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## 2 Population genetic data for 17 Y STR markers from Benghazi (East Libya)

3 Samir Elmrghni<sup>a,b,\*</sup>, Yvette M. Coulson-Thomas<sup>a</sup>, Mahmoud Kaddura<sup>b</sup>, Ron A. Dixon<sup>a</sup>,  
4 D. Ross Williams<sup>a</sup>

5 <sup>a</sup>School of Natural and Applied Sciences, University of Lincoln, Brayford Pool, Lincoln, LN6 7TS, UK  
6 <sup>b</sup>Department of Forensic and Toxicology, Faculty of Medicine, University of Garyounis, Benghazi, Libya

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### ABSTRACT

The seventeen Y-STR loci included in the AmpF/STR<sup>®</sup> Yfiler<sup>™</sup> PCR Amplification kit (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a/b, DYS438, DYS439, DYS437, DYS448, DYS458, DYS456, DYS635, and Y-GATA-H4) were used to type a sample population of 238 males from eastern Libya (Benghazi region). Of 238 observed haplotypes, 214 were unique (90%) and 24 (10%) were found more than once. The 17 loci gave a discriminating power of 0.999. DYS458 showed the highest diversity as a single-locus marker (0.73). Allelic frequencies and gene diversities for each Y-STR locus were determined. The high haplotype diversity and discrimination capacity (0.996) demonstrate the utility of these loci for human identification in forensic applications. Comparative analysis with Y-STR datasets of relevant populations and submission of the haplotypes to the Y-STR Haplotype Reference Database (YHRD) was undertaken.

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### 1. Introduction

9 Y-STR (short tandem repeats) haplotype is shared by males  
10 in the same paternal lineage [1]. Studying the ability of Y-STR  
11 markers to differentiate between DNA samples from unrelated  
12 male donors is crucial to the forensic science community [2]. Since  
13 the beginning of the nineties (1992), when Lutz Roewer and  
14 colleagues described the first polymorphic Y-chromosome marker  
15 Y-27H39 – now better known as the STR locus DYS19 [3] the field  
16 of forensic chromosome analysis has been successfully developed  
17 [4]. National DNA databases collectively house millions of  
18 STR profiles around the world [5]. Global population databases  
19 have been established [6] yet studies focusing on Middle Eastern  
20 and North African countries are still rare. In this study, seventeen  
21 Y-STR loci included in the AmpF/STR<sup>®</sup> Yfiler<sup>™</sup> PCR Amplification  
22 kit (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393,  
23 DYS385a/b, DYS438, DYS439, DYS437, DYS448, DYS458, DYS456,  
24 DYS635, and Y-GATA-H4) were used to type a sample population of  
25 238 males from eastern Libya (Benghazi region).

### 1.1. Population

26 Libya, a Northern African country, was first inhabited by  
27 Berbers, followed by Phoenicians, Greeks, Romans, Arabs and  
28 Ottomans. Libya became independent in 1951 after a brief period  
29 as an Italian colony; it had been invaded by Italy in 1911. In  
30 February 2011 an uprising against the government occurred in the  
31 city. Benghazi is the second largest city in Libya and the main city  
32 (or capital) of the Cyrenaica region (or ex-Province), located in the  
33 North of Africa. Benghazi is located half way between Tripoli in the  
34 West (a distance of approximately 1000 km between these cities)  
35 and Cairo in the East (also approximately 1000 km) (Fig. 1).  
36 Cyrenaica is surrounded by desert on three sides; hence in ancient  
37 times the most accessible civilization was to the North, across the  
38 Mediterranean, in Crete and Greece, only 400 km away. The  
39 population of Benghazi was 500,120 in 1995 (census) and  
40 increased to 670,797 in the 2006 census. As with other cities in  
41 Libya, there is a reasonable amount of ethnic diversity in Benghazi.  
42 The people of eastern Libya, Benghazi included, have in the past  
43 always been of predominantly Arab descent. In recent times,  
44 however, there has been an influx of African immigrants into  
45 Benghazi. There are also many Egyptian immigrants in Benghazi. A  
46 small Greek community also exists in Benghazi; the Greek island of  
47 Crete is a short distance from Benghazi and many families in  
48 Benghazi today bear Cretian surnames.

In modern times, Benghazi has seen a lot of Libyans from  
50 different parts of the country move into the city, especially since  
51 the Kingdom era (1951–1969). Many Libyans came to Benghazi  
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\* Corresponding author at: School of Natural and Applied Sciences, University of  
Lincoln, Brayford Pool, Lincoln, LN6 7TS, UK. Tel.: +44 01522 886875;  
fax: +44 01522 886875.  
E-mail address: [selmrghni@lincoln.ac.uk](mailto:selmrghni@lincoln.ac.uk) (S. Elmrghni).



Fig. 1. Map of Libya showing the city of Benghazi (<http://en.wikipedia.org/wiki/Benghazi>).

from Misrata (about 60% of the population have roots from Misrata, West of Benghazi).

2. Material and methods

Informed consent was obtained from 238 unrelated Libyan male individuals (Benghazi region).

2.1. DNA extraction

DNA was extracted from blood stains collected on FTA® cards (Whatman, Kent, UK) using FTA® Purification Reagent (Whatman) following the manufacturer's protocol and from buccal swabs using QIAamp® DNA Blood Mini Kit (QIAGEN, Hilden, Germany). DNA was quantified using a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, USA).

2.2. PCR amplification

About 1.2 mm FTA® disc and 1 ng of DNA purified from buccal swabs was used to amplify 17 STR loci using the AmpF/STR® Yfiler™ PCR kit in accordance with the manufacturer's instructions.

2.3. Typing

Amplified products were separated and detected using the ABI Prism 310xl Genetic Analyzer (Applied Biosystems) according to the manufacturer's recommended protocol. The data were analysed using GeneMapper ID v3.2 (Applied Biosystems). Alleles were assigned according to the International Society of Forensic Genetics (ISFG) guidelines for forensic Y-STR [7].

2.4. Quality control

The laboratory has participated in the Y-STR Haplotyping Quality Assurance Exercise (Certified at 2010-5-20). The data were submitted to YHRD ([www.yhrd.org](http://www.yhrd.org)) and received the accession number: YA003680.

2.5. Analysis of data

Gene and haplotype diversities were estimated according to Nei [8]. The discrimination capacity (DC) was calculated as the proportion of different haplotypes in the sample. Population pairwise genetic distances were carried out based on *Fst* and the significance tested with 1000 permutations using AMOVA and the distances were visualized in two-dimensional space using the multi-dimensional scaling (MDS) analysis included in the YHRD software package ([www.yhrd.org](http://www.yhrd.org)).

Access to the data: see Tables S1 and S2

3. Results and discussion

This is, to our knowledge, the first in-depth large study among Afro-Asiatic metapopulation (238 samples) of genetic diversity in Y-STR haplotypes in an eastern Libyan population. Two previous studies, of a Tripoli population (West of Libya) and a Fezzan population (South Libya) analysed a smaller number of inhabitants (63 samples and 47 samples, respectively) [9,10].

A total of 238 haplotypes were identified; 214 were unique (90%) and 24 (10%) were observed more than once. Of these 24

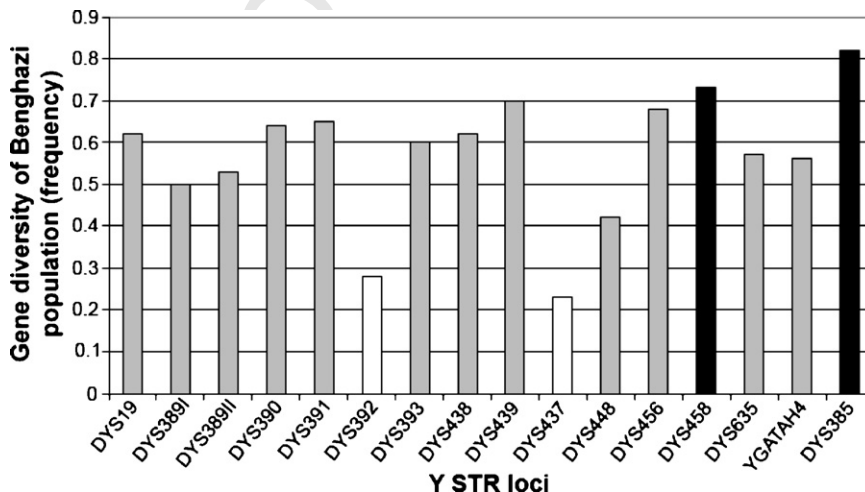


Fig. 2. Gene diversity of the 17 loci of the Y-filer kit. Black bars represent the highest diversities (DYS385 and DYS458), white bars represent the lowest diversities (DYS437 and DYS392) and grey bars represent remaining diversities.

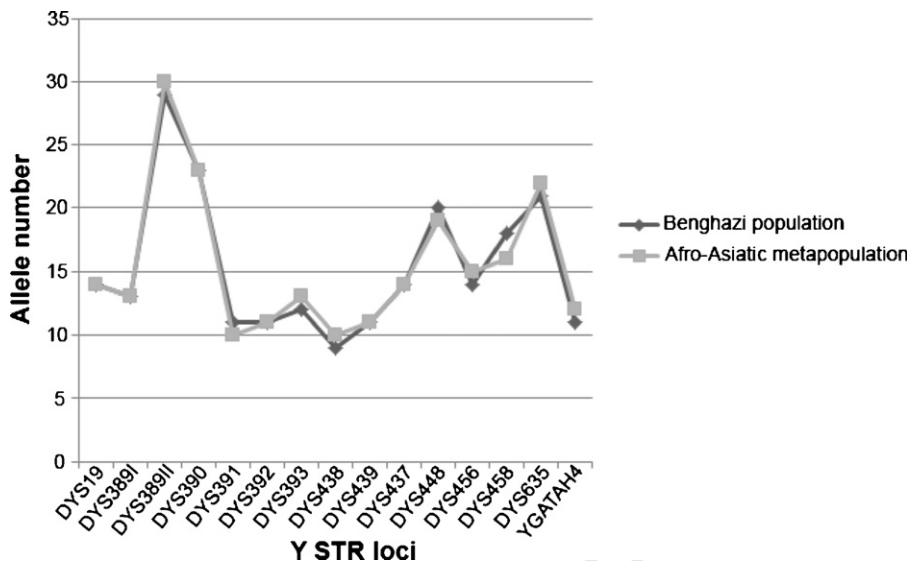


Fig. 3. Similarity between Benghazi population and Afro-Asiatic metapopulation (YHRD database) regarding allele number frequencies using the same Y filer kit.

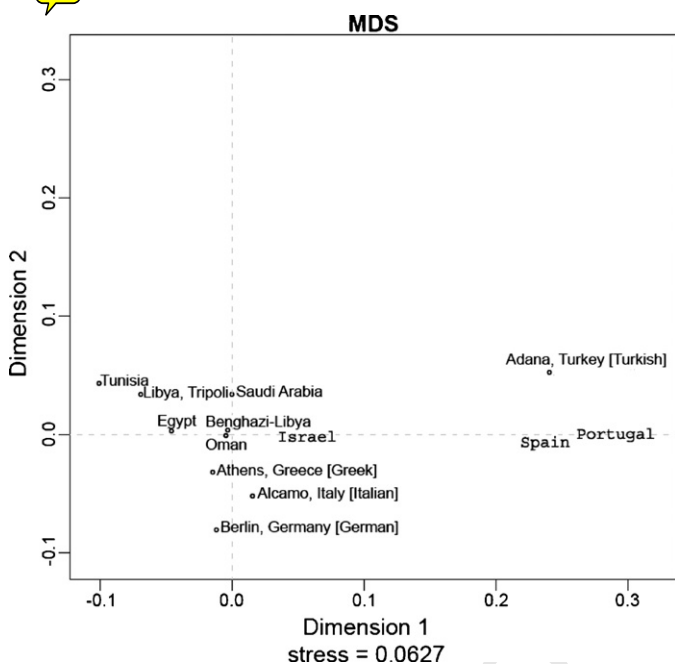


Fig. 4. MDS based on Fst genetic distances and pair-wise analysis of molecular variance (AMOVA) of Eastern Libyans (Benghazi region), geographically nearby Middle-Eastern, North African and European populations.

repeated haplotypes, none matched any haplotypes listed in the YHRD database suggesting that these haplotypes are specific for Benghazi populations. Most of these haplotypes were from individuals bearing the same surname but are not first-degree related. The total gene diversity (equivalent to the power of discrimination) of the 17 loci was high (0.9998), making them suitable for use in forensic practice. Individually, DYS385a/b showed the highest diversity ( $h = 0.82$ ) followed by DYS458 ( $h = 0.73$ ) as a single-locus marker and when compared with other populations, these gene diversities were the same as those observed for an Egyptian population (Fig. 2).

When Benghazi population was compared with the Afro-Asiatic metapopulation (in the YHRD database), minimal differences were revealed regarding allele number frequencies when using the same Y filer kit indicating that Benghazi population shares the same

common alleles as Arabic (Semitic) populations which belong to the Afro-Asiatic metapopulation (Fig. 3).

A Fst genetic distances and pair-wise analysis of molecular variance (AMOVA) test carried out through YHRD by Calculating P-values with 10,000 permutations ( $P$ -value < 0.05) revealed significant differences between populations from Northern Libya (Tripoli and Benghazi); Tripoli ( $P = 0.2385$ ) showed similarities to Tunisia whilst Benghazi to Egypt, when comparing North African countries (Fig. 4). Similarities between Egypt ( $P = 0.0485$ ) and Libya have also been previously reported by Omran et al. [11]. The AMOVA analysis also revealed similarities to Israel and Palestinian Authority Area, recently reported ( $P = 0.0000$ ) [12]. Both of these studies analysed the same 17 markers evaluated for Benghazi population in the current study. This genetic affinity may be due to the geographical proximity of these countries to Benghazi.

Similarities in AMOVA analysis of Benghazi with Yemen ( $P = 0.0562$ ) [13], Oman ( $P = 0.0246$ ), Saudi Arabia ( $P = 0.0489$ ) and other Gulf countries using minimal haplotypes (9 markers which are included in the 17 marker Y filer kit) may be due to the historical Islamic migration towards North African countries. On the other hand, similarity with Greece ( $P = 0.0321$ ) [14] may be due to old trade and architectural history in North Africa (Fig. 4). Our results differ significantly from the results reported for a western Libyan population (Tripoli) [9], in which Y-STR polymorphisms across 9 loci were analysed. These 9 markers are included in the Y filer kit used in our study, however, we analysed an additional 8 Y-STR markers included in the kit, thus providing further population data for eastern Libyan men. Tripoli population has been shown to be similar to Tunisian ( $P = 0.168$ ) [15] and other Western North African populations, whilst we observed that Benghazi population is similar to geographically nearby Middle Eastern populations.

Geographically nearby European populations (Spain and Portugal) differ significantly from Benghazi Population P-values were (0.3689 and 0.3006) respectively recorded by AMOVA (all population data compared with Benghazi was chosen from YHRD).

In conclusion, the 17 Y-STR analysis of a population from eastern Libya (Benghazi region) suggests that based on the high haplotype diversity and discrimination capacity observed, these loci can be used for human identification in forensic applications.

This paper follows the guidelines suggested for publication of population data in Forensic Science International [16].

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165 **Appendix A. Supplementary data**

166 Supplementary data associated with this article can be found, in  
167 the online version, at doi:10.1016/j.fsigen.2011.05.001.

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