando et al. 1999) and also show the highest NW African contribution (62%) estimated with our AB0 data.

In conclusion, although the data presented here are based only on one locus, they fit extremely well into the general picture of the peopling of the Canary Islands obtained from previous studies.

Acknowledgements This research was supported by grants of the Spain Ministry of Science and Technology BMC2001–3511 and Gobierno de Canarias COF2002/015 to V.M.C., and R.F. is supported by a CajaCanarias predoctoral fellowship. The experiments complied with current Spanish law.

Reference

Afonso JM, Hernández M, Larruga JM, Cabrera VM, González AM (1989) Human enzyme polymorphism in the Canary Islands. III. Tenerife island population. Hum Hered 61:543–549

Almeida TA, Cabrera VM, Miranda JG (1998) Improved detection and characterization of mutations by primer addition in nonradioisotopic SSCP and direct PCR sequencing. BioTechniques 24:220–221

Bertorelle G, Excoffier L (1998) Inferring admixture proportions from molecular data. Mol Biol Evol 15:1298–1311

Chakraborty R, Kamboh MI, Nwankwo M, Ferrell RE (1992) Caucasian genes in American Blacks: new data. Am J Hum Genet 50:145–155

Doi Y, Yamamoto Y, Inagaki S, Shigeta Y, Miyaishi S, Ishizu H (2004) A new method for ABO genotyping using a multiplex single-base primer extension reaction and its application to forensic casework samples. Leg Med (Tokyo) 6:213–223

Flores C, Larruga JM, González AM, Hernández M, Pinto FM, Cabrera VM (2001a) The origin of the Canary Island aborigines and their contribution to the modern population: a molecular genetics perspective. Curr Anthropol 42:749–755

Flores C, Maca-Meyer N, Pérez JA, Cabrera VM (2001b) The peopling of the Canary Islands: A CD4/Alu microsatellite haplotype perspective. Hum Immunol 62:949–953

Flores C, Maca-Meyer N, Perez JA, Gonzalez AM, Larruga JM, Cabrera VM (2003) A predominant European ancestry of paternal lineages from Canary islanders. Ann Hum Genet 67:138–152

Guo S, Thompson E (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48:361–372

Hirszfeld L, Hirszfeld H (1919) Serological differences between the blood of different races. The result of researches on the Macedonian front. Lancet ii:675–679

Hummel S, Schmidt D, Kahle M (2002) AB0 blood group genotyping of ancient DNA by PCR-RFLP. Int J Leg Med 116:327–333

Landsteiner K (1901) Über Agglunationserscheinungen normalen menschlichen Blutes. Wien Klin Wochenschr 46:1132–1134

Long JC (1991) The genetic structure of admixed populations. Genetics 127:417–428

Maca-Meyer N, Arnay M, Rando JC, Flores C, González AM, Cabrera VM, Larruga JM (2004a) Ancient mtDNA analysis and the origin of the Guanches. Eur J Hum Genet 12:155–162

Maca-Meyer N, Villar J, Pérez-Méndez L, Cabrera de León A, Flores C (2004b) A tale of aborigines, conquerors and slaves: Alu insertion polymorphisms and the peopling of Canary Islands. Ann Hum Genet 68:600–605

Maca-Meyer N, Cabrera VM, Arnay M, Flores C, Fregel R, González AM, Larruga JM (2005) Mitochondrial DNA diversity in 17th–18th century remains from Tenerife (Canary Islands). Am J Phys Anthropol 127(4):418–426. DOI 10.1002/ajpa.20148

Morilla JM, Afonso JM, Hernández M, Pestano JJ, Larruga JM (1988) Human enzyme polymorphism in the Canary Islands. II. African influence. Hum Hered 38(2):101–105

Ohashi J, Naka I, Ohtsuka R, Inaoka T, Ataka Y, Nakazawa M, Tokunaga K, Matsumura Y (2004) Molecular polymorphism of ABO blood group gene in Austronesian and non-Austronesian populations in Oceania. Tissue Antigens 63:355–361

Olsson ML, Guerreiro JF, Zago MA, Chester MA (1997) Molecular analysis of the 0 alleles at the blood group ABO locus in populations of different ethnic origin reveals novel crossing-over events and point mutations. Biochem Biophys Res Commun 234:779–782

Pinto F, Cabrera VM, Gonzalez AM, Larruga JM, Noya A, Hernandez M (1994) Human enzyme polymorphism in the Canary Islands. VI. Northwest African influence. Hum Hered 44:156–161

Pinto F, Rando JC, López M, Morilla JM, Larruga JM (1996) Blood group polymosphism in the Canary Islands. Gene Geogr 10:171–179

Rando JC, Cabrera VM, Larruga JM, Hernandez M, Gonzalez AM, Pinto F, Bandelt HJ (1999) Phylogeographic patterns of mtDNA reflecting the colonization of the Canary Islands. Ann Hum Genet 63:413–428

Roberts D, Hiorns R (1965) Methods of analysis of the genetic composition of a hybrid population. Hum Biol 37:38–43

Roubinet F, Kermarrec N, Despiau S, Apoil PA, Dugoujon JM, Blancher A (2001) Molecular polymorphism of O alleles in five populations of different ethnic origins. Immunogenetics 53:95–104

Rudbeck L, Dissing J (1998) Rapid, simple alkaline extraction of human genomic DNA from whole blood, buccal epithelial cells, semen and forensic stains for PCR. BioTechniques 25:588–592

Schneider S, Roessli D, Excoffier L (2000) Arlequin ver. 2000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland

Villa A, Drago F, Misto R, Morelati F, Poli F, Sirchia G (1996) ABO genotyping in Italian blood donors. Haematologica 81:492–496

Watanabe G, Umetsu K, Yuasa I, Suzuki T (1997) Amplified product length polymorphism (APLP): a novel strategy for genotyping the ABO blood group. Hum Genet 99:34–37

Yamamoto F (2000) Molecular genetics of ABO. Vox Sang 78 (Suppl 2):91–103

Yamamoto F, Clausen H, White T, Hakomori S (1990) Molecular genetic basis of the histo-blood group ABO system. Nature 345:229–233

Yip SP (2000) Single-tube multiplex PCR-SSCP analysis distinguishes 7 common ABO alleles and readily identifies new alleles. Blood 95:1487–1492

4.7. Evolución temporal de las frecuencias del alelo AB0 en las Islas Canarias: El impacto de la colonización europea

La población aborigen de las Islas Canarias sufrió un profundo cambio cultural y un gran impacto genético tras la conquista europea en el siglo XV. El estudio de marcadores uniparentales, ADNmt y cromosoma Y en las poblaciones actuales detectó una marcada asimetría sexual debida a que los varones aborígenes fueron desplazados por los conquistadores. El estudio de la evolución temporal de un marcador autosómico como el locus AB0 nos permitirá obtener una visión complementaria del efecto de la conquista en la población prehispánica. Para ello, hemos analizado el locus AB0 en la población aborigen y una población histórica (S.XVII-XVIII) de las Islas Canarias.

Al comparar las frecuencias del grupo sanguíneo AB0 de los aborígenes canarios con las poblaciones del norte de África, encontramos que no existen diferencias significativas, lo que reafirma un origen norteafricano para la población prehispánica. De la misma forma, tampoco presenta diferencias significativas con la población histórica ni con La Gomera actual, caracterizada por una mayor aportación norteafricana en el locus ABO. Por el contrario, la población aborigen es significativamente diferente de la Península Ibérica y del resto de poblaciones actuales de las Islas Canarias.

La composición genética para el locus AB0 de la población indígena, refleja su origen norteafricano ya que presenta la frecuencia más baja para el alelo *A101*, la más alta para el alelo B101 y valores similares para los alelos *O01* y *O02*. Además, muestra una frecuencia relativamente alta para el alelo *O03*, que es más abundante en África que en la Península Ibérica.

En la población histórica del S.XVII-XVIII se observa una congruente posición intermedia que testifica un fuerte impacto europeo, ya que, comparada con la población aborigen, posee una frecuencia dos veces mayor para el alelo *A101* y frecuencias menores para los alelos *B101* y *O03*. Sin embargo, también se asemeja al norte de África al presentar una proporción 1:1 en la frecuencias de los alelos *O01* y *O02*.

Si observamos la evolución temporal del locus AB0 en Canarias, el impacto europeo, que ya es patente en la muestra de La Concepción, ha ido en aumento desde entonces hasta la actualidad, afectando a todas las islas con la importante excepción de La Gomera.

Los resultados obtenidos en este trabajo fueron publicados en la revista *Immunogenetics*, con el título "Temporal evolution of the AB0 allele frequencies in the Canary Islands: the impact of the European colonization".

Immunogenetics
DOI 10.1007/s00251-009-0393-8

ORIGINAL PAPER

Temporal evolution of the ABO allele frequencies in the Canary Islands: the impact of the European colonization

Rosa Fregel · Eva Betancor · Nicolás M. Suárez · Vicente M. Cabrera · Jose Pestano · Jose M. Larruga · Ana M. González

Received: 1 June 2009 / Accepted: 30 July 2009

Abstract The indigenous Canary Islands population suffered a strong cultural and genetic impact when they were colonized by Europeans in the fifteenth century. The molecular analysis of the ABO blood group gene on aboriginal and seventeenth to eighteenth century remains confirms the demographic history of the islands depicted by previous archaeological, anthropological, and genetic studies. ABO allele frequencies clearly related Canarian aborigines with North African Berber populations, its most probable source of origin, and is far related to Iberian and to the current population of the archipelago. The historical sample shows a congruent intermediate position testifying already a strong European influence that would go in augment since then to present times, affecting all the islands with the important exception of La Gomera.

Keywords Canary Islands · Colonization · Guanches · ABO group · Ancient DNA

Introduction

In the fifteenth century, as a prelude of their discoveries and colonizations along Africa and the Americas, Portuguese and Spaniards occupied several Northwest African archipelagos as Azores, Madeira, Canary Islands, and Cape Verde, but only the Canary Islands were already found inhabited by white people with a fairly advanced Neolithic culture (Hooton 1916).

The origin and affinities of the indigenous Canary islanders, known as Guanches, were object of scientific curiosity since the beginning of the Spaniard conquest (Viera y Clavijo 1772). Curiously, from a population genetic perspective, the first analysis performed in the Canary Islands was on mummified Guanche remains, not on their living inhabitants. It consisted in an ABO blood group study carried out on tissues from mummies of the main islands of Tenerife and Gran Canaria (Schwarzfischer and Liebrich 1963). For that, authors applied serological techniques previously tested on Amerindian and Egyptian mummies (Boyd and Boyd 1934). The frequency found for the *O* allele was exceptionally high (97% in Gran Canaria and 91% in Tenerife), only comparable to those found in Meknes (91%) and the Ait Haddidu (89%) Berber tribes of the Atlas Mountains of Southern-Central Morocco (Gaud 1942; Messerlin and Lorho 1951), corroborating archaeological and linguistic studies that also showed close affinities between the Guanche and Berber cultures (Navarro 1997). Posterior serological studies on the living Canary population, sampled at different times, showed that ABO allele frequencies kept rather stable (Pinto et al. 1996; Roberts et al. 1966; Rösing 1967). However, the *O* allele frequency ranged only from 60% up to 70% in all the islands, with the exception of La Gomera that displayed the highest frequency of the archipelago (78%). Differences in frequencies between the ancient remains and the living population were explained as result of the European gene flow on the islands after the conquest and/or to technical and contamination problems in the typing of the mummified specimens (Paabo et al. 1989).

Electronic supplementary material The online version of this article (doi:10.1007/s00251-009-0393-8) contains supplementary material, which is available to authorized users.

R. Fregel (✉) · V. M. Cabrera · J. M. Larruga · A. M. González
Department of Genetics, University of La Laguna,
Avda. Astrofísico Fco. Sánchez, s/n,
38271 La Laguna, Tenerife, Spain
e-mail: rfregel@gmail.com

E. Betancor · N. M. Suárez · J. Pestano
Genetics Laboratory, Faculty of Medicine,
University of Las Palmas de Gran Canaria,
Las Palmas, Gran Canaria, Spain

Published online: 20 August 2009

New DNA-based techniques have allowed a more refined analysis of the ABO gene (Doi et al. 2004; Watanabe et al. 1997; Yip 2000). Such a type of analysis was recently applied to the modern Canary Islands population (Fregel et al. 2005), but the allele frequencies obtained were rather similar to those detected in past serological studies, with La Gomera again displaying the highest *O* allele frequency (72%). These techniques have been successfully applied to perform ABO typing on ancient material (Hummel et al. 2002). Its robustness has been recently confirmed with the genetic characterization of the ABO blood group on Neanderthal remains (Lalueza-Fox et al. 2008).

In the present study, these molecular techniques currently used to analyze the ABO blood group system, have been adapted to be assayed on Guanche and historical Canarian remains to follow the temporal variation of this gene in the Canary Islands since pre-Hispanic times to present.

Material and methods

Samples

As recommended for ancient DNA (aDNA) studies, archaeological samples consisted, in all cases, of well-preserved teeth. A total of 643 teeth, belonging to 493 different aboriginal individuals, were sampled in several burials from six of the seven Canary Islands: Fuerteventura (13 teeth from ten individuals), Gran Canaria (230 teeth from 115 individuals), Tenerife (45 teeth from 39 individuals), La Gomera (62 teeth from 52 individuals), El Hierro (44 teeth from 44 individuals), and La Palma (43 teeth from 38 individuals). Care was taken to choose teeth without fractures, and whenever possible, they were directly taken from its mandible alveolus. Radiocarbon analysis performed on Gran Canaria, La Gomera, El Hierro, and Tenerife samples gave dates from 1,100 to 1,700 years BP. Although the Fuerteventura and La Palma materials were not directly C-14 dated, ceramic types co-excavated with the remains indicate they are not older than 1,000 years BP. The historical sample, exhumed from La Concepción Church in Tenerife, is dated to the seventeenth to eighteenth centuries and consisted of 206 teeth from 195 individuals.

Contamination prevention and authentication

Strict measures were taken to prevent and monitor contamination (Cooper and Poinar 2000; Paabo et al. 2004). Lab coats, face shields, hats, and multiple pairs of gloves were used for all personnel involved in the aDNA work. All sample manipulations were carried out in laminar flow cabinets, with dedicated pipettes and sterile filter tips (Tip One, Star Lab). Solutions were commercially acquired whenever possible; otherwise, they were autoclaved and UV-treated. All metallic material was sterilized in an oven at 200°C overnight. Work areas were constantly irradiated with UV lamps and cleaned with bleach. DNA extractions and PCR reactions were performed in three independently dedicated aDNA laboratories. In the first, the ancient material was decontaminated and powdered. In the second, the DNA extraction and pre-PCR steps were performed, and in the third, PCR amplifications were carried out. To authenticate results, duplicate samples were independently analyzed in physically isolated laboratories, using restriction fragment length polymorphisms (RFLPs) in La Laguna and SNaPshot in Las Palmas.

Ancient DNA extraction

Before extraction, teeth were washed with 15% HCl, rinsed with UV-treated ddH_2O, and exposed to UV light for 10 min. After that, they were transversely cut through the midline with a dentist electric saw and the dentin drilled out using a dental drill. The powder was collected in 1.5-ml sterile tubes, and DNA was extracted according to a modified GuSCN-silica-based protocol (Maca-Meyer et al. 2004).

Previous mitochondrial DNA, amelogenin, and Y chromosome analyses

Previous to the ABO study, all the samples were analyzed for mitochondrial DNA (mtDNA). As it is expected that there are approximately 3,000 mtDNA molecules per cell (Iborra et al. 2004), all samples that failed to amplify mtDNA were discarded. The successfully amplified samples (65%) were then analyzed for the amelogenin gene (Arnay et al. 2007; Maca-Meyer et al. 2005), and only those that gave positive results were taken into account for posterior analysis. For both aboriginal and historical remains, the male individuals were used to perform a Y-chromosome study (unpublished results), and the females were analyzed for the ABO blood group in the present study. Moreover, several males, who left enough DNA extract, were also included. Finally, a total of 60 individuals (46 female and 14 male) from the aboriginal population and 62 individuals (44 female and 18 male) from the historical sample were included in this study.

Real-time PCR quantification

The number of molecules used as template for PCR amplification (Alonso et al. 2004) were determined by real-time PCR quantification using iQ™ SYBR® Green Supermix (BioRad) in an iCycler Thermal Cycler (BioRad). Primers and thermal cycling conditions were

as described for ABO amplifications. Tenfold serial dilutions of a purified and quantified standard were included in all the assays to determine the standard curve in order to estimate the initial number of DNA molecules in each sample.

ABO genotyping

As there is no consensus terminology for ABO alleles, we followed the nomenclature proposed by the Blood Group Antigen Gene Mutation Database (http://www.ncbi.nlm.nih.gov/projects/mhc/xslcgi.fcgi?cmd=bgmut/home). At DNA level, ABO blood groups are defined by polymorphisms on exons 6 and 7 (Yamamoto et al. 1990). Following a previous ABO genotyping on ancient remains, two fragments of 103/104 bp and 64 bp from exons 6 and 7, respectively, were amplified in order to distinguish the *A101*, *B101*, *O01*, *O02*, and *O03* alleles (Hummel et al. 2002).

Multiplex preamplification

Due to the limited amount of DNA substrate left after the mtDNA and amelogenin analysis and the high frequency of PCR inhibition problems, caused by the high amount of extract necessary to directly amplify the ABO products, we included a previous multiplex preamplification step. The multiplex PCR was carried out in 10-μl reaction, containing 1 μl of 10× Tris–HCl buffer, 200 μM of each dNTP, 2 pmol of previously designed primers (Hummel et al. 2002), 5 mM $MgCl_2$, 15 ng of BSA, 1 unit of Taq polymerase (Ecogen), and 5 μl of DNA extract. Reactions were submitted to 40 amplification cycles with denaturation at 94°C for 10 s, annealing at 56°C for 10 s, and extension at 72°C for 10 s. Extraction and PCR controls were included to detect modern DNA contamination.

Nested-PCR reamplification

Multiplex products were reamplified using a nested PCR for each exon. In these amplifications, one of the previous PCR primers was used together with a new designed nested primer (Supplementary Material 1). Both PCRs were carried out in a 50-μl reaction, containing 5 μl of 10× Tris–HCl buffer, 200 μM of each dNTP, 5 pmol of each primer, 5 mM $MgCl_2$, 3 U of Taq polymerase (Ecogen), and 10 μl of 1:100 diluted multiplex PCR product. For exon 6 reamplification, 40 cycles with denaturation at 94°C for 15 s, annealing at 56°C for 15 s, and extension at 72°C for 15 s were used. For exon 7, reactions were submitted to 40 amplification cycles with denaturation at 94°C for 10 s, annealing at 55°C for 10 s, and extension at 72°C for 10 s. An aliquot of each PCR product was loaded in 10% acrylamide/bis-acrylamide (19:1) gels, stained with ethidium bromide, and visualized under UV to assess the amplification yield. Typing was repeated twice for heterozygous samples in order to confirm their genotypes. Homozygous samples were retyped five times to avoid false genotyping due to allelic dropout.

RFLP analysis

For RFLP analysis, previously published conditions were followed (Hummel et al. 2002), using 0.5–1 U of the appropriate restriction enzyme to directly digest 10 μl of the nested PCR. RFLP patterns were resolved on 8% acrylamide/bis-acrylamide (19:1) in 1× Tris–Borate–EDTA buffer and stained with ethidium bromide (1 μg/ml) for 15 min.

Multiplex SNaPshot analysis

For SNaPshot analysis, products of both nested PCR were collected together in 0.5-ml sterile Eppendorf tubes at comparatively optimal amounts and ethanol precipitated to purify and concentrate the samples in an 8 μl volume. To remove any primers and dNTPs left by the previous ethanol precipitation, 1 μl of the concentrated PCR products was treated with 0.5 μl of Exo-SAP-it (USB) and incubated at 37°C for 15 min, followed by an enzyme inactivation step, consisting in heating at 85°C for 15 min. The multiplex minisequencing reactions were carried out in a 5-μl final volume containing 1 μl of SNaPshot™ Multiplex Ready Mix (Applied Biosystem), 1.5 μl of the previously treated PCR products, and different amounts of each single base extension (SBE) primers (sequences and concentrations for SBE primers are specified in Supplementary Material 1). Reactions were submitted to 25 cycles of denaturation at 96°C for 10 s, annealing at 50°C for 5 s, and extension at 60°C for 30 s. Final extension products were treated with 1 μl of SAP (USB) and incubated at 37°C for 1 h, followed by enzyme inactivation by heating at 85°C for 15 min.

For capillary electrophoresis, 0.5 μl purified extension products were mixed with 9.325 μl Hi-Di™ formamide (Applied Biosystem) and 0.125 μl of internal size standard GeneScan-120 LIZ™ (Applied Biosystem). Samples were run on an ABI Prism 3100® Genetic Analyzer (Applied Biosystem) using POP-4® (Applied Biosystem), results were visualized with GeneScan and samples typed with the GeneMapper 4.0 software (Applied Biosystem).

Cloning and sequencing

To check the specificity of the primers and to enhance heterozygous detection, in several instances, PCR products of each exon were ligated into pGEM-T vectors (Promega).

Colonies were plated on selective Amp/IPTG/X-gal plates, and white colonies were selected for amplification. In addition, after electrophoretic separation, authentic and anomalous migration bands were tooth-picked from the gel, dissolved in 10 μl sterile ddH_2O and 2 μl used in a 20-μl re-amplification PCR carried out with the same primers and conditions as those used in the nested reamplification. Clones were directly sequenced using M13 universal primers, and reamplified products were sequenced with the corresponding forward primer used in their reamplifications. In both cases, sequencing reactions were performed in 10-μl volumes using the BigDye 3.1 Terminator Cycle Sequencing kit (Applied Biosystems), and the products were run on an ABI Prism 310 Genetic Analyzer (Applied Biosystems).

Statistical analyses

Aboriginal and historical Canarian samples were compared between them and with present-day populations of the seven Canary islands, Northwest Africa (comprising Arab and Berber from Morocco), West Sahara (including Saharan and Mauritanian samples), and the Iberian peninsula, excluding Basques due to its high differentiation (Fregel et al. 2005). Analysis of molecular variance and pairwise F_{ST} genetic distances based on allele frequencies (Slatkin 1995) were performed using Arlequin 2000 package (Schneider et al. 2000). Multidimensional scaling (MDS) analysis was carried out using the SPSS statistical package 11.5 (SPSS, Inc).

Results and discussion

Authenticity of the results

Efficiency for the samples used in the present ABO group analysis was 68% for the aboriginal and 81% for La Concepción historic material. However, taking into account previous mtDNA and amelogenin analysis, only 41 (21%) and 50 (40%) of the total aboriginal and historic samples, respectively, produced positive ABO results.

Using direct ABO typing, a mean of 549±380 initial molecules was quantified by real-time PCR. The same quantity of template was added to the routine preamplification reactions. However, for several samples tenfold dilutions were necessary in order to overcome inhibition problems.

Artifactual bands with anomalous electrophoretic migration resulted in the majority of the cases in different primer dimmer combinations, although in some cases, non-human unknown sequences were cloned.

Specific products gave always known ABO alleles, except in two instances in which, in addition to the characteristic polymorphisms of *O01* and *O02* alleles, two T→C transitions at nucleotide positions 260 and 267, respectively, were observed, leading to amino acid changes of Val by Ala and Leu by Pro at protein positions 87 and 89, respectively. As these mutations have not been previously detected, we consider them as result of postmortem DNA damage (Hofreiter et al. 2001; Stiller et al. 2006).

Sporadical contamination was observed in some PCR negative controls. However, the problem was always solved repeating the assay with new reaction aliquots. In all cases, positive typing in both laboratories gave identical results, except for three cases in which samples considered homozygous by RFLP but heterozygous by SNaPshot resulted true heterozygous after RFLP product cloning.

ABO frequencies in aborigines

Genotypic and allelic frequencies found for the Guanche sample are shown in Tables 1 and 2, respectively. Genotypic frequencies are statistically in Hardy–Weinberg equilibrium; however, an excess of *A101*/*A101*, *O01*/*O01* and *O02*/*O02* homozygous and a defect for *A101*/*O01* and *O01*/*O02* heterozygous were observed. Although a methodological deficit of heterozygous detection cannot be ruled out, it must be mentioned that an excess of *A101* allele frequency in aborigines would be expected; however, they showed the lowest frequency for this allele (Table 2).

Compared to previous serological analysis carried out on mummified tissues (Schwarzfischer and Liebrich 1963), the *O* allele frequency (71%) obtained here by molecular methods from Guanche remains is significantly smaller ($p<0.0001$ for both comparisons) than those found in the serological analyses (91–97%). Although these discrepancies could be attributed to sampling differences, it has been previously suggested that past serological methods performed on ancient tissues could be inaccurate (Paabo et al. 1989). In the same vein, differences for the ABO allele frequencies between Moroccan Berber samples serotipically tested in the middle of the past century (Gaud 1942; Messerlin and Lorho 1951) and in modern times (Fernandez-Santander et al. 1999; Fregel et al. 2005) with more refined techniques showed no statistical differentiation when samples of the same period were compared. However, *O* allele frequencies were significantly higher ($p<0.0001$ for all comparisons) in former samples (88–91%) than in those obtained in recent times (66–68%).

Congruent with the supposed Berber origin of the Guanches, the Canarian aboriginal sample is not significantly different with any of the North African populations, from the historical sample of La Concepción, or from La Gomera, the island that better has retained the prehispanic gene pool (Fregel et al. 2005; Maca-Meyer et al. 2004;

Table 1 ABO allele frequencies in the studied populations

	ABG	CON	LAN	FUE	GCA	TFE	GOM	PAL	HIE	IBP	MOR	BER	MAU	SAH
A101	0.110	0.260	0.292	0.298	0.208	0.307	0.212	0.246	0.378	0.330	0.217	0.226	0.184	0.227
B101	0.183	0.100	0.069	0.089	0.083	0.073	0.068	0.031	0.024	0.082	0.145	0.107	0.133	0.152
O01	0.317	0.300	0.361	0.315	0.445	0.404	0.386	0.485	0.293	0.370	0.362	0.310	0.333	0.311
O02	0.329	0.310	0.271	0.298	0.264	0.204	0.311	0.238	0.281	0.209	0.254	0.333	0.333	0.295
O03	0.061	0.030	0.007	0.000	0.000	0.012	0.023	0.000	0.024	0.009	0.022	0.024	0.017	0.015
N	82	100	144	124	144	260	132	130	82	440	138	84	60	132

ABG aborigines, *CON* seventeenth- to eighteenth-century population, *LAN* Lanzarote, *FUE* Fuerteventura, *GCA* Gran Canaria, *TFE* Tenerife, *GOM* Gomera, *PAL* Palma, *HIE* Hierro, *CAN* Canary Islands, *IBP* Iberian Peninsula, *MOR* Moroccans, *BER* Berbers, *MAU* Mauritanian, *SAH* Saharawis

Rando et al. 1999). On the contrary, it is significantly different from the Iberian Peninsula and the rest of the present-day Canary Islands populations that have received a stronger European genetic input than La Gomera (Table 3).

At ABO level, North African populations are differentiated from the Iberian Peninsula because they have higher frequencies for *B101* allele, lower frequencies for *A101* allele, and 1:1 ratio between *O01* and *O02* alleles (Fregel et al. 2005). The aboriginal ABO profile clearly reflects its affinity with the North African populations, as they show the lowest *A101* allele frequency, the highest *B101* allele frequency, and equal frequencies for *O01* and *O02* alleles. In addition, they also show the highest frequency for *O03* allele that has been consistently detected in North Africa but only sporadically in the Iberian Peninsula (Fregel et al. 2005; Roubinet et al. 2001). Sometimes founder effects strongly biased the parental population allele frequencies during the settlement event. However, for the ABO gene, it seems that the Canarian colonization process has exacerbated the parental Berber allele frequency peculiarities in the Guanches. This might suggest that strong bottlenecks did not occur during the colonization event, as was previously deduced from aboriginal mtDNA data from different islands (Fregel et al. 2009; Maca-Meyer et al. 2004). The extreme position of the ABO Guanche sample is graphically depicted in a bidimensional plot based on allele frequency F_{ST} distances (Fig. 1). Canarian aborigines are related to North West African samples and show the farthest distances from the Iberian Peninsula and modern Canary Islands populations.

ABO frequencies in the seventeenth to eighteenth century population

Genotypic and allelic ABO frequencies (Tables 1 and 2) in the historical population show the impact of the European gene flow, when compared to the aboriginal sample. For instance, the *A101* allele frequency is twofold higher, and the *B101* and *O03* are half of those found in Guanches. However, it still has more affinities with North African populations than to the Iberian Peninsula and the present-day Canary Islands inhabitants (Fig. 1). The clear 1:1 ratio between *O01* and *O02* alleles further reinforces its North African proximity. F_{ST}-based distances between populations (Table 3) show that the historical sample has only significant differences with the Iberian Peninsula and the extant populations of Tenerife and La Palma islands.

Previous studies performed on the same sample, using mtDNA (Maca-Meyer et al. 2005) and Y-chromosome markers (unpublished results), detected that in the seventeenth to eighteenth centuries, the Canarian population had a larger sub-Saharan African genetic input than today as consequence of slave trade. Moreover, a notable sexual

Table 2 Observed and expected ABO genotypic frequencies for all studied populations

		A101/ A101	*A101/ B101*	*A101/ O01*	*A101/ O02*	*A101/ O03*	*B101/ B101*	*O01/ B101*	*O02/ B101*	*B101/ O03*	*O01/ O01*	*O01/ O02*	*O01/ O03*	*O02/ O02*	*O02/ O03*	*O03/ O03*	N
ABG	OBS.	0.049	0.024	0.000	0.073	0.024	0.049	0.146	0.098	0.000	0.146	0.123	0.073	0.171	0.024	0.000	41
	EXP.	0.012	0.040	0.070	0.072	0.013	0.033	0.117	0.120	0.022	0.101	0.209	0.039	0.108	0.040	0.004	
CON	OBS.	0.100	0.040	0.100	0.160	0.020	0.000	0.140	0.000	0.020	0.080	0.180	0.020	0.140	0.000	0.000	50
	EXP.	0.068	0.052	0.156	0.161	0.016	0.010	0.060	0.062	0.006	0.090	0.186	0.018	0.096	0.019	0.001	
LAN	OBS.	0.097	0.028	0.250	0.111	0.000	0.000	0.056	0.056	0.000	0.083	0.236	0.014	0.069	0.000	0.000	72
	EXP.	0.085	0.041	0.211	0.158	0.004	0.005	0.050	0.038	0.001	0.130	0.196	0.005	0.073	0.004	0.000	
FUE	OBS.	0.081	0.065	0.177	0.194	0.000	0.032	0.032	0.016	0.000	0.145	0.129	0.000	0.129	0.000	0.000	62
	EXP.	0.089	0.053	0.188	0.178	0.000	0.008	0.056	0.053	0.000	0.099	0.188	0.000	0.089	0.000	0.000	
GCA	OBS.	0.083	0.028	0.167	0.056	0.000	0.000	0.139	0.000	0.000	0.153	0.278	0.000	0.097	0.000	0.000	72
	EXP.	0.043	0.035	0.185	0.110	0.000	0.007	0.074	0.044	0.000	0.198	0.235	0.000	0.070	0.000	0.000	
TFE	OBS.	0.115	0.031	0.223	0.115	0.015	0.008	0.054	0.046	0.000	0.177	0.169	0.008	0.038	0.000	0.000	130
	EXP.	0.095	0.045	0.249	0.125	0.007	0.005	0.059	0.030	0.002	0.163	0.165	0.009	0.042	0.005	0.000	
GOM	OBS.	0.091	0.000	0.091	0.121	0.030	0.015	0.030	0.076	0.000	0.197	0.258	0.000	0.076	0.015	0.000	66
	EXP.	0.045	0.029	0.164	0.132	0.010	0.005	0.053	0.042	0.003	0.149	0.240	0.018	0.096	0.014	0.001	
PAL	OBS.	0.062	0.015	0.292	0.062	0.000	0.015	0.000	0.015	0.000	0.215	0.246	0.000	0.077	0.000	0.000	65
	EXP.	0.061	0.015	0.239	0.117	0.000	0.001	0.030	0.015	0.000	0.235	0.231	0.000	0.057	0.000	0.000	
HIE	OBS.	0.146	0.024	0.220	0.195	0.024	0.000	0.000	0.024	0.000	0.098	0.171	0.000	0.073	0.024	0.000	41
	EXP.	0.143	0.018	0.221	0.212	0.018	0.001	0.014	0.014	0.001	0.086	0.164	0.014	0.079	0.014	0.001	
IBP	OBS.	0.114	0.059	0.250	0.123	0.000	0.000	0.077	0.027	0.000	0.123	0.163	0.005	0.045	0.014	0.000	220
	EXP.	0.109	0.054	0.244	0.138	0.006	0.007	0.061	0.034	0.001	0.137	0.155	0.007	0.044	0.004	0.000	
MOR	OBS.	0.029	0.072	0.159	0.145	0.000	0.014	0.130	0.058	0.000	0.101	0.188	0.043	0.058	0.000	0.000	69
	EXP.	0.047	0.063	0.158	0.110	0.009	0.021	0.105	0.074	0.006	0.131	0.184	0.016	0.064	0.011	0.000	
BER	OBS.	0.095	0.048	0.119	0.095	0.000	0.000	0.071	0.071	0.024	0.119	0.190	0.000	0.143	0.024	0.000	42
	EXP.	0.051	0.048	0.140	0.151	0.011	0.011	0.066	0.071	0.005	0.096	0.206	0.015	0.111	0.016	0.001	
MAU	OBS.	0.000	0.033	0.167	0.167	0.000	0.000	0.100	0.133	0.000	0.133	0.133	0.000	0.100	0.033	0.000	30
	EXP.	0.034	0.049	0.122	0.122	0.006	0.018	0.089	0.089	0.004	0.111	0.222	0.011	0.111	0.011	0.000	
SAH	OBS.	0.034	0.049	0.122	0.122	0.006	0.018	0.089	0.089	0.004	0.111	0.222	0.011	0.111	0.011	0.000	66
	EXP.	0.052	0.069	0.141	0.134	0.007	0.023	0.094	0.090	0.005	0.096	0.184	0.009	0.087	0.009	0.000	

Codes as in Table 1

Table 3 F_{ST} distances between populations based on ABO allelic frequencies

	ABG	CON	LAN	FUE	GCA	TFE	GOM	PAL	HIE	IBP	MOR	BER	MAU	SAH
ABG	–													
CON	0.010	–												
LAN	0.028*	0.000	–											
FUE	0.023*	0.000	0.000	–										
GCA	0.021*	0.013	0.003	0.011	–									
TFE	0.046***	0.014*	0.000	0.006	0.006	–								
GOM	0.011	0.000	0.000	0.002	0.000	0.009	–							
PAL	0.049***	0.028**	0.007	0.020*	0.000	0.004	0.006	–						
HIE	0.058***	0.004	0.001	0.000	0.031*	0.010	0.018*	0.032*	–					
IBP	0.048***	0.011*	0.000	0.003	0.012*	0.000	0.013*	0.012*	0.005	–				
MOR	0.005	0.001	0.001	0.002	0.001	0.007	0.000	0.014*	0.022*	0.008*	–			
BER	0.002	0.000	0.000	0.000	0.008	0.016*	0.000	0.024*	0.011	0.015*	0.000	–		
MAU	0.000	0.000	0.003	0.000	0.003	0.019*	0.000	0.022*	0.023	0.019*	0.000	0.000	–	
SAH	0.002	0.000	0.002	0.000	0.010	0.015*	0.001	0.027**	0.017*	0.014*	0.000	0.000	0.000	–

Codes as in Table 1

*$p<0.05$, **$p<0.01$, ***$p<0.001$

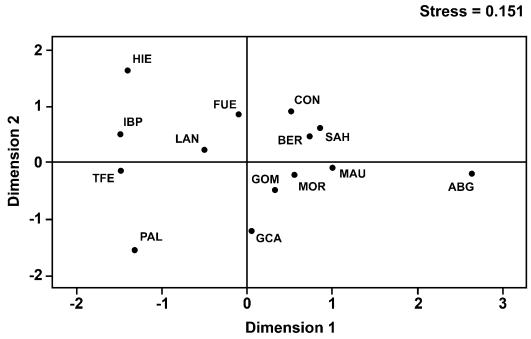


Fig. 1 MDS plots based on ABO allelic F_{ST} distances. Codes are as in Table 1

asymmetry was detectable with those uniparental markers in the present-day population, in such a way that, whereas maternal Guanche lineages had high reproductive success, Guanche and sub-Saharan male lineages were at disadvantage compared with European male lineages (Flores et al. 2003; Rando et al. 1999). Regrettably, there are only slight differences in ABO allele frequencies between West African and North African populations, with the former presenting higher *O* allele and smaller *A* allele frequencies (Livingstone et al. 1960; Roubinet et al. 2001). Therefore, those peculiarities cannot be confirmed in this ABO study. However, as in the Guanche sample case, although the historical population is also in Hardy–Weinberg equilibrium, it is near significance ($p=0.06$), presenting a defect of *A101*/*O01* and an excess of *O01*/*B101* heterozygous genotypes and an excess of *O02*/*O02* genotypes. This result could be a hint of lack of panmixis in that multiethnic population.

Finally, it deserves to mention that, although only an autosomal gene has been analyzed in this study, it confirms the temporal evolution of the Canary Islands population, drawn from previous archaeological, historical, and genetic studies that proposed a Berber origin for the indigenous population and an important and sex-biased European influence after the Spaniard conquest (Flores et al. 2001; González and Tejera 1990; Hooton 1916; Schwidetzky 1975). On the other hand, although our main conclusions are statistically supported, we would like to point out that greater sample sizes would be necessary, not only to further confirm the Berber origin of the Canarian aborigines but also to assess the colonization process in each island. However, these future projects have to wait until new archaeological material could be available.

Acknowledgments We gratefully acknowledge P. Hernández Carpio and A. González and J. Espino from Servicio de Secuenciación y Genotipado de ADN (SEGAI) for technical assistance. Samples were generously donated by J. Pais, M. Arnay, A. Gámez, J.C. Hernández,

J. Velasco, and T. Delgado. This research was supported by grants of the Spain Ministry of Science and Technology BFU2006-04490 to J. M. Larruga, and R. Fregel is supported by a Gobierno de Canarias predoctoral fellowship.

References

Alonso A, Martin P, Albarran C, Garcia P, Garcia O, de Simon LF, Garcia-Hirschfeld J, Sancho M, de La Rua C, Fernandez-Piqueras J (2004) Real-time PCR designs to estimate nuclear and mitochondrial DNA copy number in forensic and ancient DNA studies. Forensic Sci Int 139:141–149

Arnay M, González-Reimers E, Fregel R, Velasco-Vázquez J, Delgado-Darias T, González AM, Larruga JM (2007) Canary islands aborigin sex determination based on mandible parameters contrasted by amelogenin analysis. J Archaeol Sci 34:1515–1522

Boyd WC, Boyd LG (1934) An attempt to determine the blood groups of mummies. Proc Soc Exp Biol Med 31:671

Cooper A, Poinar HN (2000) Ancient DNA: do it right or not at all. Science 289:1139

Doi Y, Yamamoto Y, Inagaki S, Shigeta Y, Miyaishi S, Ishizu H (2004) A new method for ABO genotyping using a multiplex single-base primer extension reaction and its application to forensic casework samples. Leg Med (Tokyo) 6:213–223

Fernandez-Santander A, Kandil M, Luna F, Esteban E, Gimenez F, Zaoui D, Moral P (1999) Genetic relationships between southeastern Spain and Morocco: new data on ABO, RH, MNSs, and DUFFY polymorphisms. Am J Hum Biol 11:745–752

Flores C, Larruga JM, Gonzalez AM, Hernandez M, Pinto FM, Cabrera VM (2001) The origin of the Canary Island aborigines and their contribution to the modern population: a molecular genetics perspective. Curr Anthropol 42:749–755

Flores C, Maca-Meyer N, Perez JA, Gonzalez AM, Larruga JM, Cabrera VM (2003) A predominant European ancestry of paternal lineages from Canary Islanders. Ann Hum Genet 67:138–152

Fregel R, Maca-Meyer N, Cabrera VM, Gonzalez AM, Larruga JM (2005) Description of a simple multiplex PCR-SSCP method for AB0 genotyping and its application to the peopling of the Canary Islands. Immunogenetics 57:572–578

Fregel R, Pestano J, Arnay M, Cabrera VM, Larruga JM, González AM (2009) The maternal aborigine colonization of La Palma (Canary Islands). Eur J Hum Genet (in press). doi:10.1038/ejhg.2009.46

Gaud J (1942) Contribution à l'étude ethnographique du Maroc. Répartition des groupes sanguins parmi les tribus des environs de Meknès. Bull Inst Hyg Maroc 2:37–52

González R, Tejera A (1990) Los aborígenes canarios: Gran Canaria y Tenerife. Ediciones Itsmo, Oviedo

Hofreiter M, Jaenicke V, Serre D, Haeseler AvA, Paabo S (2001) DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. Nucleic Acids Res 29:4793–4799

Hooton EA (1916) Preliminary remarks on the archaeology and physical anthropology of Tenerife. Am Anthropol New Series 18:358–365

Hummel S, Schmidt D, Kahle M, Herrmann B (2002) AB0 blood group genotyping of ancient DNA by PCR-RFLP. Int J Legal Med 116:327–333

Iborra FJ, Kimura H, Cook PR (2004) The functional organization of mitochondrial genomes in human cells. BMC Biol 2:9

Lalueza-Fox C, Gigli E, de la Rasilla M, Fortea J, Rosas A, Bertranpetit J, Krause J (2008) Genetic characterization of the ABO blood group in Neandertals. BMC Evol Biol 8:342

Livingstone FB, Gershowitz H, Neel JV, Zuelzer WW, Solomon MD (1960) The distribution of several blood group genes in Liberia, the Ivory Coast and Upper Volta. Am J Phys Anthropol 18:161–178

Maca-Meyer N, Arnay M, Rando JC, Flores C, Gonzalez AM, Cabrera VM, Larruga JM (2004) Ancient mtDNA analysis and the origin of the Guanches. Eur J Hum Genet 12:155–162

Maca-Meyer N, Cabrera VM, Arnay M, Flores C, Fregel R, Gonzalez AM, Larruga JM (2005) Mitochondrial DNA diversity in 17th–18th century remains from Tenerife (Canary Islands). Am J Phys Anthropol 127:418–426

Messerlin A, Lorho Y (1951) Groupes Sanguins et Facteur Rhésus au Maroc. Bull Inst Hyg Maroc 11:97–119

Navarro JF (1997) Arqueología de las Islas Canarias. Espacio tiempo y forma. Serie I, Prehistoria y Arqueología 10:447–478

Paabo S, Higuchi RG, Wilson AC (1989) Ancient DNA and the polymerase chain reaction. The emerging field of molecular archaeology. J Biol Chem 264:9709–9712

Paabo S, Poinar H, Serre D, Jaenicke-Despres V, Hebler J, Rohland N, Kuch M, Krause J, Vigilant L, Hofreiter M (2004) Genetic analyses from ancient DNA. Annu Rev Genet 38:645–679

Pinto F, Rando JC, Lopez M, Morilla JM, Larruga JM (1996) Blood group polymorphisms in the Canary Islands. Gene Geogr 10:171–179

Rando JC, Cabrera VM, Larruga JM, Hernandez M, Gonzalez AM, Pinto F, Bandelt HJ (1999) Phylogeographic patterns of mtDNA reflecting the colonization of the Canary Islands. Ann Hum Genet 63:413–428

Roberts DF, Evans M, Ikin EW, Mourant AE (1966) Blood groups and the affinities of the Canarian islanders. Man 1:512–525

Rösing I (1967) AB0-Blutgruppen und Rh-Faktoren auf Teneriffa unter besonderer Berücksichtigung des Vergleichs zwischen vorspanischen und heutiger Bevölkerung. Homo 18:96–104

Roubinet F, Kermarrec N, Despiau S, Apoil PA, Dugoujon JM, Blancher A (2001) Molecular polymorphism of O alleles in five populations of different ethnic origins. Immunogenetics 53:95–104

Schneider S, Roessli D, Excoffier L (2000) Arlequin ver. 2000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland

Schwarzfischer F, Liebrich K (1963) Serologische Untersuchungen an prähistorischen Bevölkerungen, insbesondere an altkanarischen Mumien. Homo 14:129–133

Schwidetzky I (1975) Investigaciones antropológicas en las Islas Canarias: Estudio comparativo entre la población actual y la prehispánica. Publicaciones del Museo Arqueológico de Tenerife, Tenerife

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462

Stiller M, Green RE, Ronan M, Simons JF, Du L, He W, Egholm M, Rothberg JM, Keates SG, Ovodov ND, Antipina EE, Baryshnikov GF, Kuzmin YV, Vasilevski AA, Wuenschell GE, Termini J, Hofreiter M, Jaenicke-Despres V, Paabo S (2006) Patterns of nucleotide misincorporations during enzymatic amplification and direct large-scale sequencing of ancient DNA. Proc Natl Acad Sci U S A 103:13578–13584

Viera y Clavijo J (1772) Historia de la Islas Canarias. Editorial Cupsa, Madrid

Watanabe G, Umetsu K, Yuasa I, Suzuki T (1997) Amplified product length polymorphism (APLP): a novel strategy for genotyping the ABO blood group. Hum Genet 99:34–37

Yamamoto F, Clausen H, White T, Marken J, Hakomori S (1990) Molecular genetic basis of the histo-blood group ABO system. Nature 345:229–233

Yip SP (2000) Single-tube multiplex PCR-SSCP analysis distinguishes 7 common ABO alleles and readily identifies new alleles. Blood 95:1487–1492

DISCUSIÓN

5. DISCUSIÓN

5.1. Evolución temporal de la composición genética de la población canaria deducida a partir de marcadores uniparentales y autosómicos.

Aunque la población canaria actual había sido estudiada previamente para el ADNmt (Rando y col., 1999), en el presente trabajo, se ha ampliado el número de individuos analizados de 300 a 800. Este aumento nos ha permitido profundizar, con más detalle, en el origen de los linajes maternos de la población canaria y en su distribución en las diferentes islas. La mayoría de los linajes detectados en Canarias tienen origen europeo o norteafricano. Las distancias genéticas interinsulares, calculadas a partir de las frecuencias haplotípicas y de haplogrupos, muestran que, en general, las islas son relativamente homogéneas en su composición para el ADNmt, siendo la mayor excepción la isla de La Gomera, que posee una frecuencia del haplogrupo autóctono de las Islas Canarias U6b1 (Rando y col., 1999) significativamente más alta que el resto del archipiélago.

Además, en este estudio, hemos comparado la aportación relativa de las poblaciones norteafricanas y subsaharianas a la población canaria actual, usando haplogrupos del ADNmt y del cromosoma Y con clara asignación geográfica. De esta forma, podemos determinar si existen diferencias significativas en las contribuciones de estos linajes maternos y paternos, comparando su frecuencia en Canarias con las que se observan en el norte de África y en la región subsahariana. Como se esperaba, las aportaciones norteafricanas masculinas y femeninas son significativamente diferentes, confirmando la asimetría sexual detectada en la población canaria actual usando estimas de mezcla (Flores y col., 2001b). Del mismo modo, la aportación subsahariana de linajes maternos es mayor que la de los paternos. Estos resultados apuntan a que, aunque los esclavos traídos a Canarias fueron mayoritariamente varones, a lo largo del tiempo se incorporaron a la población canaria actual con una eficacia menor que la de los linajes maternos.

El estudio directo de restos aborígenes de cuatro de las Islas Canarias (El Hierro, La Gomera, Tenerife y Gran Canaria) evidenció la presencia del haplogrupo autóctono U6b1 en los Guanches, confirmando un origen prehispánico para este linaje. Además, la comparación de las frecuencias de los aborígenes con las de las poblaciones del noroeste africano mostró una mayor similitud con los bereberes de Marruecos (Maca-Meyer y col., 2004a). Por otro lado, el análisis de una muestra histórica del S.XVII-XVIII, mostró una importante heterogeneidad étnica. Aunque la mayoría de los linajes son ya de origen europeo, la aportación aborigen y la frecuencia de linajes subsaharianos, como consecuencia del tráfico de esclavos, era muy superior a la detectada en la actualidad.

En la presente tesis, hemos contribuido al conocimiento genético de la población aborigen analizado una muestra pre-hispánica de la isla de La Palma para la región control del ADNmt y posiciones diagnóstico de la región codificante. Si comparamos los resultados obtenidos para La Palma con los ya publicados de Tenerife (Maca-Meyer y col., 2004a), encontramos que tanto en sus frecuencias haplotípicas como en sus diversidades genéticas, ambas islas son bastante similares. Este resultado sugiere una composición genética similar en la población aborigen de dos islas geográficamente distantes, como son Tenerife y La Palma.

El estudio del cromosoma Y en la población actual de las Islas Canarias indicó que sus linajes paternos son mayoritariamente de origen europeo (Flores y col., 2003). Tal como ocurre en otras poblaciones de Europa, en Canarias aproximadamente el 50% de los varones pertenecen al haplogrupo R-M269, aunque aparecen también algunos marcadores asociados a poblaciones norteafricanas, como el haplogrupo E-M81. Sin embargo, dado que el E-M81 también se encuentra en la Península Ibérica como consecuencia de la ocupación árabe durante los siglos VII-XV, sólo el estudio de muestras aborígenes podría confirmar su origen prehispánico.

En el presente estudio se observa, tal como se esperaba, que el marcador norteafricano E-M81 es el subtipo más frecuente en la población aborigen de las Islas Canarias. Otros marcadores, prominentes en el norte de África, como el E-M78 y el J-M267, también aparecen en la población aborigen de las Islas, reforzando el origen

norteafricano de los indígenas canarios. Por el contrario, en la población histórica de la Iglesia de La Concepción, como ocurre con la población canaria actual, el marcador europeo R-M269 es ya el más frecuente, con la consiguiente disminución de los haplogrupos E-M78, E-M81 y J-M267. Este resultado apunta a un reemplazo de los varones aborígenes por europeos desde los comienzos de la colonización hispánica. Además, al igual que ocurre con el ADNmt, se detectan linajes del cromosoma Y de origen subsahariano, como el E-M2, que atestiguan el impacto del tráfico de esclavos en Canarias.

Desde el punto de vista del locus autosómico AB0, la composición genética actual de las Islas Canarias es similar a la de la población peninsular, aunque a nivel de ADN se observa una influencia norteafricana, indetectable en estudios previos de polimorfismos enzimáticos (Afonso y col., 1989; Cabrera y col., 1996; Larruga y col., 1992; Martell y col., 1986; Morilla y col., 1988; Pérez y col., 1991; Pinto y col., 1996c) y de grupos sanguíneos a nivel serológico (Bravo y De las Casas, 1958; Guasch y col., 1952; Parejo, 1966; Pinto y col., 1996b; Rösing, 1967; Schwidetzky, 1970), en los que solo se sugirió un posible componente subsahariano. Así, el alelo *A101*, que es más frecuente en la Península Ibérica que en el norte de África, aparece en Canarias con frecuencias que cubren los valores medios de ambas áreas continentales. Lo mismo ocurre con el alelo *B101*, que siendo más abundante en el norte de África que en la Península, también aparece en Canarias con frecuencias intermedias. Respecto al alelo *O*, la proporción relativa entre *O01* y *O02* tiene también en Canarias (1,7) un valor intermedio entre el del norte de África (1,2) y el de la Península Ibérica (1,9). Además, los alelos *O03 y O12*, que solo habían sido detectados en bereberes y vascos en baja frecuencia, también han sido encontrados en Canarias.

En el caso de la población aborigen de las Islas Canarias, las frecuencias para AB0 apoyan nuevamente su origen norteafricano. Esta relación con el norte de África se debe a una frecuencia baja para el alelo *A101*, una frecuencia alta para *B101* y frecuencias similares para los alelos *O01* y *O02*, así como por la presencia del alelo *O03*, más frecuente en el norte de África que en la Península Ibérica. Por el contrario, las frecuencias en la población histórica se acercan más a las de las europeas, ya que posee una frecuencia para el alelo *A101* que es el doble de la encontrada en los aborígenes,

mientras que, los alelos *B101* y *O03* tienen frecuencias reducidas a la mitad respecto a esa misma población. Sin embargo, a pesar de que la muestra histórica de Tenerife presenta unas frecuencias similares a las de la población actual, aún conserva ciertas características que la relacionan con el norte de África, como una proporción equivalente de los alelos *O01* y *O02*. Como señalábamos antes, tanto el estudio del ADNmt como el del cromosoma Y, detectaron una influencia subsahariana en la población histórica debido al tráfico de esclavos que afectó a Canarias tras la conquista. En el caso del locus AB0, existen pocas diferencias entre el norte de África y la región subsahariana, por lo que esta influencia no puede ser determinada mediante el estudio de este marcador. Sin embargo, aunque la población histórica presenta frecuencias que estadísticamente están todavía en equilibrio de Hardy-Weinberg, las mismas se hallan cerca de los límites de la significación, lo que podría explicarse por una falta de panmixia ocasionada por la mezcla multiétnica de la población histórica de Tenerife.

En conclusión, la población aborigen de las Islas Canarias presenta un perfil similar al de las poblaciones del norte de África, tanto desde el punto de vista de los linajes uniparentales, como del locus autosómico ABO. Cuando éstos se comparan con los de la población actual, los marcadores uniparentales muestran resultados contradictorios, con un reemplazo casi total de los linajes prehispánicos para el cromosoma Y y una pervivencia considerable de los mismos para el ADNmt. Como cabría esperar, el locus autosómico AB0 presenta contribuciones intermedias. Respecto a la población histórica, en el caso del ADNmt solo muestra un moderado descenso de la contribución aborigen y una presencia relativamente alta de marcadores subsaharianos. Por el contrario, para el cromosoma Y y el locus AB0, ya se observan valores más cercanos a la población actual, evidenciando el reemplazo progresivo de la población aborigen por la europea.

5.2. Estimas de mezcla en la población histórica y actual de Canarias

Las estimas de mezcla calculadas para la población actual de las Islas Canarias, usando la Península Ibérica, el noroeste de África y el África subsahariana como parentales, mostraron un 33% de aportación aborigen para los linajes maternos (Rando y col., 1999) y sólo un 7% para los paternos (Flores y col., 2003). Esta asimetría sexual fue atribuida a una migración mayoritaria de conquistadores europeos varones, que desplazaron a la población aborigen masculina, pero que se mezclaron con las hembras indígenas.

Dado que el ADNmt y el cromosoma Y representan a los linajes maternos y paternos, respectivamente, el uso de un marcador autosómico puede ser útil para aportar una visión complementaria de la evolución de la población canaria. Con esa intención, se realizó un análisis molecular del locus AB0 en el archipiélago y en sus dos poblaciones parentales, el norte de África y la Península Ibérica. Las estimas de mezcla en la población canaria actual muestran que la contribución ibérica es aproximadamente del 82%, valor que no difiere significativamente de los obtenidos usando polimorfismos autosómicos Alu, 70% (Maca-Meyer y col., 2004b) y marcadores autosómicos clásicos, 65-75% (Flores y col., 2001b). Lógicamente, al existir una asimetría sexual calculada a partir de marcadores uniparentales (Flores y col., 2003; Rando y col., 1999), un locus autosómico como el AB0 da lugar a estimas de contribución intermedias. Hay que señalar que La Gomera, caracterizada por el mayor porcentaje del haplotipo U6b1 del ADNmt (Rando y col., 1999), muestra también la mayor aportación norteafricana (62%) para el locus AB0. Aunque estos resultados pueden deberse a una mejor conservación del sustrato aborigen, también pueden estar causados por fenómenos de deriva genética debido al aislamiento geográfico sufrido por esta población.

Sin embargo, en todas las estimas de mezcla calculadas a partir de poblaciones actuales, se ha utilizado a la norteafricana como representativa de la aborigen. Pero sólo mediante el análisis genético de restos arqueológicos se pudo obtener un valor directo de la aportación indígena. La caracterización de la composición genética del ADNmt de la

población aborigen (Maca-Meyer y col., 2004a), permitió estimar que los linajes maternos de la población canaria actual están compuestos por un 55% de aportación europea, un 42% de linajes aborigenes y sólo un 3% de aportación subsahariana. En el caso de la población histórica de Tenerife (Maca-Meyer y col., 2005), la aportación norteafricana fue similar a la actual (40%), mientras que la subsahariana (12%) fue significativamente mayor.

Para poder completar estos resultados desde el punto de vista de los linajes paternos, hemos determinado la composición de las poblaciones aborigen e histórica para el cromosoma Y. Las estimas de mezcla, utilizando como población parental a la aborigen, confirman la existencia de una marcada asimetría sexual. La contribución europea a los linajes paternos aumentó desde el 63% al 83% desde los siglos XVII-XVIII hasta la actualidad, con la consiguiente disminución de la aportación indígena, de un 31% a un 17%, y subsahariana, de un 6% a un 1%. Si lo comparamos con los cambios en la composición materna, observamos que tanto los linajes europeos (48% vs 55%) como los aborígenes (40% vs 42%) son bastante similares desde los siglos XVII-XVIII hasta la actualidad y que, sólo los linajes maternos subsaharianos han sufrido un marcado descenso desde tiempos históricos (12% vs 3%). Estos resultados señalan que los indígenas varones tuvieron una menor aportación a la población canaria actual, no sólo por la alta mortalidad sufrida durante la conquista y la posterior venta como esclavos, sino tras ella, debido probablemente a una discriminación basada en el mejor estatus social de los colonizadores europeos. En el caso de los linajes subsaharianos, el descenso de la aportación en los dos marcadores uniparentales, muestra que esta desventaja afectó por igual a ambos sexos.

En conclusión, el estudio de las poblaciones aborigen, histórica y actual de Canarias, usando marcadores uniparentales y el autosómico AB0, confirman que la conquista y posterior colonización de las islas por parte de los europeos afectó de forma diferente a la población masculina y a la femenina, introduciendo una marcada asimetría sexual de forma que los linajes aborígenes maternos pervivieron en la población actual en un número significativamente mayor que los paternos. Por otra parte, el impacto de la introducción de esclavos en Canarias tras la conquista ha ido disminuyendo desde

tiempos históricos hasta la actualidad, afectando por igual a los linajes maternos y paternos.

5.3. El proceso de colonización aborigen de las Islas Canarias deducido a partir del estudio de marcadores autosómicos y uniparentales

El estudio de la composición genética de las Islas Canarias ha permitido aportar ciertos datos, no siempre coincidentes, sobre el proceso de colonización aborigen de las islas. En un trabajo previo sobre el ADNmt, se encontró una correlación negativa entre la heterocigosidad de este marcador en las islas y sus distancias al continente africano (Rando y col., 1999). Estos resultados se interpretaron como consecuencia de una colonización basada en un único evento migratorio con saltos sucesivos de este a oeste, desde el noroeste africano. En otro estudio, basado en haplotipos CD4/Alu, también se detectó una única migración apoyada, nuevamente, por la correlación negativa hallada entre la heterocigosidad insular y la distancia al continente (Flores y col., 2001b).

En la presente tesis, el estudio del grupo sanguíneo AB0 en las poblaciones actuales de Canarias, también ha aportado ciertos datos que pueden reflejar el proceso de colonización. En este caso, existen dos alelos del locus AB0, el *B101* y el *O12*, que son más abundantes en el noroeste de África que en la Península Ibérica. Aunque ambos alelos presentan una correlación negativa entre la distancia al continente africano y la frecuencia del alelo en las islas (*B101*, R = -0.822; *O12*, R = -0.589), sólo el *B101* alcanzó un valor significativo (p = 0.023). Este resultado puede explicarse nuevamente por una sola colonización aborigen, en pasos sucesivos, que comenzó desde el noroeste de África ocupando consecutivamente las islas desde las más orientales a las más occidentales, aunque también podría explicarse por un mayor impacto de la colonización europea en las islas del oeste.

Por el contrario, en un estudio previo del cromosoma Y en la población canaria actual, se detectó un proceso de colonización en dos fases, basado en distribución y edad respectiva de los linajes africanos presentes en las islas (Flores y col., 2003). Estos

resultados coinciden además con evidencias antropológicas, arqueológicas y lingüísticas (Martínez, 1996; Navarro, 1992; Onrubia-Pintado, 1987; Reyes-García, 2000) que apuntan, al menos, a dos eventos colonizadores.

En el presente trabajo, a pesar de que no hemos detectado grandes diferencias interinsulares en la composición para el ADNmt de la población actual, si exceptuamos La Gomera, existen peculiaridades asociadas a ciertos grupos de islas que pueden ser útiles para inferir el proceso colonizador aborigen. Por ejemplo, un haplogrupo derivado del U6 y también autóctono, el U6c1, y el haplogrupo T2c, considerado previamente como uno de los fundadores (Rando y col., 1999), aparecen con frecuencias significativamente mayores en las islas de Lanzarote y Fuerteventura. Curiosamente, ambos haplogrupos han sido detectados en poblaciones aborígenes (Maca-Meyer y col., 2004a). Esta distribución asimétrica de los haplogrupos U6c1 y T2c, y la uniforme del haplogrupo U6b1, podría apuntar a una colonización en dos fases, como ya había sido sugerido para el cromosoma Y (Flores y col., 2003) y estudios antropológicos, arqueológicos y lingüísticos.

El estudio del ADNmt en aborígenes de la isla de La Palma también ha aportado pistas sobre el proceso de colonización del archipiélago. Dado que La Palma ocupa una de las posiciones más alejadas del continente africano, la comparación de su población aborigen con la de la isla central de Tenerife, estudiada previamente (Maca-Meyer y col., 2004a), pudo servir para determinar qué modelo de migración se ajusta más a la variabilidad observada. La gran diversidad genética encontrada en la isla de La Palma (95,2% ± 2,3%), similar a la de Tenerife (92,4 ± 2,8) no coincide con un modelo restrictivo de saltos consecutivos de isla a isla y posterior aislamiento genético. Dada la gran similitud de la población aborigen de La Palma y Tenerife, tampoco parece probable una colonización marítima independiente para cada isla. Además, si la colonización se produjo en varias oleadas, ambas debieron afectar a las islas de Tenerife y La Palma. Nuestros resultados parecen indicar que la colonización aborigen del archipiélago canario se produjo siguiendo un modelo de islas con migración entre ellas.

Por otro lado, la composición genética de la población aborigen de las Islas Canarias para el locus AB0, bastante similar a las presentes en el norte de África, parece indicar igualmente que el proceso de colonización de las islas no implicó grandes cuellos

de botella. Por lo tanto, estos resultados tampoco apoyan un modelo de colonización por saltos sucesivos de isla a isla, con posterior aislamiento entre las mismas.

Otro aspecto que queda pendiente en la prehistoria de Canarias es determinar, con una mayor exactitud, la procedencia de los primeros colonizadores. En la caracterización del ADNmt de la población canaria actual Rando y col. (1999) propusieron, basándose en criterios probabilísticos, ciertos linajes norteafricanos como fundadores, es decir, como representantes de la influencia aborigen prehispánica. Una gran parte de estos linajes están presentes en la población indígena de La Palma y de Tenerife, y tienen parejas exactas en el norte de África, confirmando, una vez más, la procedencia norteafricana de los aborígenes. Sin embargo, el haplogrupo autóctono canario U6b1 aún no ha sido detectado en África. Por otro lado, el subhaplogrupo 16260, perteneciente al haplogrupo H1, que aparece en alta frecuencia en la población indígena de La Palma, aparece en baja frecuencia en el norte de África (Ennafaa y col., 2009). Teniendo en cuenta que las dataciones más antiguas del material arqueológico excavado en las islas están en torno al cambio de era (Arco y col., 1981; Arco y col., 1997; Martín de Guzmán, 1978; Martín, 2000; Navarro, 1997; Velasco-Vázquez y col., 2002), parece improbable que el haplogrupo U6b1 y el subgrupo 16260 del haplogrupo H1 se originaran en las islas tras la colonización aborigen, ya que las edades de coalescencia calculadas para ambos dan estimas de 5.911 ± 3.536 y 6.263 ± 2.869 años, respectivamente. Debido a que estas fechas preceden a la de colonización, propuesta mediante criterios antropológicos y dataciones de C-14, lo más probable es que dichos linajes procedan del norte de África y que su ausencia o baja frecuencia en esa zona se deba a que el área exacta desde donde migraron los primeros colonizadores no ha sido aún muestreada o a que migraciones posteriores han modificado las frecuencias de estos linajes en el norte de África.

En el estudio del cromosoma Y de la población aborigen de las Islas Canarias, la presencia de haplogrupos de clara asignación europea como el I-M170 y el R-M269, podría sugerir que oleadas secundarias de origen europeo, fundamentalmente mediterráneo, afectaron a las islas. Sin embargo, este resultado se explicaría, igualmente, por una presencia de estos marcadores en el norte de África cuando los primeros colonizadores migraron hacia las islas. En este último caso, la presencia de R-M269 en África antecedería a la propuesta por otros investigadores (Bosch y col., 2001). En este

mismo sentido, el haplogrupo J-M267, cuya presencia en el norte de África había sido relacionada con la expansión árabe en el siglo VII (Semino y col., 2004), tuvo, igual que I-M170 y R-M269, que haber migrado a las islas en oleadas secundarias o haber existido en el norte de África en fechas anteriores a las propuestas.

En resumen, aunque serán necesarios futuros trabajos para esclarecer el proceso de colonización de las Islas Canarias, el estudio de los marcadores uniparentales y el marcador autosómico AB0 en las poblaciones aborigen, histórica y actual parece señalar que este proceso no se basó en un único evento migratorio, siguiendo un modelo de salto escalonado desde el continente. Nuestros resultados, en cambio, son más concordantes con la hipótesis de que se produjeron, al menos, dos oleadas migratorias desde el noroeste africano y que éstas no tuvieron fuertes efectos fundadores ya que, la ausencia de deriva y la similitud entre las poblaciones aborígenes, apuntan a la existencia de migraciones secundarias entre islas.

DISCUSSION

6. DISCUSSION

6.1. Temporal evolution of the Canary Islands genetic composition deduced from uniparental and autosomal markers.

Although the present-day population had been previously characterized for mtDNA (Rando et al. 1999), in the present work, we have increased the number of individuals analysed from 300 to 800. This has allowed us to more accurately determine the origin of maternal lineages and their distribution on the different islands. Most of the lineages found in the Canaries have European or N-African origin. Inter-island genetic distances, calculated from haplotype and haplogroup frequencies, show that the Canary Islands are, in general, relatively homogeneous in their mtDNA composition. The major exception is the island of Gomera with an autochthonous haplogroup U6b1 frequency (Rando et al. 1999), which is significantly higher with respect to the rest of the archipelago.

Furthermore, we have compared the relative contribution of the North and sub-Saharan African populations to the present-day islanders. For this, we used mtDNA and Y-chromosome lineages with clear geographic allocation, in order to determine whether there are significant differences between the maternal and paternal contributions from a particular geographic area. As expected, the N-African male and female inputs are significantly different, confirming the sexual asymmetry detected in the current population when using admixture estimations (Flores et al. 2001b). Likewise, the contribution of sub-Saharan maternal lineages is greater than the paternal one. This last result suggests that although the slaves brought to the islands were mostly men, over time they contributed to the present-day population less effectively than the maternal lineages.

The direct study of Guanche remains from four of the Canary Islands: Hierro, Gomera, Tenerife and Gran Canaria, showed the presence of the U6b1 haplogroup, thus confirming a pre-conquest origin for this lineage. Moreover, when the aboriginal mtDNA frequencies were compared with those of NW-African populations, they showed a greater similarity with the Berbers of Morocco (Maca-Meyer et al. 2004a). In addition, the analysis of the 17^{th}-18^{th} C. historical samples revealed a significant ethnic heterogeneity. Although

most of the lineages were of European origin, the indigenous contribution and notably the sub-Saharan (derived from the slave trade), were much higher than those observed in the present-day population.

In this thesis, we have contributed to the genetic knowledge of the indigenous population of La Palma, analysing a sample for the mtDNA control region and diagnostic coding region positions. When these results were compared with those already published for Tenerife (Maca-Meyer et al. 2004a), we find that both haplotype frequencies and genetic diversities are quite similar for these two geographically well-separated islands.

The analysis of the Y-chromosome in the current Canary Island population indicated that the paternal lineages are of European origin in 90% of the cases (Flores et al. 2003). As in other European populations, about 50% of their paternal lineages belong to haplogroup R-M269. Some N-African markers were also detected, like E-M81, which is also found in the Iberian Peninsula due to the Arab occupation between the 7^{th} and 15^{th} C.

Indeed, the present study confirms, as expected, that the E-M81 marker is the most common subtype in our samples of the prehistoric population. Other prominent N-African Y-chromosome markers, like E-M78 and J-M267, also appear; these strengthen the case for its possible Berber origin. On the other hand, in the historic sample, as occurs with the present-day Canary population, the European R-M269 marker is already the most common type, with the consequent decrease of E-M78, E-M81 and J-M267 haplogroups. This points to a replacement of indigenous males from the beginning of the European colonization. Moreover, as with the mtDNA, sub-Saharan Y-chromosome lineages such as E-M2 are detected, testifying to the impact of the slave trade in the Canary Islands.

From the perspective of the autosomal AB0 locus, the present-day genetic composition of the Canaries is similar to that of the modern Iberian population, although a certain N-African influence is observed, undetectable in previous studies using enzyme polymorphisms (Afonso et al. 1989; Cabrera et al. 1996; Larruga et al. 1992; Martell et al. 1986; Morilla et al. 1988; Pérez et al. 1991; Pinto et al. 1996c) and blood group serological analysis (Bravo & De las Casas 1958; Guasch et al. 1952; Parejo 1966; Pinto et al. 1996b; Rösing 1967; Schwidetzky 1970), in which only a possible sub-Saharan component was

suggested. So the *A101* allele, more frequent in the Iberian Peninsula than in N Africa, appears in the Canaries with frequencies overlapping the average values of the two continental areas. The same applies to the *B101* allele, which being more abundant in N Africa than in the Peninsula, also appears in the Canaries with intermediate frequencies. Regarding the *O* allele, the relative proportion of *O01* and *O02* has an intermediate value in the Canaries (1.7), between that of N Africa (1.2) and the Peninsula (1.9). Furthermore, *O03* and *O12* alleles were also found in the Canaries, which had only been detected in Berbers and Basques at a low frequency.

The pre-conquest population has an AB0 locus with frequencies similar to those of N Africa. This affinity with N Africa is based on a low frequency for the *A101* allele, a high frequency for *B101* and similar frequencies for *O01* and *O02*, as well as the presence of allele *O03*, more common in N Africa than the Iberian Peninsula. In contrast, the frequencies in the historical population are closer to those of Europe, since it has an *A101* allelic frequency twice as high as the pre-conquest samples, whereas *B101* and *O03* are half as frequent. However, despite the historical sample from Tenerife being so close to the current population, it still retains certain affinities to N Africa, such as the similar *O01* and *O02* frequencies. As stated before, both the mtDNA and the Y-chromosome analyses of the historical population, revealed Saharan influence due to the slave trade in the Canary Islands after the Conquest. There are few differences in the AB0 locus between sub-Saharan and N-Africans, so that this contribution cannot be determined using this autosomal marker. However, although the historical population is at Hardy-Weinberg equilibrium, it is near significance, which might be due to a lack of panmixia in this multi-ethnic population.

In conclusion, the indigenous population of the Canary Islands shows similar profiles to that of N-African populations, both for uniparental lineages and for the AB0 autosomal marker. When these are compared with those of the current population, uniparental markers show discrepant results, with an almost total replacement of Y-chromosome lineages by European ones, and considerable persistence of the indigenous mtDNA pool. As expected, the contributions calculated from the autosomal AB0 locus are intermediate. In the historical population, the mtDNA shows only a moderate decline in the indigenous contribution and a relatively high presence of sub-Saharan African markers.

However, for Y-chromosome and the AB0 locus, the observed values are closest to the current population, suggesting a progressive replacement by Europeans.

6.2. Admixture analysis in the historical and present-day population of the Canary Islands

Admixture analysis taking the Iberian (modern Spanish and Portuguese), and, Northwest and sub-Saharan W-African populations as parental sources of the present-day Canary Islanders showed that the indigenous contribution was 33% for maternal (Rando et al. 1999) and only 7% for paternal lineages (Flores et al. 2003). This strong sex-bias is attributed to an asymmetrical migration of the European conquerors, which consisted mostly of men who displaced the indigenous males, but mixed with the females.

Since the mtDNA and the Y-chromosome represent the paternal and maternal lineages, respectively, the use of an autosomal marker provided a complementary view of the evolution of the Canary Island population. This involved molecular analysis of the AB0 locus in the archipelago and its two parent populations, NW Africa and the Iberian Peninsula. Admixture estimations with samples from the current Canary Islanders show an Iberian Peninsula contribution of approximately 82%, a value not significantly different from those obtained using autosomal Alu polymorphisms, 70% (Maca-Meyer et al. 2004b) and classical autosomal markers, 65-75% (Flores et al. 2001b). Logically, intermediate estimates of their contribution are obtained with autosomal markers when there is sexual asymmetry, calculated from uniparental markers (Flores et al. 2003; Rando et al. 1999). It should be noted that Gomera, characterized by the highest percentage of the mtDNA U6b1 haplotype (Rando et al. 1999), shows the greatest N-African contribution (62%) for the AB0 locus. Although these results may reflect a better conservation of native substrate, they may also be caused by genetic drift due to geographical isolation.

However, in all the admixture estimations calculated from current populations, the NW-African population has been used as representative of the aboriginal genetic pool. But a direct value of the indigenous contribution can be obtained only through genetic analysis

of archaeological remains. The study of their mtDNA composition (Maca-Meyer et al. 2004a), allowed us to estimate the maternal lineages of the current population as 55% European, 42% NW-African and only 3% sub-Saharan. In the historical population of Tenerife (Maca-Meyer et al. 2005), the NW-African contribution was similar to the present-day population (40%), whereas sub-Saharan African (12%) was significantly higher.

To complete these results from the standpoint of paternal lineages, we determined the genetic composition of the indigenous and historical populations for the Y-chromosome. The admixture estimations, using the pre-historic sample directly as parental population, confirm the existence of a strong sexual asymmetry. The European contribution to the paternal lineages increased from the 17^{th}-18^{th} C. to the present (63% vs 83%), with the consequent reduction of the indigenous (31% vs 17%) and sub-Saharan inputs (6% vs 1%). Comparing them to the maternal lineages, we observed that both European (48% vs 55%) and indigenous (40% vs. 42%) lineages are quite similar from the 17^{th}-18^{th} century to the present, and that only the sub-Saharan maternal lineages have suffered a sharp decline since historical times (12% vs 3%). These results suggest that indigenous males made a lesser contribution to the current population, not only due to their high mortality during the Conquest, and subsequent sale as slaves, but also after that, probably due to a mating discrimination based on the better social status held by the European settlers. In the case of sub-Saharan lineages, the decline in the contribution in both uniparental markers shows that this disadvantage affected both men and women.

In conclusion, the study of the original, historical and current populations of the Canary Islands, using uniparental and autosomal markers, confirms that the Conquest and subsequent colonization of the islands by Europeans, caused a strong sexual asymmetry in which the indigenous maternal lineages survived in the present-day population in a significantly higher proportion than the paternal lineages. Moreover, the impact of the slave-trade in the islands after the Conquest has declined since historical times to the present, affecting maternal and paternal lineages equally.

6.3. The pre-historic settlement process of the Canary Islands deduced from autosomal and uniparental markers

The genetic characterization of the Canaries provides information, although sometimes contradictory, about the early colonization. The detection of significant correlations between relative frequencies and/or diversity values for mtDNA and CD4/Alu haplotypes, and geographical distances of the islands from Africa up to now have been explained assuming a single main colonization event (Flores et al. 2001b; Rando et al. 1999).

In this thesis, the study of AB0 blood groups in the present-day population of the Canary Islands has also provided some data that may reflect the colonization process. Two AB0 alleles are significantly more abundant in NW Africa (*B101* and *O12*) than in the Iberian Peninsula. Although Pearson correlations between the islands' distance from Africa and insular frequencies showed negative values for both alleles (*B101*, R=−0.822; *O12*, R=−0.589), only the *B101* reached significance (p=0.023; two-tailed test). This result can be explained supposing a colonization beginning in the nearest island to Africa, continuing westwards following a stepping-stone model, but can also be explained by a proportionally greater Iberian Peninsula gene-flow to the western islands.

In contrast, in a previous Y-chromosome study in the current population, based on distribution and age of African lineages present in the islands, two opposite correlations were found (Flores et al. 2003). This result was explained by at least two independent waves of colonists from NW Africa, still detectable today, congruent with previous anthropological, archaeological and linguistic data (Martínez 1996; Navarro 1992; Onrubia-Pintado 1987; Reyes-García 2000).

Except for Gomera, we have not detected significant differences beween the mtDNA composition of the present-day populations at intra-archipelago level. However, some affinities were found on the easternmost islands of Lanzarote and Fuerteventura. The higher frequencies on these islands for the autochthonous U6c1 lineage and for the T2c subgroup, both considered founder haplotypes (Rando et al. 1999), compared with the

homogenous distribution of the autochthonous U6b1 subtype, suggest possible secondary waves of colonization from N Africa, mainly affecting these closer islands. Indeed, these lineages were later directly detected in Guanche remains, confirming their presence in the indigenous genetic pool (Maca-Meyer et al., 2004). Although this asymmetrical distribution of haplogroups U6c1 and T2c could be attributed to isolation and drift effects, the fact that they resemble similar asymmetrical distributions found for Y chromosome haplogroups (Flores et al., 2003) reinforces the 'two waves' colonization hypothesis.

The analysis of mtDNA in the pre-conquest population of La Palma has also provided clues to the early human colonization. Since La Palma is one of the two westernmost islands of the archipelago, comparison of its pre-conquest population at the level of mtDNA gene diversity and structure with the previously published data on that of Tenerife proved useful to test the alternative models of colonization directly. The great genetic diversity found on the island of La Palma (95.2% ± 2.3%), similar to that obtained on Tenerife (92.4 ± 2.8), is opposed to the assumption of a settlement process through island-hopping and posterior isolation. Moreover, the great overall similarity found between these two populations also rebuts the idea of an independent island-by-island maritime colonization without secondary contacts. In addition, if the Canary Islands were colonized in several waves, it indicates that Tenerife and La Palma were affected likewise by these events. In conclusion, our data fit better with a colonization model including frequent migrations between islands, requiring some kind of marine transport.

Furthermore, the genetic composition of the sample of the pre-conquest population for the AB0 locus is similar to those found in N Africa, and suggesting that heavy bottlenecks did not occur during the colonization event/process. Therefore, these results again do not support a restrictive stepping-stone model, with subsequent isolation.

Another aspect that remains unresolved in the prehistory of the Canary Islands is to determine the exact origin of the aboriginal population. On the basis of the probabilistic criteria, several sequences of putative NW-African ancestry were proposed as pre-Hispanic founder haplotypes for the Islands (Rando et al. 1999). Some of these lineages are present in the indigenous populations of La Palma and Tenerife and have exact matches in NW Africa. However, the autochthonous U6b1 haplogroup has not yet been

detected in Africa. Furthermore, the H1 (16260) subhaplogroup, highly frequent in the ancient population of La Palma, is detected at a low frequency in N Africa (Ennafaa et al. 2009). Due to the relatively recent radiocarbon dates estimated for the first settlement of the archipelago (Arco et al. 1997a; Arco et al. 1981; Martín de Guzmán 1978; Martín 2000; Navarro 1997; Velasco-Vázquez et al. 2002), it seems improbable that the U6b1 haplogroup and all the derived 16260 types originated in the islands after their colonization. In fact, coalescence ages calculated for 16260 H1 and U6b1 gave dates of 6,263 ± 2,869 and 5,911 ± 3,536 years ago, respectively. These dates, not statistically different, clearly precede the proposed dating of the indigenous settlement at the beginning of the first millennium (Arco et al. 1997; Arco et al. 1981; Martín de Guzmán 1978; Martín 2000; Navarro 1997; Velasco-Vázquez et al. 2002), so their absence or rareness in N Africa must be because the precise site from which the islanders' ancestors originated has not yet been sampled or because they have been replaced by later human migrations.

In the Y-chromosome study of the indigenous population, the detection of markers like I-M170 and R-M269, of clear European ascription, might suggest that other secondary waves also reached the archipelago, most likely from the Mediterranean basin. However, as these markers are also present in N Africa, albeit in low frequencies, they may also have arrived in the islands with the first settlers. If so, their presence in Africa may be older than previously proposed for R-M269 (Bosch et al. 2001). In the same vein, haplogroup J-M267, whose presence in N Africa had been related to the Arab expansion in the 7th century A.D. (Semino et al. 2004), must have reached the island along with a second wave of colonists, like M170 and M269, or otherwise its presence in NW Africa would have to be previous to its Arab occupation.

In summary, although further analysis will be necessary to clarify the colonization process of the Canary Islands, the present study of uniparental and autosomal markers in the indigenous, historical and present-day populations seems to indicate that this process was not based on a single migration event, following a stepping-stone model with subsequent genetic isolation between islands. Our results are more consistent with at least two colonization waves from NW Africa without strong drift effects afterwards. Furthermore,

the similarity in mtDNA frequencies and diversity found between aboriginal people from Tenerife and La Palma are evidence of secondary migration between islands.

Conclusiones

8. CONCLUSIONES

Las principales conclusiones de este trabajo son:

- Como promedio, la composición genética del locus AB0 en la población canaria actual es similar a la de la Península Ibérica, pero con cierta mezcla norteafricana detectable por la frecuencia del alelo O12 y la proporción entre los alelos O01:O02, que en Canarias muestran valores intermedios entre ambas regiones.

- Todavía se detecta en la población canaria actual una correlación negativa entre la distancia a África y la frecuencia del alelo B101 en cada una de las islas, lo que apoyaría la hipótesis de una colonización aborigen de este a oeste desde el continente africano, siguiendo un modelo escalonado.

- Los valores de estima de mezcla para la población actual de Canarias, basados en el locus AB0, muestran asimismo una aportación europea dominante, pero con una contribución norteafricana aún detectable.

- La isla de La Gomera, caracterizada por una mayor frecuencia del haplogrupo U6b1 del ADNmt, destaca igualmente por presentar una aportación norteafricana mayoritaria con un valor del 62%.

- Las frecuencias del locus AB0 en los aborígenes son similares a las de las poblaciones del noroeste africano, señalando nuevamente esta área como el origen de los indígenas canarios.

- La diversidad genética del locus AB0 en la población aborigen es similar a la de la población actual y a la del norte de África, lo que indica que la colonización de las islas no implicó grandes cuellos de botella que afectaran a este locus.

- También para el locus AB0, la población histórica muestra una composición alélica intermedia entre la aborigen y la actual, indicando que el impacto de la colonización europea ha sido progresivo.

- Aunque los linajes de ADNmt muestran una composición relativamente homogénea en la población actual de las islas, la de La Gomera destaca por su alta frecuencia para el haplogrupo autóctono U6b1, lo que puede atribuirse a una mayor conservación del sustrato aborigen femenino o a afectos de deriva genética provocados por su geografía.

- La distribución asimétrica de los haplogrupos fundadores T2c y U6c1 del ADN mitocondrial en la población actual de Canarias, con frecuencias significativamente más altas en las islas orientales, parece reflejar un proceso colonizador en, al menos, dos fases.

- La asimetría sexual observada en las frecuencias de haplogrupos uniparentales con asignación geográfica norteafricana y subsahariana, en la población canaria actual, apunta a una aportación significativamente mayor de los linajes maternos de ambas regiones geográficas, comparadas con las contribuciones paternas.

- La composición y alta diversidad genética observada para el ADN mitocondrial en la población aborigen de la isla de La Palma, una de las más alejadas del continente africano, y su similitud con la población indígena de la isla de Tenerife, está en contradicción con un modelo basado en la colonización secuencial de las islas y su posterior aislamiento.

- La ausencia en el Norte de África del haplogrupo autóctono canario U6b1 y la baja frecuencia del subhaplogrupo fundador H1 (16260), indica que la región exacta de la que partieron los primeros pobladores de Canarias no ha sido localizada todavía o que su composición

genética ha sido modificada por posteriores eventos migratorios. La posibilidad de que dichos haplogrupos se originaran en las islas es muy baja si comparamos sus edades de coalescencia con las dataciones obtenidas de yacimientos aborígenes.

- La alta frecuencia de los haplogrupos E-M81, E-M78 y J-M267 del cromosoma Y en los aborígenes canarios confirma también el origen norteafricano de los linajes masculinos de la población indígena.

- La presencia en la población aborigen masculina de los haplogrupos mayoritariamente europeos I-M170 y R-M269 puede indicar la existencia de migraciones directas a las islas de colonizadores mediterráneos prehispánicos, o bien, la presencia de estos haplogrupos en el norte de África en fechas anteriores a las propuestas hasta ahora.

- La respectiva alta y baja frecuencia del haplogrupo europeo R-M269 y del norteafricano E-M81 en la población histórica, comparada con la aborigen, apunta a una sustitución temprana de los linajes masculinos indígenas por los de los nuevos colonizadores.

- La presencia de linajes masculinos de origen subsahariano como el E-M2 en la población histórica, confirma el impacto del tráfico de esclavos en esa época.

- La estima de mezcla de los linajes masculinos en la población canaria actual y en la histórica, usando la muestra aborigen como contribución norteafricana, muestra que la influencia europea fue notable desde tiempos históricos y que ha aumentado significativamente hasta la actualidad. Esto indica que el reemplazo de los linajes aborígenes por los europeos no sólo se produjo durante la conquista, sino que fue aumentando de forma progresiva en periodos posteriores.

- En resumen, el conjunto de los datos parece señalar que más que un único evento migratorio, siguiendo un modelo de salto escalonado desde el continente, la colonización se produjo en, al menos, dos oleadas migratorias desde el noroeste africano, sin fuertes efectos fundadores y con migraciones secundarias entre islas.

CONCLUSIONS

8. CONCLUSIONS

The main conclusions of the present work are:

- In general terms, the genetic composition of the AB0 locus in the current population is similar to that of the Iberian peninsula, but a N African admixture detected by the O12 allele frequency and the ratio of alleles O01 : O02 in the Canaries show intermediate values between the two regions.

- The negative correlation between the distance from Africa and the B101 allele frequency still detectable in the current population supports the hypothesis of a main colonization event from East to West following a stepping-stone model.

- AB0 locus admixture estimations calculated for the present Canary Islands population show a greater European contribution, although some NW African influence is still detectable.

- Gomera was however notable for its higher N African contribution of 62%.

- The AB0 locus frequencies in the pre-conquest population are similar to those of NW Africa, supporting its origin in this area.

- The genetic diversity of the AB0 locus in the indigenous population is similar to those of the current population and North Africa, suggesting that colonization of the islands did not involve bottlenecks affecting this locus.

- The AB0 locus of the historical population has an intermediate composition between the pre-conquest and current populations, showing that the impact of the European colonization has been progressive.

- Although mtDNA lineages show a relatively homogeneous composition in the current Canary Island population, Gomera stands out for its high frequency for the autochthonous U6b1 haplogroup, which can be attributed to greater conservation of the female indigenous substrate or to genetic drift effects.

- The asymmetric distribution of the mtDNA founder haplogroups T2c and U6c1 in the current population, with significantly higher frequencies in the eastern islands, seems to reflect a colonization process in at least two waves.

- The sexual asymmetry observed in the frequencies of uniparental haplogroups with North African and sub-Saharan geographical assignation in the current population, points to a significantly greater contribution of the maternal rather than paternal lineages of both geographical regions.

- The composition and high genetic diversity observed for the pre-conquest mtDNA in La Palma, one of the most distant islands from the African continent, and its similarity to the indigenous population of the island of Tenerife, is in contradiction with a model based on sequential colonization of the islands and subsequent isolation.

- The absence of the autochthonous U6b1 haplogroup and the low frequency of the founder 16260 H1 subhaplogroup in NW Africa indicate that the current inhabitants of the exact region from which the ancestors of the original Canary Islanders came have not yet been sampled or that their genetic composition has been modified by the intervening human migrations. The possibility that these haplogroups originated in the

islands is very low if we compare their coalescence age with the datings obtained from archaeological sites.

- The high frequency of E-M81, E-M78 and J-M267 haplogroups in the Y-chromosome lineages extracted from archaeological remains of the indigenous population also confirm their N African origin.

- The presence in the male indigenous population of the European I-M170 and R-M269 haplogroups may point to a direct migration of other pre-Hispanic settlers to the islands from the Mediterranean area, or the presence of these haplogroups in N Africa at earlier dates than those previously proposed.

- The respectively high and low frequencies of the European R-M269 and N African E-M81 haplogroups in the historical, and pre-conquest, populations point to an early replacement of the indigenous male lineages by the European settlers.

- The presence of sub-Saharan male lineages, like E-M2, in the historical population confirms the impact of the slave-trade at that time.

- The admixture estimation of the male lineages present in the historical and current populations, using the aboriginal sample as N African parental source, shows that European influence was notable during historical times and has significantly increased up to the present. This indicates that replacement of native lineages by European ones not only took place during the Conquest, but progressively increased in subsequent periods.

- In conclusion, all the data seems to indicate that this process was not based on a single migration event, following a stepping-stone model with subsequent genetic isolation between islands. Our results are more

consistent with at least two colonization waves from NW Africa without strong drift effects afterwards.

Bibliografía

9. BIBLIOGRAFÍA

Afonso, J. M., Hernandez, M., Larruga, J. M., Cabrera, V. M. y González, A. M. 1989 Human enzyme polymorphism in the Canary Islands. III. Tenerife Island population. *Hum Biol* **61**, 543-9.

Almeida, T. A., Cabrera, V. M. y Miranda, J. G. 1998 Improved detection and characterization of mutations by primer addition in nonradioisotopic SSCP and direct PCR sequencing. *Biotechniques* **24**, 220-1.

Alonso, A., Martin, P., Albarran, C., Garcia, P., Garcia, O., de Simon, L. F., Garcia-Hirschfeld, J., Sancho, M., de La Rua, C. y Fernandez-Piqueras, J. 2004 Real-time PCR designs to estimate nuclear and mitochondrial DNA copy number in forensic and ancient DNA studies. *Forensic Sci Int* **139**, 141-9.

Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J., Staden, R. y Young, I. G. 1981 Sequence and organization of the human mitochondrial genome. *Nature* **290**, 457-65.

Andrews, R. M., Kubacka, I., Chinnery, P. F., Lightowlers, R. N., Turnbull, D. M. y Howell, N. 1999 Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat Genet* **23**, 147.

Arco, M. C. y Navarro, J. F. 1987 *Historia popular de Canarias. Vol. 1. Los aborígenes*. Santa Cruz de Tenerife: Centro de la Cultura Popular Canaria.

Arco, M. C., Hernández, M., Jiménez, M. C. y Navarro, J. F. 1981 Nuevas fechas de C-14 en la prehistoria de Gran Canaria. *El Museo Canario* **II**, 51-87.

Arco, M. C., Del Arco, M. M., Atiénzar, E., Atoche, P., Martín, M., Rodríguez, C. y Rosario, C. 1997 Dataciones absolutas en la Prehistoria de Tenerife. *Homenaje a Celso Martín de Guzman (1946-1994)* **Las Palmas**, 65-77.

Arnay, M. y Pérez, A. R. 2002 Estudio de un espacio sepulcral del Siglo XVIII en la Iglesia de la Concepción de Santa Cruz de Tenerife. *Tabona* **11**, 131-167.

Arnay, M. 2009 La arqueología histórica en Canarias. El yacimiento sepulcral de la Iglesia de Nuestra Señora de La Concepción de Santa Cruz de Tenerife. *Arqueología Iberoamericana* **3**, 21-36.

Arredi, B., Poloni, E. S., Paracchini, S., Zerjal, T., Fathallah, D. M., Makrelouf, M., Pascali, V. L., Novelletto, A. y Tyler-Smith, C. 2004 A predominantly neolithic origin for Y-chromosomal DNA variation in North Africa. *Am J Hum Genet* **75**, 338-45.

Arriaza, B. T., Salo, W., Aufderheide, A. C. y Holcomb, T. A. 1995 Pre-Columbian tuberculosis in northern Chile: molecular and skeletal evidence. *Am J Phys Anthropol* **98**, 37-45.

Atoche, P., Paz, J. A., Ramírez, M. A. y Ortiz, M. E. 1995 *Evidencias arqueológicas del mundo romano en Lanzarote*. Arrecife: Servicio de publicaciones del Excmo. Cabildo Insular de Lanzarote.

Aznar-Vallejo, E. 1991 *Población y sociedad en la época realenga. Historia de Canarias. I.* Editorial Prensa Ibérica S.A.

Aznar-Vallejo, E. 1992 *La integración de las Islas Canarias en la Corona de Castilla (1478-1526).* Las Palmas de Gran Canaria: Cabildo Insular de Gran Canaria.

Aznar-Vallejo, E. 1996 Colonización. En *Gran Enciclopedia Canaria, IV*, pp. 1053-1056. Las Palmas de Gran Canaria: Edirca.

Bailey, J. F., Richards, M. B., Macaulay, V. A., Colson, I. B., James, I. T., Bradley, D. G., Hedges, R. E. y Sykes, B. C. 1996 Ancient DNA suggests a recent expansion of European cattle from a diverse wild progenitor species. *Proc Biol Sci* **263**, 1467-73.

Bandelt, H. J., Forster, P. y Rohl, A. 1999 Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* **16**, 37-48.

Bandelt, H. J. 2005 Mosaics of ancient mitochondrial DNA: positive indicators of nonauthenticity. *Eur J Hum Genet* **13**, 1106-12.

Bensasson, D., Zhang, D., Hartl, D. L. y Hewitt, G. M. 2001 Mitochondrial pseudogenes: evolution's misplaced witnesses. *Trends Ecol Evol* **16**, 314-321.

Betancor, G. 2002 *Los canarios en la formación de la moderna sociedad tinerfeña. Integración y aculturación de los indígenas de Gran Canaria (1946)*. Las Palmas de Gran Canaria: Ediciones del Cabildo de Gran Canaria.

Betancor, G. 2003 Los indígenas en la formación de la moderna sociedad canaria. Integración y aculturación de canarios, gomeros y guanches (1496-1525). Tesis doctoral: Universidad de Las Palmas de Gran Canaria.

Béthencourt, A. 1995 *Historia de Canarias*. Las Palmas de Gran Canaria: Cabildo Insular de Gran Canaria.

Bosch, E., Calafell, F., Comas, D., Oefner, P. J., Underhill, P. A. y Bertranpetit, J. 2001 High-resolution analysis of human Y-chromosome variation shows a sharp discontinuity and limited gene flow between northwestern Africa and the Iberian Peninsula. *Am J Hum Genet* **68**, 1019-29.

Brakez, Z., Bosch, E., Izaabel, H., Akhayat, O., Comas, D., Bertranpetit, J. y Calafell, F. 2001 Human mitochondrial DNA sequence variation in the Moroccan population of the Souss area. *Ann Hum Biol* **28**, 295-307.

Bravo, A. M. y De las Casas, M. T. 1958 Distribución de grupos sanguíneos en los habitantes de la Isla de La Palma. *Acta Médica de Tenerife* **10**, 114-117.

Brion, M., Sobrino, B., Blanco-Verea, A., Lareu, M.V., Carracedo, A. 2005 Hierarchical analysis of 30 Y-chromosome SNPs in European populations. *Int J Legal Med* **119**, 10-15.

Brotherton, P., Endicott, P., Sanchez, J. J., Beaumont, M., Barnett, R., Austin, J. y Cooper, A. 2007 Novel high-resolution characterization of ancient DNA reveals C → U-type base modification events as the sole cause of post mortem miscoding lesions. *Nucleic Acids Res* **35**, 5717-28.

Brotherton, P., Sanchez, J. J., Cooper, A. y Endicott, P. 2009 Preferential access to genetic information from endogenous hominin ancient DNA and accurate quantitative SNP-typing via SPEX. *Nucleic Acids Res*.

Burger, J., Hummel, S., Hermann, B. y Henke, W. 1999 DNA preservation: a microsatellite-DNA study on ancient skeletal remains. *Electrophoresis* **20**, 1722-8.

Cabrera, J. C. 1991 El Resdescubrimiento. En *Historia de Canarias*. Prensa Ibérica S.A.

Cabrera, J. C. 1992a *La prehistoria de Canarias. Vol. 4. Lanzarote y Los Majos.* Santa Cruz de Tenerife: Centro de la Cultura Popular Canaria.

Cabrera, J. C. 1992b *La Prehistoria de Canarias. Vol. 7. Fuerteventura y los Majoreros.* Santa Cruz de Tenerife: Centro de la Cultura Popular Canaria.

Cabrera, V. M., González, P. y Salo, W. L. 1996 Human enzyme polymorphism in the Canary Islands. VII. G6PD Seattle in Canarians and North African Berbers. *Hum Hered* **46**, 197-200.

Cann, R. L., Stoneking, M. y Wilson, A. C. 1987 Mitochondrial DNA and human evolution. *Nature* **325**, 31-6.

Cano, R. J., Poinar, H. N., Pieniazek, N. J., Acra, A. y Poinar, G. O., Jr. 1993 Amplification and sequencing of DNA from a 120-135-million-year-old weevil. *Nature* **363**, 536-8.

Carlyle, S. W., Parr, R. L., Hayes, M. G. y O'Rourke, D. H. 2000 Context of maternal lineages in the Greater Southwest. *Am J Phys Anthropol* **113**, 85-101.

Casas, M. J., Hagelberg, E., Fregel, R., Larruga, J. M. y González, A. M. 2006 Human mitochondrial DNA diversity in an archaeological site in al-Andalus: genetic impact of migrations from North Africa in medieval Spain. *Am J Phys Anthropol* **131**, 539-51.

Castellano, J. M. y Macías, F. J. 1997 *Historia de Canarias*. Santa Cruz de Tenerife: Centro de la Cultura Popular Canaria.

Cioranescu, A. 1998 *Historia de Santa Cruz de Tenerife I (1494-1803)*. Servicio de publicaciones de la Caja General de Ahorros de Canarias (Nº 207).

Cooper, A., Mourer-Chauvire, C., Chambers, G. K., von Haeseler, A., Wilson, A. C. y Pääbo, S. 1992 Independent origins of New Zealand moas and kiwis. *Proc Natl Acad Sci U S A* **89**, 8741-4.

Cooper, A. y Poinar, H. N. 2000 Ancient DNA: do it right or not at all. *Science* **289**, 1139.

Crosby, A. W. 1988 *Imperialismo ecológico. La expansión biológica de Europa 900-1900*. Barcelona: Ed. Crítica.

Cruciani, F., La Fratta, R., Santolamazza, P., Sellitto, D., Pascone, R., Moral, P., Watson, E., Guida, V., Colomb, E. B., Zaharova, B., Lavinha, J., Vona, G., Aman, R., Cali, F., Akar, N., Richards, M., Torroni, A., Novelletto, A. y Scozzari, R. 2004 Phylogeographic analysis of haplogroup E3b (E-M215) Y chromosomes reveals multiple migratory events within and out of Africa. *Am J Hum Genet* **74**, 1014-22.

Curry, G. B. 1988 Amino acids and proteins from fossils. En *Molecular evolution and the fossil record. Short courses in Paleontology* (ed. B. Runnegard y J. W. Schopf). Knoxville, USA: Paleontological Society.

Daniels, G. 1995 *Human Blood Groups*. Oxford: Blackwell Science.

Delgado, J. A. 2009 Canarias en la Antigüedad como problema histórico. Consideraciones teóricas, metodológicas y documentales. En *Actas del VI Congreso de Patrimonio Histórico: Investigación arqueológica: territorio y sociedad*. Arrecife, Lanzarote.

Destro-Bisol, G., Maviglia, R., Caglia, A., Boschi, I., Spedini, G., Pascali, V., Clark, A. y Tishkoff, S. 1999 Estimating European admixture in African Americans by using microsatellites and a microsatellite haplotype (CD4/Alu). *Hum Genet* **104**, 149-57.

Díaz, G. y Rodríguez, J. M. 1990 *El señorío en las Canarias Occidentales. La Gomera y El Hierro hasta 1700*. Santa Cruz de Tenerife: Cabildo Insular de La Gomera, Cabildo Insular de El Hierro.

Drancourt, M., Aboudharam, G., Signoli, M., Dutour, O. y Raoult, D. 1998 Detection of 400-year-old Yersinia pestis DNA in human dental pulp: an approach to the diagnosis of ancient septicemia. *Proc Natl Acad Sci U S A* **95**, 12637-40.

Dupanloup, I. y Bertorelle, G. 2001 Inferring admixture proportions from molecular data: extension to any number of parental populations. *Mol Biol Evol* **18**, 672-5.

Endicott, P., Gilbert, M. T., Stringer, C., Lalueza-Fox, C., Willerslev, E., Hansen, A. J. y Cooper, A. 2003 The genetic origins of the Andaman Islanders. *Am J Hum Genet* **72**, 178-84.

Ennafaa, H., Cabrera, V. M., Abu-Amero, K. K., González, A. M., Amor, M. B., Bouhaha, R., Dzimiri, N., Elgaaied, A. B. y Larruga, J. M. 2009 Mitochondrial DNA haplogroup H structure in North Africa. *BMC Genet* **10**, 8.

Eshleman, J. y Smith, D. G. 2001 Use of DNase to eliminate contamination in ancient DNA analysis. *Electrophoresis* **22**, 4316-9.

Estévez, F. 1987 *Indigenismo, raza y evolución. El pensamiento antropológico canario (1750-1900)*. Santa Cruz de Tenerife: Cabildo Insular de Tenerife.

Faerman, M., Filon, D., Kahila, G., Greenblatt, C. L., Smith, P. y Oppenheim, A. 1995 Sex identification of archaeological human remains based on amplification of the X and Y amelogenin alleles. *Gene* **167**, 327-32.

Farrujía de la Rosa, A. J. 2004 *Ab Initio (1342-1969). Análisis historiográfico y arqueológico del primitivo poblamiento de Canarias*. La Laguna: Artemisa Ediciones.

Farrujía de la Rosa, A. J. 2007 *Arqueología y franquismo en Canarias. Política, poblamiento e identidad (1939-1969)*. Canarias Arqueológica 2. Santa Cruz de Tenerife: Organismo Autónomo de Museos y Centros. Cabildo de Tenerife.

Ferembach, D. 1970 Les cromagnoides de l´Afrique du Nord. En *L´Homme de Cro-Magnon. Anthropologie et Archaeologie* (ed. G. Camps y G. Olivier), pp. 81-91. Paris: Arts et Metiers Graphiques.

Ferembach, D. 1986 Homo Sapiens en Afrique: des origes du néolithique. En *L´Homme, son evolution sa diversité. Manuel de Anthropologie Physique* (ed. D. Ferembach, C. Sussane y M. C. Chamla), pp. 245-256. Paris: Editions du CNRS.

Flores, C., Maca-Meyer, N., González, A. M. y Cabrera, V. M. 2000 Northwest African distribution of the CD4/Alu microsatellite haplotypes. *Ann Hum Genet* **64**, 321-7.

Flores, C. 2001 Composición genética y posible origen paterno de las poblaciones humanas canarias, deducidos de su polimorfismo en el cromosoma Y. Tesis Doctoral: Universidad de La Laguna.

Flores, C., Maca-Meyer, N., Pérez, J. A. y Cabrera, V. M. 2001a The peopling of the Canary Islands: a CD4/Alu microsatellite haplotype perspective. *Hum Immunol* **62**, 949-53.

Flores, C., Larruga, J. M., González, A. M., Hernández, H., Pinto, F. M. y Cabrera, V. M. 2001b The origin of the Canary Island aborigines and their contribution to the modern population: A molecular genetics perspective. *Current Anthropology* **42**, 749-754.

Flores, C., Maca-Meyer, N., Pérez, J. A., González, A. M., Larruga, J. M. y Cabrera, V. M. 2003 A predominant European ancestry of paternal lineages from Canary Islanders. *Ann Hum Genet* **67**, 138-52.

Flores, C., Maca-Meyer, N., González, A. M., Oefner, P. J., Shen, P., Pérez, J. A., Rojas, A., Larruga, J. M. y Underhill, P. A. 2004 Reduced genetic structure of the Iberian peninsula revealed by Y-chromosome analysis: implications for population demography. *Eur J Hum Genet* **12**, 855-63.

Forster, P., Harding, R., Torroni, A. y Bandelt, H. J. 1996 Origin and evolution of Native American mtDNA variation: a reappraisal. *Am J Hum Genet* **59**, 935-45.

Gaud, J. 1942 Contribution à l'étude ethnographique du Maroc. Répartition des groupes sanguins parmi les tribus des environs de Meknès. *Bull Inst Hyg Maroc* **2**, 37-52.

Gilbert, M. T., Bandelt, H. J., Hofreiter, M. y Barnes, I. 2005 Assessing ancient DNA studies. *Trends Ecol Evol* **20**, 541-4.

Gilbert, M. T., Binladen, J., Miller, W., Wiuf, C., Willerslev, E., Poinar, H., Carlson, J. E., Leebens-Mack, J. H. y Schuster, S. C. 2007 Recharacterization of ancient DNA miscoding lesions: insights in the era of sequencing-by-synthesis. *Nucleic Acids Res* **35**, 1-10.

Gill, P., Whitaker, J., Flaxman, C., Brown, N. y Buckleton, J. 2000 An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Sci Int* **112**, 17-40.

Ginther, C., Issel-Tarver, L. y King, M. C. 1992 Identifying individuals by sequencing mitochondrial DNA from teeth. *Nat Genet* **2**, 135-8.

Golenberg, E. M., Giannasi, D. E., Clegg, M. T., Smiley, C. J., Durbin, M., Henderson, D. y Zurawski, G. 1990 Chloroplast DNA sequence from a miocene Magnolia species. *Nature* **344**, 656-8.

González, A. M., Cabrera, V. M., Larruga, J. M., Tounkara, A., Noumsi, G., Thomas, B. N. y Moulds, J. M. 2006 Mitochondrial DNA variation in Mauritania and Mali and their genetic relationship to other Western Africa populations. *Ann Hum Genet* **70**, 631-57.

González, R. y Tejera, A. 1990 *Los aborígenes canarios: Gran Canaria y Tenerife*. Oviedo: Ediciones Itsmo.

Green, R. E., Krause, J., Ptak, S. E., Briggs, A. W., Ronan, M. T., Simons, J. F., Du, L., Egholm, M., Rothberg, J. M., Paunovic, M. y Pääbo, S. 2006 Analysis of one million base pairs of Neanderthal DNA. *Nature* **444**, 330-6.

Guasch, J., Florensa, A., Díaz de Yraola, G., Gavilanes, C., Del Rio, R., Tabuenca, J. y Raichs, A. 1952 Los factores hemáticos en España, excepto en el País Vasco. *Med Clin* **18**, 268-271.

Guo, S. W. y Thompson, E. A. 1992 Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* **48**, 361-72.

Gutiérrez, G. y Marin, A. 1998 The most ancient DNA recovered from an amber-preserved specimen may not be as ancient as it seems. *Mol Biol Evol* **15**, 926-9.

Handt, O., Hoss, M., Krings, M. y Pääbo, S. 1994 Ancient DNA: methodological challenges. *Experientia* **50**, 524-9.

Hanni, C., Brousseau, T., Laudet, V. y Stehelin, D. 1995 Isopropanol precipitation removes PCR inhibitors from ancient bone extracts. *Nucleic Acids Res* **23**, 881-2.

Harris, H. 1966 Enzyme polymorphisms in man. *Proc R Soc Lond B Biol Sci* **164**, 298-310.

Hernández, P. 1999 *Natura y cultura de las Islas Canarias*. La Laguna: Tafor publicaciones.

Higuchi, R., Bowman, B., Freiberger, M., Ryder, O. A. y Wilson, A. C. 1984 DNA sequences from the quagga, an extinct member of the horse family. *Nature* **312**, 282-4.

Hofreiter, M., Poinar, H. N., Spaulding, W. G., Bauer, K., Martin, P. S., Possnert, G. y Pääbo, S. 2000 A molecular analysis of ground sloth diet through the last glaciation. *Mol Ecol* **9**, 1975-84.

Hofreiter, M., Serre, D., Poinar, H. N., Kuch, M. y Pääbo, S. 2001a Ancient DNA. *Nat Rev Genet* **2**, 353-9.

Hofreiter, M., Jaenicke, V., Serre, D., Haeseler Av, A. y Pääbo, S. 2001b DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. *Nucleic Acids Res* **29**, 4793-9.

Hofreiter, M., Mead, J. I., Martin, P. y Poinar, H. N. 2003 Molecular caving. *Curr Biol* **13**, R693-5.

Hooton, E. A. 1916 Preliminary remarks on the Archaeology and Physical Anthropology of Tenerife. *Am Anthropol New Series* **18**, 358-365.

Hoss, M., Jaruga, P., Zastawny, T. H., Dizdaroglu, M. y Pääbo, S. 1996 DNA damage and DNA sequence retrieval from ancient tissues. *Nucleic Acids Res* **24**, 1304-7.

Hubby, J. L. y Lewontin, R. C. 1966 A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in Drosophila pseudoobscura. *Genetics* **54**, 577-94.

Hummel, S., Schmidt, D., Kahle, M. y Herrmann, B. 2002 AB0 blood group genotyping of ancient DNA by PCR-RFLP. *Int J Legal Med* **116**, 327-33.

Hurles, M. E., Veitia, R., Arroyo, E., Armenteros, M., Bertranpetit, J., Pérez-Lezaun, A., Bosch, E., Shlumukova, M., Cambon-Thomsen, A., McElreavey, K., Lopez De Munain, A., Rohl, A., Wilson, I. J., Singh, L., Pandya, A., Santos, F. R., Tyler-Smith, C. y Jobling, M. A. 1999 Recent male-mediated gene flow over a linguistic barrier in Iberia, suggested by analysis of a Y-chromosomal DNA polymorphism. *Am J Hum Genet* **65**, 1437-48.

INE. 2008 Censos de población y viviendas. España: Instituto Nacional de Estadística.

Jiménez, J. J. 2005 *Canarii. La génesis de los canarios desde el Mundo Antiguo*. Taller de Historia 32. Santa Cruz de Tenerife: Centro de la Cultura Popular Canaria.

Jobling, M. A. y Tyler-Smith, C. 1995 Fathers and sons: the Y chromosome and human evolution. *Trends Genet* **11**, 449-56.

Jobling, M. A. y Tyler-Smith, C. 2003 The human Y chromosome: an evolutionary marker comes of age. *Nat Rev Genet* **4**, 598-612.

Jorde, L. B., Bamshad, M. y Rogers, A. R. 1998 Using mitochondrial and nuclear DNA markers to reconstruct human evolution. *Bioessays* **20**, 126-36.

Jorge, S. 1996 *Las navegaciones por la costa atlántica africana y las Islas Canarias en la Antigüedad. Estudios prehispánicos 4*. Santa Cruz de Tenerife: Dirección General de Patrimonio Histórico.

Karafet, T. M., Mendez, F. L., Meilerman, M. B., Underhill, P. A., Zegura, S. L. y Hammer, M. F. 2008 New binary polymorphisms reshape and increase resolution of the human Y chromosomal haplogroup tree. *Genome Res* **18**, 830-8.

Krings, M., Stone, A., Schmitz, R. W., Krainitzki, H., Stoneking, M. y Pääbo, S. 1997 Neandertal DNA sequences and the origin of modern humans. *Cell* **90**, 19-30.

Krings, M., Geisert, H., Schmitz, R. W., Krainitzki, H. y Pääbo, S. 1999 DNA sequence of the mitochondrial hypervariable region II from the neandertal type specimen. *Proc Natl Acad Sci U S A* **96**, 5581-5.

Krings, M., Capelli, C., Tschentscher, F., Geisert, H., Meyer, S., von Haeseler, A., Grossschmidt, K., Possnert, G., Paunovic, M. y Pääbo, S. 2000 A view of Neandertal genetic diversity. *Nat Genet* **26**, 144-6.

Lalueza, C., Pérez-Pérez, A., Prats, E., Cornudella, L. y Turbon, D. 1997 Lack of founding Amerindian mitochondrial DNA lineages in extinct aborigines from Tierra del Fuego-Patagonia. *Hum Mol Genet* **6**, 41-6.

Landsteiner, K. 1901 Über Agglutinationserscheinnungen normalen menschlichen Blutes. *Wien Klin Wochenschr* **14**, 1132-1134.

Larraz, A. y González, M. C. 1995 Estudio documental de la Parroquia de Nuestra Señora de la Concepción de Santa Cruz de Tenerife. En *Excavación Arqueológica en la Iglesia de Nuestra Señora de la Concepción*, pp. 232-246. Santa Cruz de Tenerife.

Larruga, J. M., Pinto, F., Hernandez, M., González, A. M. y Cabrera, V. M. 1992 Human enzyme polymorphism in the Canary Islands. V. Western Islands. *Gene Geogr* **6**, 159-66.

León, J. y Perera, M. A. 1996 Manifestaciones rupestres de Fuerteventura. *Manifestaciones rupestres de las Islas Canarias* **1**.

Lewontin, R. C. y Hubby, J. L. 1966 A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of

heterozygosity in natural populations of Drosophila pseudoobscura. *Genetics* **54**, 595-609.

Lindahl, T. 1993a Instability and decay of the primary structure of DNA. *Nature* **362**, 709-15.

Lindahl, T. 1993b Recovery of antediluvian DNA. *Nature* **365**, 700.

Linderholm, A., Malmstrom, H., Liden, K., Holmlund, G. y Gotherstrom, A. 2008 Cryptic contamination and phylogenetic nonsense. *PLoS ONE* **3**, e2316.

Lobo-Cabrera, M. 1993 La esclavitud en Fuerteventura en los siglos XVI y XVII. *V Jornadas de estudios sobre Fuerteventura y Lanzarote* **I**, 13-40.

Maca-Meyer, N., González, A. M., Larruga, J. M., Flores, C. y Cabrera, V. M. 2001 Major genomic mitochondrial lineages delineate early human expansions. *BMC Genet* **2**, 13.

Maca-Meyer, N., González, A. M., Pestano, J., Flores, C., Larruga, J. M. y Cabrera, V. M. 2003a Mitochondrial DNA transit between West Asia and North Africa inferred from U6 phylogeography. *BMC Genet* **4**, 15.

Maca-Meyer, N., Carranza, S., Rando, J. C., Arnold, E. N. y Cabrera, V. M. 2003b Status and relationships of the extinct giant Canary Island lizard Gallotia goliath (Reptilia : Lacertidae), assessed using ancient mtDNA from its mummified remains. *Biological Journal of the Linnean Society* **80**, 659-670.

Maca-Meyer, N., Arnay, M., Rando, J. C., Flores, C., González, A. M., Cabrera, V. M. y Larruga, J. M. 2004a Ancient mtDNA analysis and the origin of the Guanches. *Eur J Hum Genet* **12**, 155-62.

Maca-Meyer, N., Villar, J., Pérez-Mendez, L., Cabrera de Leon, A. y Flores, C. 2004b A tale of aborigines, conquerors and slaves: Alu insertion polymorphisms and the peopling of Canary Islands. *Ann Hum Genet* **68**, 600-5.

Maca-Meyer, N., Cabrera, V. M., Arnay, M., Flores, C., Fregel, R., González, A. M. y Larruga, J. M. 2005 Mitochondrial DNA diversity in 17th-18th century remains from Tenerife (Canary Islands). *Am J Phys Anthropol* **127**, 418-26.

Macías, A. 1988 Fuentes y principales problemas metodológicos de la demografía histórica de Canarias. *Anuario de Estudios Atlánticos* **34**, 51-157.

Macías, A. 1992 Expansión europea y demografía aborigen. El ejemplo de canarias (1400-1505). *Boletín de la Asociación de Demografía Histórica* **2**, 9-45.

Macías, A. y Bravo de Laguna, J. M. 1997 Conquista. En *Gran Enciclopedia Canaria, V*. Las Palmas de Gran Canaria: Edirca.

Malmstrom, H., Svensson, E. M., Gilbert, M. T., Willerslev, E., Gotherstrom, A. y Holmlund, G. 2007 More on contamination: the use of asymmetric molecular behavior to identify authentic ancient human DNA. *Mol Biol Evol* **24**, 998-1004.

Manak, M. M. 1993 Sample preparation. En *DNA probes* (ed. G. H. Keller y M. M. Manak). New York: Stockton Press.

Maniatis, T., Fritsch, E. F. y Sambrook, J. 1982 *Molecular cloning: A laboratory manual.* Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

Margulies, M., Egholm, M., Altman, W. E., Attiya, S., Bader, J. S., Bemben, L. A., Berka, J., Braverman, M. S., Chen, Y. J., Chen, Z., Dewell, S. B., Du, L., Fierro, J. M., Gomes, X. V., Godwin, B. C., He, W., Helgesen, S., Ho, C. H., Irzyk, G. P., Jando, S. C., Alenquer, M. L., Jarvie, T. P., Jirage, K. B., Kim, J. B., Knight, J. R., Lanza, J. R., Leamon, J. H., Lefkowitz, S. M., Lei, M., Li, J., Lohman, K. L., Lu, H., Makhijani, V. B., McDade, K. E., McKenna, M. P., Myers, E. W., Nickerson, E., Nobile, J. R., Plant, R., Puc, B. P., Ronan, M. T., Roth, G. T., Sarkis, G. J., Simons, J. F., Simpson, J. W., Srinivasan, M., Tartaro, K. R., Tomasz, A., Vogt, K. A., Volkmer, G. A., Wang, S. H., Wang, Y., Weiner, M. P., Yu, P., Begley, R. F. y Rothberg, J. M. 2005 Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**, 376-80.

Martell, M., Padron, G., Hernandez, M., Afonso, J. M. y Cabrera, V. M. 1986 Human enzyme polymorphism on the Canary Islands. I. Gran Canaria Island population. *Hum Hered* **36**, 41-4.

Martín de Guzmán, C. 1978 Dataciones C-14 para la prehistoria de las Islas Canarias. En *C-14 y Prehistoria de la Península Ibérica*, vol. 77 (ed. M. Almagro y M. Fernández-Miranda), pp. 144-150. Madrid: Fundación Juan March. Serie Universitaria.

Martín, E. 1992 *La Prehistoria de Canarias. Vol. 3. La Palma y los auaritas.* Santa Cruz de Tenerife: Centro de la Cultura Popular Canaria.

Martín, E. 2000 Dataciones absolutas para los yacimientos de Risco Chirimique (Tejeda) y Playa de Aguadulce (Telde). *Vegueta* **5**, 29-46.

Martínez, M. 1992 Historia *Popular de Canarias. Vol. 11. Canarias en la Mitología.* Santa Cruz de Tenerife: Centro de la Cultura Popular Canaria.

Martínez, V. M. F. 1996 *Arqueología prehistórica de África*. Madrid: Editorial Síntesis.

Matsuoka, Y., Vigouroux, Y., Goodman, M. M., Sanchez, G. J., Buckler, E. y Doebley, J. 2002 A single domestication for maize shown by multilocus microsatellite genotyping. *Proc Natl Acad Sci U S A* **99**, 6080-4.

Messerlin, A. y Lorho, Y. 1951 Groupes Sanguins et Facteur Rhésus au Maroc. *Bull Inst Hyg Maroc* **11**, 97-119.

Morilla, J. M., Afonso, J. M., Hernandez, M., Pestano, J. J. y Larruga, J. M. 1988 Human enzyme polymorphism in the Canary Islands. II. African influence. *Hum Hered* **38**, 101-5.

Morral, N., Bertranpetit, J., Estivill, X., Nunes, V., Casals, T., Gimenez, J., Reis, A., Varon-Mateeva, R., Macek, M., Jr., Kalaydjieva, L. y et al. 1994 The origin of the major cystic fibrosis mutation (delta F508) in European populations. *Nat Genet* **7**, 169-75.

Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G. y Erlich, H. 1986 Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring Harb Symp Quant Biol* **51 Pt 1**, 263-73.

Navarro, J. F. 1992 *La Prehistoria de Canarias. Vol. 5. La Gomera y Los Gomeros.* Santa Cruz de Tenerife: Centro de la Cultura Popular Canaria.

Navarro, J. F. 1997 Arqueología de las Islas Canarias. *Espacio, tiempo y forma. Serie I, Prehistoria y Arqueología* **10**, 447-478.

Navarro, J. F. 2005a *Todo sobre Canarias: Los aborígenes*. Santa Cruz de Tenerife: Centro de la Cultura Popular Canaria.

Navarro, J. F. 2005b Un recorrido histórico sobre el papel de la arqueología y los aborígenes en la construcción de una identidad canaria. En *I-dentidad canaria. Los antiguos*. Santa Cruz de Tenerife: Artemisa Ediciones.

Nei, N. 1987 *Molecular evolutionary genetics*. New York: Columbia University Press.

Noonan, J. P., Hofreiter, M., Smith, D., Priest, J. R., Rohland, N., Rabeder, G., Krause, J., Detter, J. C., Pääbo, S. y Rubin, E. M. 2005 Genomic sequencing of Pleistocene cave bears. *Science* **309**, 597-9.

Noonan, J. P., Coop, G., Kudaravalli, S., Smith, D., Krause, J., Alessi, J., Chen, F., Platt, D., Pääbo, S., Pritchard, J. K. y Rubin, E. M. 2006 Sequencing and analysis of Neanderthal genomic DNA. *Science* **314**, 1113-8.

Onrubia-Pintado, J. 1987 Les cultures préhistoriques des Îles Canaries, état de la question. *L'Anthropologie* **91**, 653-678.

Onrubia-Pintado, J., Rodríguez, C. G., Sáenz, J. I., González, M. C. y Olmo, S. 1998 Los materiales históricos de la Cueva Pintada de Gáldar (Gran Canaria). Una primera aproximación al contexto de las series coloniales bajomedievales y modernas (S.XV-XVI). En *XII Coloquio de Historia Canario-Americana (1996)*, vol. I, pp. 643-663. Las Palmas de Gran Canaria: Ediciones del Cabildo Insular de Gran Canaria.

Onrubia-Pintado, J. 2003 *La Isla de los Guanartemes. Territorio, sociedad y poder en la Gran Canaria indígena (siglos XIV-XV)*. Las Palmas de Gran Canaria: Ediciones del Cabildo de Gran Canaria.

Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K. y Sekiya, T. 1989 Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Natl Acad Sci U S A* **86**, 2766-70.

Ovchinnikov, I. V., Gotherstrom, A., Romanova, G. P., Kharitonov, V. M., Liden, K. y Goodwin, W. 2000 Molecular analysis of Neanderthal DNA from the northern Caucasus. *Nature* **404**, 490-3.

Pääbo, S. 1985 Molecular cloning of Ancient Egyptian mummy DNA. *Nature* **314**, 644-5.

Pääbo, S., Gifford, J. A. y Wilson, A. C. 1988 Mitochondrial DNA sequences from a 7000-year old brain. *Nucleic Acids Res* **16**, 9775-87.

Pääbo, S. y Wilson, A. C. 1988 Polymerase chain reaction reveals cloning artefacts. *Nature* **334**, 387-8.

Pääbo, S. 1989 Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. *Proc Natl Acad Sci U S A* **86**, 1939-43.

Pääbo, S., Higuchi, R. G. y Wilson, A. C. 1989 Ancient DNA and the polymerase chain reaction. The emerging field of molecular archaeology. *J Biol Chem* **264**, 9709-12.

Pääbo, S., Irwin, D. M. y Wilson, A. C. 1990 DNA damage promotes jumping between templates during enzymatic amplification. *J Biol Chem* **265**, 4718-21.

Pääbo, S. y Wilson, A. C. 1991 Miocene DNA sequences - a dream come true? *Curr Biol* **1**, 45-6.

Pääbo, S., Poinar, H., Serre, D., Jaenicke-Despres, V., Hebler, J., Rohland, N., Kuch, M., Krause, J., Vigilant, L. y Hofreiter, M. 2004 Genetic analyses from ancient DNA. *Annu Rev Genet* **38**, 645-79.

Parejo, M. 1966 El sistema AB0 en la poblacion actual de las Islas Canarias. *Actas del V Congreso Panafricano de prehistoria y estudios del Cuaternario* **II**.

Pérez, J. 1991 *Los portugueses en Canarias. Portuguesismos*. Las Palmas de Gran Canaria: Ediciones del Cabildo Insular de Gran Canaria.

Pérez, M. J., Hierro, J. M., Marrero, M. J., González, A. M. y Cabrera, V. M. 1991 Human enzyme polymorphism in the Canary Islands. IV. Eastern islands. *Hum Hered* **41**, 385-90.

Pinto, F., Cabrera, V. M., González, A. M., Larruga, J. M., Noya, A. y Hernandez, M. 1994 Human enzyme polymorphism in the Canary Islands. VI. Northwest African influence. *Hum Hered* **44**, 156-61.

Pinto, F., Rando, J. C., Lopez, M., Morilla, J. M. y Larruga, J. M. 1996a Blood group polymorphisms in the Canary Islands. *Gene Geogr* **10**, 171-9.

Pinto, F. M., González, A. M., Hernandez, M., Larruga, J. M. y Cabrera, V. M. 1996b Sub-Saharan influence on the Canary Islands population deduced from G6PD gene sequence analysis. *Hum Biol* **68**, 517-22.

Pinto, F., González, A. M., Hernandez, M., Larruga, J. M. y Cabrera, V. M. 1996c Genetic relationship between the Canary Islanders and their African and Spanish ancestors inferred from mitochondrial DNA sequences. *Ann Hum Genet* **60**, 321-30.

Poinar, G. O., Jr. y Hess, R. 1982 Ultrastructure of 40-Million-Year-Old insect tissue. *Science* **215**, 1241-1242.

Poinar, H. N., Hofreiter, M., Spaulding, W. G., Martin, P. S., Stankiewicz, B. A., Bland, H., Evershed, R. P., Possnert, G. y Pääbo, S. 1998 Molecular coproscopy: dung and diet of the extinct ground sloth Nothrotheriops shastensis. *Science* **281**, 402-6.

Poinar, H. N. 2002 The genetic secrets some fossils hold. *Acc Chem Res* **35**, 676-84.

Pruvost, M. y Geigl, E. M. 2004 Real-time quantitative PCR to assess the authenticity of ancient DNA amplification. *Journal of Archaeological Science* **31**, 1191-1197.

Pusch, C. M. y Bachmann, L. 2004 Spiking of contemporary human template DNA with ancient DNA extracts induces mutations under PCR and generates nonauthentic mitochondrial sequences. *Mol Biol Evol* **21**, 957-64.

Rando, J. C., Pinto, F., González, A. M., Hernandez, M., Larruga, J. M., Cabrera, V. M. y Bandelt, H. J. 1998 Mitochondrial DNA analysis of northwest African populations

reveals genetic exchanges with European, near-eastern, and sub-Saharan populations. *Ann Hum Genet* **62**, 531-50.

Rando, J. C., Cabrera, V. M., Larruga, J. M., Hernandez, M., González, A. M., Pinto, F. y Bandelt, H. J. 1999 Phylogeographic patterns of mtDNA reflecting the colonization of the Canary Islands. *Ann Hum Genet* **63**, 413-28.

Reid, A. H., Fanning, T. G., Hultin, J. V. y Taubenberger, J. K. 1999 Origin and evolution of the 1918 "Spanish" influenza virus hemagglutinin gene. *Proc Natl Acad Sci U S A* **96**, 1651-6.

Relethford, J. H. 1998 Genetics of modern human origins and diversity. *Ann Rev Anthropol* **27**, 1-23.

Reyes-García, I. 2000 Consideraciones metodológicas en torno al estudio de la antigua lengua de Canarias. En *Acta del XIII coloquio de Historia Canario Americana*. Las Palmas de Gran Canaria: Cabildo de Gran Canaria.

Richards, M., Macaulay, V., Hickey, E., Vega, E., Sykes, B., Guida, V., Rengo, C., Sellitto, D., Cruciani, F., Kivisild, T., Villems, R., Thomas, M., Rychkov, S., Rychkov, O., Rychkov, Y., Golge, M., Dimitrov, D., Hill, E., Bradley, D., Romano, V., Cali, F., Vona, G., Demaine, A., Papiha, S., Triantaphyllidis, C., Stefanescu, G., Hatina, J., Belledi, M., Di Rienzo, A., Novelletto, A., Oppenheim, A., Norby, S., Al-Zaheri, N., Santachiara-Benerecetti, S., Scozari, R., Torroni, A. y Bandelt, H. J. 2000 Tracing European founder lineages in the Near Eastern mtDNA pool. *Am J Hum Genet* **67**, 1251-76.

Roberts, D. F., Evans, M., Ikin, E. W. y Mourant, A. E. 1966 Blood groups and the affinities of the Canarian islanders. *Man* **1**, 512-525.

Rodríguez, C. y Hernández, J. C. 2005 *El enigma de la modorra. La epidemia de los guanches*. Santa Cruz de Tenerife: Ediciones Idea.

Rösing, I. 1967 AB0-Blutgruppen und Rh-Faktoren auf Teneriffa unter besonderer Berücksichtigung des Vergleichs zwischen vorspanischen und heutiger Bevölkerung. *Homo* **18**.

Roubinet, F., Kermarrec, N., Despiau, S., Apoil, P. A., Dugoujon, J. M. y Blancher, A. 2001 Molecular polymorphism of O alleles in five populations of different ethnic origins. *Immunogenetics* **53**, 95-104.

Rudbeck, L. y Dissing, J. 1998 Rapid, simple alkaline extraction of human genomic DNA from whole blood, buccal epithelial cells, semen and forensic stains for PCR. *Biotechniques* **25**, 588-90, 592.

Saillard, J., Forster, P., Lynnerup, N., Bandelt, H. J. y Norby, S. 2000 MtDNA variation among Greenland Eskimos: the edge of the Beringian expansion. *Am J Hum Genet* **67**, 718-26.

Salido, E. C., Yen, P. H., Koprivnikar, K., Yu, L. C. y Shapiro, L. J. 1992 The human enamel protein gene amelogenin is expressed from both the X and the Y chromosomes. *Am J Hum Genet* **50**, 303-16.

Sampietro, M. L., Gilbert, M. T., Lao, O., Caramelli, D., Lari, M., Bertranpetit, J. y Lalueza-Fox, C. 2006 Tracking down human contamination in ancient human teeth. *Mol Biol Evol* **23**, 1801-7.

Sanger, F. y Coulson, A. R. 1975 A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J Mol Biol* **94**, 441-8.

Sanger, F., Nicklen, S. y Coulson, A. R. 1977 DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A* **74**, 5463-7.

Sanz de Magallanes, J. M. 2001 *Enterramientos en la Parroquia Matriz de La Concepción*. Santa Cruz de Tenerife: Imprenta Editora Católica.

Schmidt, T., Hummel, S. y Herrmann, B. 1995 Evidence of contamination in PCR laboratory disposables. *Naturwissenschaften* **82**, 423-31.

Schneider, S., Roessli, D. y Excoffier, L. 2000 Arlequin ver. 2000: A software for population genetics data analysis. Switzerland: Genetics and Biometry Laboratory, University of Geneva.

Scholz, M., Bachmann, L., Nicholson, G. J., Bachmann, J., Giddings, I., Ruschoff-Thale, B., Czarnetzki, A. y Pusch, C. M. 2000 Genomic differentiation of Neanderthals and anatomically modern man allows a fossil-DNA-based classification of morphologically indistinguishable hominid bones. *Am J Hum Genet* **66**, 1927-32.

Schwarzfischer, F. y Liebrich, K. 1963 Serologische Untersuchungen an prähistorischen Bevölkerungen, insbesondere an altkanarischen Mumien. *Homo* **14**, 129-133.

Schwidetzky, I. 1963 *La población prehispánica de las Islas Canarias*. Santa Cruz de Tenerife: Cabildo Insular de Tenerife.

Schwidetzky, I. 1970 Groupes sanguins et histoire des populations aux iles Canaries. En *Homenaje a E. Serra Ráfols*, vol. III, pp. 333-338. Santa Cruz de Tenerife: Universidad de La Laguna.

Schwidetzky, I. 1975 *Investigaciones antropológicas en las Islas Canarias: Estudio comparativo entre la población actual y la prehispánica*. Tenerife: Publicaciones del Museo Arqueológico de Tenerife.

Semino, O., Passarino, G., Oefner, P. J., Lin, A. A., Arbuzova, S., Beckman, L. E., De Benedictis, G., Francalacci, P., Kouvatsi, A., Limborska, S., Marcikiae, M., Mika, A., Mika, B., Primorac, D., Santachiara-Benerecetti, A. S., Cavalli-Sforza, L. L. y Underhill, P. A. 2000 The genetic legacy of Paleolithic Homo sapiens sapiens in extant Europeans: a Y chromosome perspective. *Science* **290**, 1155-9.

Semino, O., Magri, C., Benuzzi, G., Lin, A. A., Al-Zahery, N., Battaglia, V., Maccioni, L., Triantaphyllidis, C., Shen, P., Oefner, P. J., Zhivotovsky, L. A., King, R., Torroni, A., Cavalli-Sforza, L. L., Underhill, P. A. y Santachiara-Benerecetti, A. S. 2004 Origin, diffusion, and differentiation of Y-chromosome haplogroups E and J: inferences on the neolithization of Europe and later migratory events in the Mediterranean area. *Am J Hum Genet* **74**, 1023-34.

Slatkin, M. 1995 A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**, 457-62.

Smith, C. I., Chamberlain, A. T., Riley, M. S., Stringer, C. y Collins, M. J. 2003 The thermal history of human fossils and the likelihood of successful DNA amplification. *J Hum Evol* **45**, 203-17.

Springer, R. A. 2001 *Origen y uso de la escritura líbico-bereber en Canarias*. Santa Cruz de Tenerife: Centro de la Cultura Popular Canaria.

Stiller, M., Green, R. E., Ronan, M., Simons, J. F., Du, L., He, W., Egholm, M., Rothberg, J. M., Keates, S. G., Ovodov, N. D., Antipina, E. E., Baryshnikov, G. F., Kuzmin, Y. V., Vasilevski, A. A., Wuenschell, G. E., Termini, J., Hofreiter, M., Jaenicke-Despres, V. y Pääbo, S. 2006 Patterns of nucleotide misincorporations during enzymatic amplification and direct large-scale sequencing of ancient DNA. *Proc Natl Acad Sci U S A* **103**, 13578-84.

Stone, A. C. y Stoneking, M. 1993 Ancient DNA from a pre-Columbian Amerindian population. *Am J Phys Anthropol* **92**, 463-71.

Suárez, J. J., Rodríguez, F. y Quintero, C. L. 1988 *Historia popular de Canarias. Vol. 2. Conquista y Colonización*. Santa Cruz de Tenerife: Centro de la Cultura Popular Canaria.

Sullivan, K. M., Mannucci, A., Kimpton, C. P. y Gill, P. 1993 A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. *Biotechniques* **15**, 636-8, 640-1.

Sutlovic, D., Gamulin, S., Definis-Gojanovic, M., Gugic, D. y Andjelinovic, S. 2008 Interaction of humic acids with human DNA: proposed mechanisms and kinetics. *Electrophoresis* **29**, 1467-72.

Tejera, A. 1992 *La Prehistoria de Canarias. Vol. 1. Tenerife y Los Guanches.* Santa Cruz de Tenerife: Centro de la Cultura Popular Canaria.

Tejera, A., Chávez, M. E. y Montesdeoca, M. 2006 *Canarias y el África antigua*. Taller de historia. Santa Cruz de Tenerife: Centro de la Cultura Popular Canaria.

Thomas, R. H., Schaffner, W., Wilson, A. C. y Pääbo, S. 1989 DNA phylogeny of the extinct marsupial wolf. *Nature* **340**, 465-7.

Thomson, R., Pritchard, J. K., Shen, P., Oefner, P. J. y Feldman, M. W. 2000 Recent common ancestry of human Y chromosomes: evidence from DNA sequence data. *Proc Natl Acad Sci U S A* **97**, 7360-5.

Timmis, J. N., Ayliffe, M. A., Huang, C. Y. y Martin, W. 2004 Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. *Nat Rev Genet* **5**, 123-35.

Tishkoff, S. A., Dietzsch, E., Speed, W., Pakstis, A. J., Kidd, J. R., Cheung, K., Bonne-Tamir, B., Santachiara-Benerecetti, A. S., Moral, P. y Krings, M. 1996 Global patterns of linkage disequilibrium at the CD4 locus and modern human origins. *Science* **271**, 1380-7.

Torroni, A., Achilli, A., Macaulay, V., Richards, M. y Bandelt, H. J. 2006 Harvesting the fruit of the human mtDNA tree. *Trends Genet* **22**, 339-45.

Trujillo, G. A. 2004 Nuevos datos para la Historia de la Arqueología "histórica" en Canarias. *Faykag Revista Canaria de Arqueología* **1**, 1-18.

Underhill, P. A., Shen, P., Lin, A. A., Jin, L., Passarino, G., Yang, W. H., Kauffman, E., Bonne-Tamir, B., Bertranpetit, J., Francalacci, P., Ibrahim, M., Jenkins, T., Kidd, J. R., Mehdi, S. Q., Seielstad, M. T., Wells, R. S., Piazza, A., Davis, R. W., Feldman, M. W., Cavalli-Sforza, L. L. y Oefner, P. J. 2000 Y chromosome sequence variation and the history of human populations. *Nat Genet* **26**, 358-61.

Underhill, P. A., Passarino, G., Lin, A. A., Shen, P., Mirazon Lahr, M., Foley, R. A., Oefner, P. J. y Cavalli-Sforza, L. L. 2001 The phylogeography of Y chromosome binary haplotypes and the origins of modern human populations. *Ann Hum Genet* **65**, 43-62.

Underhill, P. A. y Kivisild, T. 2007 Use of Y chromosome and mitochondrial DNA population structure in tracing human migrations. *Annu Rev Genet* **41**, 539-64.

van Oven, M. y Kayser, M. 2009 Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Hum Mutat* **30**, E386-94.

Velasco-Vázquez, J. C., Hernández-Gómez, C. M. y Alberto-Barroso, V. 2002 Dataciones arqueológicas contra tiempos sociales. Reflexiones sobre cronología y prehistoria de Canarias. *Tabona* **10**, 31-46.

Viera y Clavijo, J. 1772 *Historia de la Islas Canarias*. Madrid: Editorial Cupsa.

Vigilant, L., Stoneking, M., Harpending, H., Hawkes, K. y Wilson, A. C. 1991 African populations and the evolution of human mitochondrial DNA. *Science* **253**, 1503-7.

Vila, C., Leonard, J. A., Gotherstrom, A., Marklund, S., Sandberg, K., Liden, K., Wayne, R. K. y Ellegren, H. 2001 Widespread origins of domestic horse lineages. *Science* **291**, 474-7.

Wang, G. C. y Wang, Y. 1997 Frequency of formation of chimeric molecules as a consequence of PCR coamplification of 16S rRNA genes from mixed bacterial genomes. *Appl Environ Microbiol* **63**, 4645-50.

Wang, G. H. y Lu, C. L. 1981 Isolation and identification of nucleic acids of the liver from a corpse from the Changssha tomb. *Shen Wu Hua Hsueh Yu Sheng Wu Chin Chan* **17**, 70-75.

Weiner, S., Lowenstam, H. A. y Hood, L. 1976 Characterization of 80-million-year-old mollusk shell proteins. *Proc Natl Acad Sci U S A* **73**, 2541-5.

Whelan, J. A., Russell, N. B. y Whelan, M. A. 2003 A method for the absolute quantification of cDNA using real-time PCR. *J Immunol Methods* **278**, 261-9.

Willerslev, E., Hansen, A. J., Binladen, J., Brand, T. B., Gilbert, M. T., Shapiro, B., Bunce, M., Wiuf, C., Gilichinsky, D. A. y Cooper, A. 2003 Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* **300**, 791-5.

Wölfel, D. J. 1930 Sind die Ureinwohner der Kanaren ausgestorben? *Zeitschrift für Ethnologie* **62**, 282-302.

Woodward, S. R., Weyand, N. J. y Bunnell, M. 1994 DNA sequence from Cretaceous period bone fragments. *Science* **266**, 1229-32.

Wyckoff, R. W. G. 1972 *The biochemistry of animal fossils*. Bristol: Scientechnica.

Y-Chromosome-Consortium. 2002 A nomenclature system for the tree of human Y-chromosomal binary haplogroups. *Genome Res* **12**, 339-48.

Yamamoto, F., Marken, J., Tsuji, T., White, T., Clausen, H. y Hakomori, S. 1990a Cloning and characterization of DNA complementary to human UDP-GalNAc: Fuc-alpha-1→ 2-Gal-alpha-1→ 3GalNAc transferase (histo-blood group A transferase) mRNA. *J Biol Chem* **265**, 1146-51.

Yamamoto, F., Clausen, H., White, T., Marken, J. y Hakomori, S. 1990b Molecular genetic basis of the histo-blood group ABO system. *Nature* **345**, 229-33.

Yamamoto, F. 2000 Molecular genetics of ABO. *Vox Sang* **78 (Suppl 2)**, 91-103.

Yang, D. Y., Eng, B. y Saunders, S. R. 2003 Hypersensitive PCR, ancient human mtDNA, and contamination. *Hum Biol* **75**, 355-64.

Yao, Y. G., Kong, Q. P., Man, X. Y., Bandelt, H. J. y Zhang, Y. P. 2003 Reconstructing the evolutionary history of China: a caveat about inferences drawn from ancient DNA. *Mol Biol Evol* **20**, 214-9.

Yip, S. P. 2002 Sequence variation at the human ABO locus. *Ann Hum Genet* **66**, 1-27.

Zar, J. H. 1984 *Biostatistical Analysis*. New Jersey: Prentice Hall.

Zischler, H., Hoss, M., Handt, O., von Haeseler, A., van der Kuyl, A. C. y Goudsmit, J. 1995 Detecting dinosaur DNA. *Science* **268**, 1192-3; author reply 1194.