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ORIGINAL ARTICLE

Y-STR variation in Albanian populations: implications on the match probabilities and the genetic legacy of the minority claiming an Egyptian descent

Gianmarco Ferri · Sergio Tofanelli · Milena Alù · Luca Taglioli · Erjon Radheshi · Beatrice Corradini · Giorgio Paoli · Cristian Capelli · Giovanni Beduschi

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Abstract Y chromosome variation at 12 STR (the Powerplex® Y system core set) and 18 binary markers was investigated in two major (the Ghegs and the Tosks) and two minor (the Gabels and the Jevgs) populations from Albania (Southern Balkans). The large proportion of haplotypes shared within and between groups makes the Powerplex 12-locus set inadequate to ensure a suitable power of discrimination for the forensic practice. At least 85% of Y lineages in the Jevgs, the cultural minority claiming an Egyptian descent, turned out to be of either Roma or Balkan ancestry. They also showed unequivocal signs of a common genetic history with the Gabels, the other Albanian minority practising social and cultural Roma traditions.

Keywords Albania · Ethnic minorities · Y chromosome lineages · Y-SNP haplogroups · Y-STR haplotypes

Gianmarco Ferri and Sergio Tofanelli contributed equally to the paper.

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G. Ferri (✉) · M. Alù · E. Radheshi · B. Corradini · G. Beduschi
Section of Legal Medicine, Department of Diagnostic and Laboratory Services and Legal Medicine,
University of Modena and Reggio Emilia,
Modena, Italy
e-mail: gianmarco.ferri@unimore.it

S. Tofanelli · L. Taglioli · G. Paoli
Department of Biology, Anthropology Unit, University of Pisa,
Pisa, Italy

C. Capelli
Department of Zoology, University of Oxford,
Oxford, UK

Introduction

The Balkan Peninsula has been a crossroads of various cultures since pre-history and still nowadays remains the target of many migration waves. Its ethnic mosaic comprises mainly Indo-European populations, as well as Altaic-speaking groups and several cultural minorities. Albanians are considered the most conservative among Balkan population and believed to be direct descendants of the Illyrians, who first appeared in the western portion of the Balkan Peninsula around 3,000 years ago. Since the 6th century AD, when migrating Slavs pushed the Illyrians into present-day Albania, the harsh mountain landscape and the resilient tribal society enabled the population to survive keeping intact their identity and their language. The greatest demographic event in historical times was caused by the Ottoman rule that forced many Christian Albanians, or Arbëreshë, to move to Southern Italy and Northern Greece in several waves of migrations from the 15th to the 18th century AD [1]. With the collapse of the communist and socialist regimes in Eastern Europe after 1989, the situation has drastically changed. No other country in the world has been so deeply affected by emigration in the last two decades [2]. Approximately 800 thousand people are thought to have emigrated out of Albania to live abroad [3], with the greatest concentration in Greece and Italy. Little is known about internal migration that should have taken place over the same period.

Nowadays, Albanian people can be divided into two major groups, the Ghegs (in the North) and the Tosks (in the South), according to the Albanian dialect they speak, plus a number of cultural minorities, including the Gabels and the Jevgs. The Gabels belong to the Roma minority, who first arrived to Albania around the 14th century AD

from present Bulgaria, and are among the most politically, economically and socially neglected groups in the country. The so-called Balkan Egyptians (Jevgs in Albanian) are a minority seeing themselves as quite distinct from the Roma community. They are widely dispersed in the Balkan area and claim an Egyptian origin. Some scholars identify their African ancestors in migrating slaves at the time of Pharaoh Ramses II (13th century BC), some others in Coptic migrants who came from Egypt in the 4th century AD or in Egyptian slaves who came to Albania in the 19th century AD [4–12].

In this research work, we primarily aimed at evaluating the forensic efficiency of Y chromosome markers in the Albanian groups. Such analyses draw importance from two reasons: (1) until recently, Albanians could be interpreted as a rural isolated population sub-structured in small clan-based groups and have been warned against the effect of ignoring the population structure when analysing the value of evidence from Y-STR haplotypes in small-sized communities [13, 14]; (2) old and recent diasporas fragmented foreign Albanians in a galaxy of small endogamous communities throughout Europe for which the use of a general Balkan database may result in a relevant overestimation of likelihood ratios.

As secondary aim, we described the Y profile of two ethnic minorities with alleged different demographic history in order to check their degree of isolation and make inference about the controversial origin of their paternal lineages.

Materials and methods

Subjects

DNA of 360 unrelated healthy Albanian males, of which 165 were Ghegs (north Albania), 121 Tosks (south Albania), 33 Jevgs (alleged Balkan Egyptians) and 41 Gabels (Roma of Albania), was purified from blood and saliva samples using either the Chelex method [15] or the QIAGEN micro Kit (Qiagen, Chatsworth, CA) according to the manufacturer's instructions.

All the participants gave their informed consent, provided detailed information on their geographical origin, and had two generations of unrelated paternal ancestry in their region of birth.

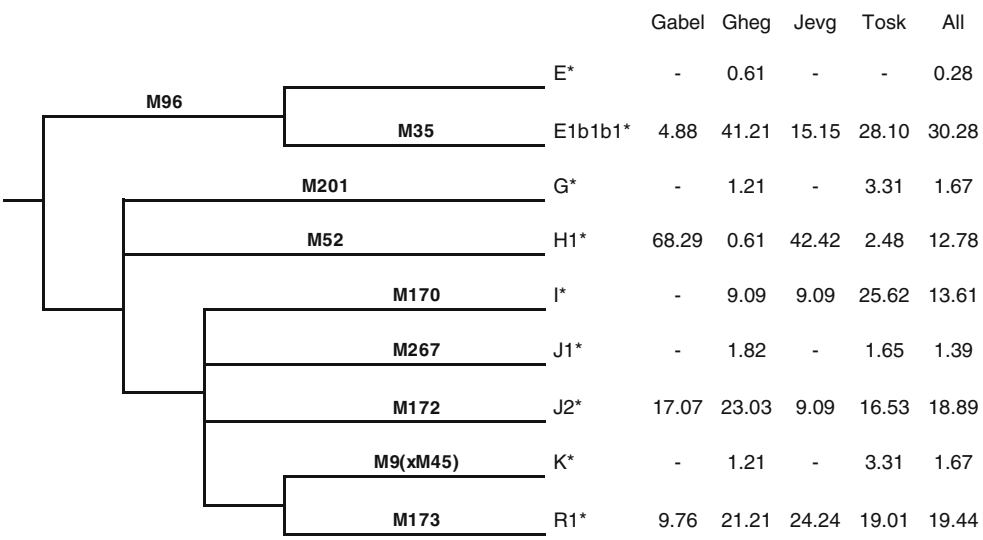
DNA analyses

The Powerplex® Y System kit was used for the amplification of 12 short tandem repeat (STR) loci (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a/b, DYS437, DYS438 and DYS439, in this order thereafter) according to manufacturer's instruction (Promega, Madison, WI). A total of 18 binary markers (M170, M172, M35, M9, M45, M173, M89, M267, M282, M304, M214, M52, M201, M96, M181, M174, M91 and M216) was typed in two multiplex PCRs following [16] (Fig. 1). Haplogroups were named according to the latest YCC nomenclature [17] and Y-STR alleles according to International Society for Forensic Genetics (ISFG) commission [18].

Genotyping of Y-STRs was achieved using an ABI 3130 Genetic Analyzer (Applied Biosystems) using the reference sequenced ladders provided by the manufacturers. Samples were analysed by GeneMapper ID v. 3.2 software (Applied Biosystems).

Quality control

In-house proficiency testing with control DNA The laboratory participated in the proficiency testing of the GeFI-ISFG working group (2006) and to the quality control test



	Gabel	Gheg	Jevg	Tosk	All
E*	-	0.61	-	-	0.28
E1b1b1*	4.88	41.21	15.15	28.10	30.28
G*	-	1.21	-	3.31	1.67
H1*	68.29	0.61	42.42	2.48	12.78
I*	-	9.09	9.09	25.62	13.61
J1*	-	1.82	-	1.65	1.39
J2*	17.07	23.03	9.09	16.53	18.89
K*	-	1.21	-	3.31	1.67
R1*	9.76	21.21	24.24	19.01	19.44

Fig. 1 Phylogenetic tree of the Y chromosome according to the YCC with SNP mutations frequencies of the Albanian populations analysed

with blind haplotyping of five DNA samples for Y-STR haplotype reference database [19].

Data analyses

Haplotype and haplogroup frequencies were estimated by gene counting. Haplotype diversity was calculated according to Nei [20] using the Arlequin software version 3.01 [21]. Diversities were also reported in terms of forensically relevant parameters (Table 1): discrimination capacity (the percentage of unique haplotypes) and match probability (the probability that two randomly chosen chromosomes are identical).

Analyses of molecular variance (AMOVA) analyses and pairwise genetic distances (R_{ST}, for STR-haplotypes and F_{ST}, for single nucleotide polymorphism (SNP)-haplogroups) were performed using the Arlequin 3.01 package. Patterns of genetic differentiation were visualised using a multidimensional scaling approach performed on pairwise R_{ST} and F_{ST} distance matrices with the STATISTICA 5.0 package (StatSoft). Allele variation at the duplicated DYS385a/b site was excluded when calculating R_{ST}. For comparative data on haplogroup and microsatellite frequencies, we referred to published sources (see Table S1).

A quantitative measure of the haplotypes shared by population pairs was performed weighting the number of matches upon the total number of diverse haplotypes.

The phylogenetic relationships between the Y-STR haplotypes within E1b1b1-M35, I*-M170 and H*-M69 lineages were elucidated through the median-joining algorithm [22] implemented in the NETWORK 4.5.1.0 software [23]. Microsatellite loci were weighted according to the inverse of their variance.

We estimated the most likely mutations between 12-locus haplotypes pairs diverged 130 (13th century BC), 65 (4th century AD) and seven (19th century AD) generations ago, from the posterior distribution of the time to the most recent common ancestor [24], considering 31 years as generation length and 2.137×10^{-3} as mean mutation rate averaged over more than 20 germ-line-based estimates [19].

Results

Y-STR diversity

The distribution and haplogroup assignment of each Powerplex haplotype in the four Albanian ethnic groups are given in Supplementary Material (Table S1). Off-ladder

Table 1 Forensic parameters of the four Albanian populations

	Gabel (*N*=41)	Gheg (*N*=165)	Jevg (*N*=33)	Tosk (*N*=121)
Minimal seven Y-STR haplotype				
Number of haplotypes	13	71	18	78
Unique haplotypes	6	42	10	51
Haplotype diversity ±SD	0.6268±0.0871	0.9469±0.0091	0.8466±0.0618	0.9825±0.0057
Discrimination capacity (%)	31.71	43.03	54.55	64.46
Match probability (%)	37.32	5.31	15.34	1.75
Minimal nine Y-STR haplotype				
Number of haplotypes	17	95	23	95
Unique haplotypes	8	73	13	73
Haplotype diversity ±SD	0.8768±0.0340	0.9756±0.0054	0.9356±0.0332	0.9945±0.0021
Discrimination capacity (%)	41.46	57.58	69.70	78.51
Match probability (%)	12.32	2.44	6.44	0.55
Minimal 11 Y-STR haplotype				
Number of haplotypes	20	107	23	102
Unique haplotypes	10	84	16	79
Haplotype diversity ±SD	0.9134±0.0289	0.9812±0.0048	0.9356±0.0332	0.9963±0.0019
Discrimination capacity (%)	48.78	64.85	69.70	84.30
Match probability (%)	8.66	1.88	6.44	0.37
Minimal 12 Y-STR haplotype				
Number of haplotypes	21	110	23	104
Unique haplotypes	11	87	16	82
Haplotype diversity ±SD	0.9171±0.0293	0.9825±0.0047	0.9356±0.0332	0.9970±0.0016
Discrimination capacity (%)	51.22	66.67	69.70	85.95
Match probability (%)	8.29	1.75	6.44	0.30

alleles were observed at DYS437 (allele 18 in one Tosk donor belonging to the R1*-M173 haplogroup) and DYS438 (allele 13 in three Gheg donors belonging to the R1*-M173 haplogroup). Duplicated or null-alleles were not observed.

Amongst the 360 Albanian males analysed, 233 diverse haplotypes (64.7%) and 196 unique haplotypes (54.4%) were observed. The most frequent haplotypes were one neighbouring pair assigned to H1-M52 (15–14–30–22–10–11–12–15,17 or 15,18-14–9–11) and one neighbouring pair assigned to E1b1b-M35 (13–13–30–24 or 25–10–11–13–16,18–14–10–12). They were found in all four groups but with different proportions: the former pair is the most common in the Gabel (39.0%) and Jevg (36.4%) pools, the latter is the most frequent pair among the Ghegs (10.9%) and the Tosks (3.3%).

The haplotype diversity (see Table 1 and Table S1) was very low in the Gabel (0.9171 for the Powerplex set of loci) and high in the Tosks (0.9970), as expected in highly endogamous and panmictic groups, respectively [25]. The Jevg diversity was low (0.9356), but at the uppermost values observed in other Roma groups. The Gheg diversity was higher (0.9825) but under the range of values observed in non-Roma Balkan populations.

The Gabels' haplotype diversity increased by 46.2% and discrimination capacity by 32.6% when using the Powerplex system instead of the minimal 7-locus set (Table 1) and a similar albeit smaller increment of the same parameters was detected in the other groups. However, the match probabilities remained high at the 12-locus resolution level within Gabel (8.29%), Jevg (6.44%) and Gheg (1.75%) pools.

Pairwise haplotype distances

Pairwise R_{ST} distances between 7-locus Y-STR haplotypes in Albanians, Roma and non-Roma host populations were summarised in Table S2 [26–46]. Almost all distance values within and between Albanians reached statistical significance (Table S2) except between the Tosks and their closest Balkan neighbours (Greek, Macedonians, Bulgarians) and between the Gabels or the Jevgs and some Roma groups (i.e. Bulgarian and Slovakian Roma).

The populations with Balkan and Italian origin grouped into separate clusters on the graph. The genetic distances among Romani-speaking groups were generally higher and did not correlate with geography in accordance with the complex history of fragmentation since the arrival of proto-gypsy groups in Europe and with previous analyses [26]. The existence of a clear-cut genetic sub-structure was further confirmed by AMOVA considering three hierarchical levels and three population groups (Roma/Balkan/Italian). The difference between

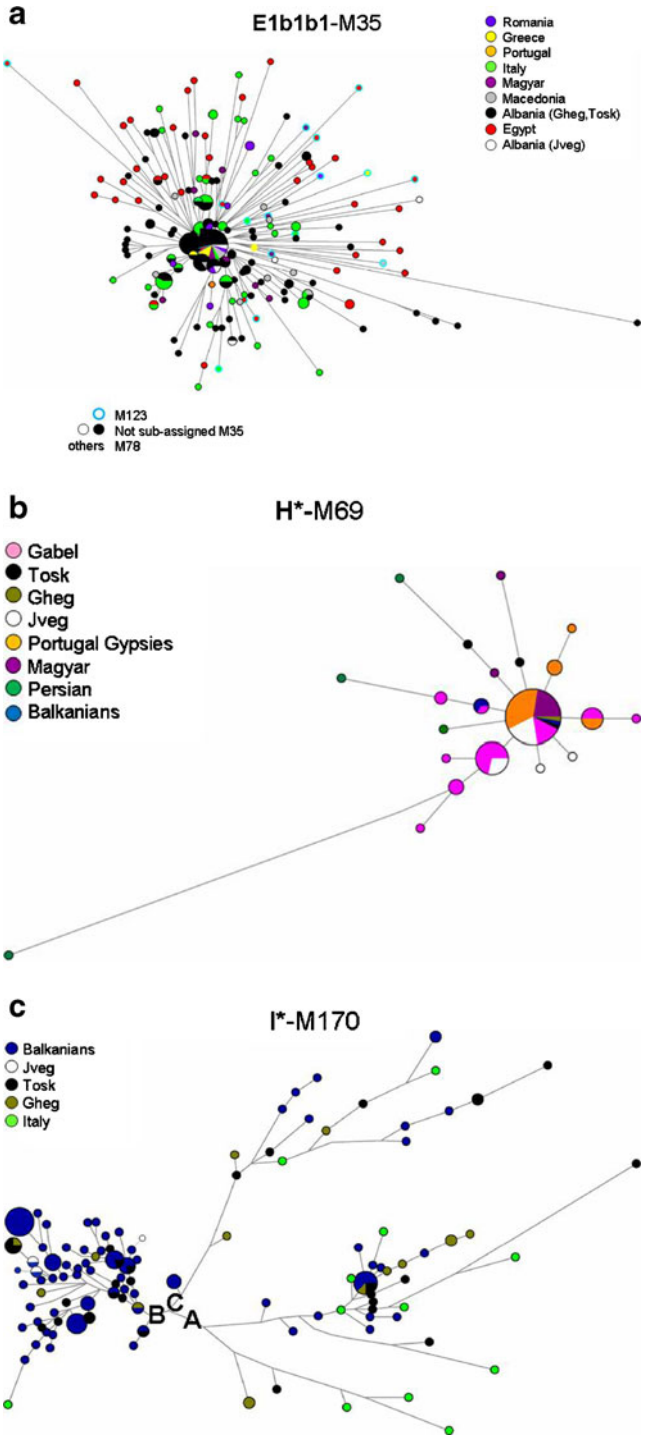


Fig. 2 Median-joining network of haplogroups E1b1b1-M35 (**a**), H*-M69 (**b**) and I*-M170 (**c**). *Circles* represent haplotypes with an area proportional to frequency, and *colours* indicate the population of origin

groups accounted for 10.6% of the total genetic variance ($p<0.00001$) and was larger than the difference within groups (4.9%, $p<0.00001$).

The Tosks fell well within the Balkan cluster and the Gabels within the Roma cluster, whereas the Gheg and the

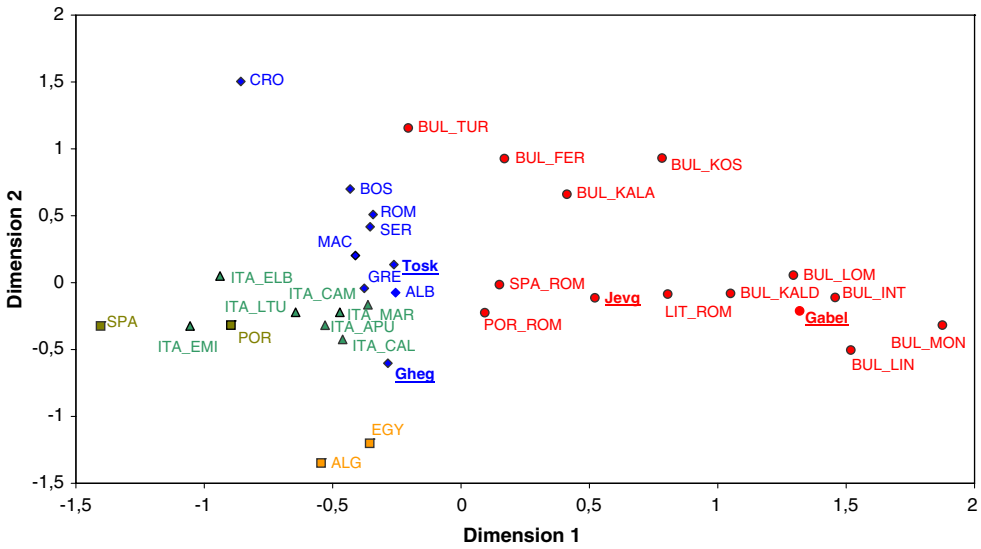


Fig. 3 MDS analysis based on the frequencies of the Y chromosome haplogroups E-M35, G-M201, H-M69, I-M170, J1-M267, J2-M172, K*-M9, P-P27 and R-M17 in the examined Albanians and other European populations (stress index 0.1225, $p<0.01$ [56])

Jevg samples took an outlier position. In order to better understand the cause of the intermediate genetic profile of the two latter groups, we calculated the Y-STR haplotype sharing between-sample pairs and surveyed SNP-based variability. Such data should disclose genetic relationships among populations, at the most recent and a more ancient time scale, respectively.

Haplotype sharing

The proportion of haplotypes shared between population samples is shown in Table S2. As for diversity values, the results can be paired for Ghegs and Tosks and for Gabels and Jevgs. At 7-locus haplotypes, the highest proportion was between the two Albanian major groups (55.6%) whereas the second highest proportions were between them and the two Albanian minorities (34.4–41.6% as average). Whatever the level of resolution, the Gabels and the Jevgs showed the highest matching proportion between them (60.8% at seven loci; 40.5% at 12 loci), and their affinity with Roma tribes (40.5–51.4% and 27.8–28.0% as average) was always much higher than with either major Albanians or non-Roma Balkans.

Y-SNP variation

The binary profile (Fig. 2a–c) mirrored the dual structure (Ghegs/Tosks vs Gabels/Jevgs) observed at Y-STR variation. Haplogroup H1-M52, which is strongly associated with European Roma tribes and to an Indian proto-gypsy ancestry [26, 29, 47], was found at very low frequency in the major Albanian groups (0.6–2.5%) but was by far the dominant haplogroup in the Gabels (68.3%) and the Jevgs (42.4%). Conversely, haplogroups I-M170 and E1b1b1-M35, which are common lineages in Balkan populations [48, 49], summed up to over the 50% of binary variability in the Ghegs and the Tosks but were less common in the Jevgs (24.2%) and even rarer in the Gabels (4.9%). Haplogroups J2-M172 and R1-M173 were observed at substantial frequencies in all the four Albanian groups. It has been suggested that J2 chromosomes were acquired by the proto-gypsy founder population through a Turkish–Aegean route and that R1 chromosomes entered in the Roma gene pool by admixture with local European populations [29]. For J2 and R1 lineages, no matching or neighbour haplotypes with Egyptian chromosomes were found in the YHRD (release 30, 23,979 Powerplex haplotypes from 221 populations worldwide) and in a manually edited archive of published and unpublished data (17,351 Powerplex haplotypes from 136 Eurasian populations). Chromosomes bearing mutations G-201, K-M9 and J1-M267 were observed only in the Gheg/Tosk pair at frequencies as low (4.2 in the Ghegs, 8.3 in the Tosks) as in the rest of the Balkan area [30, 50].

The dissection of the main haplogroups into networks of 12-locus haplotypes is given in Fig. 2a–c. The network of H-M69 haplotypes is in accordance with mode (strong founder effect) and times (800–1,100 yBP) [51, 52] at the origin of proto-gypsy migration from Asia to Europe and points to a deep common ancestry for Gabel and Jevg variation (rho-based TMRCA=1,110±582 yBP).

The network of I-M170 haplotypes is structured into at least three sub-clades that we inferred to correspond to I1-M253 (A branch), I2a-P37 (B branch) and I2b-M223 (C branch) chromosomes [48] on the basis of SNP-typed haplotypes (data not shown). Gheg and Tosk haplotypes are closely associated with Balkan chromosomes in all the three sub-clades, whereas Jevg chromosomes appeared to be much more linked to Balkan than to Albanian chromosomes on the B branch only. The latter

feature suggests that the flow of I chromosomes to the Jevg Y pool might not have been mediated by the Ghegs or the Tosks and most likely is to be traced back to an early phase of permanence of Roma tribes in the Balkan area.

The absence of close links between Jevg and North African haplotypes in the network of E1b1b1-M35 chromosomes makes unlikely a recent common ancestry in Egypt. However, Y chromosomes with up to two mutational differences at 12 STR haplotypes can be considered compatible with putative Egyptian ancestors. Following this, we searched haplotypes differing 0–2 mutational steps from Jevg E chromosomes on databases. Three out of five haplotypes fully matched with a total of 76 chromosomes of Albanian (31.6%), Balkan (30.3%), Central European (28.9%), Italian (1.3%) but also North African (5.3%) origin. Whether the African matches (two haplotypes from Sohag, Egypt; one haplotype from El Minia, Egypt; two haplotypes from Sfax, Tunisia) are identical by descent to Jevg chromosomes or they are the effect of convergence, a phenomenon commonly observed among haplotypes belonging to E-M35 subgroups [36] could be checked only by typing a larger set of population samples for M35 downstream mutations.

Discussion

The determination of the population of origin of a crime-scene sample would be very useful information in forensics and the combined analysis of SNP and STR Y chromosome markers is a critical step for making inferences on the geographic or ethnic ancestry of an unknown sample because of some populations may show a proportion of shared haplotypes as a results of recent gene flow [53]. However, such inferences should be always carefully evaluated given the complex historical demographic dynamics that have characterised European history [54, 55]. When generating reference databases for forensic purpose, in particular for culturally isolated populations such as Roma groups, the population structure should always be investigated for a correct evaluation of haplotype frequencies, and the use of a general database instead of the specific one may result in a relevant overestimation of the likelihood ratio.

The present research contributed to define the first SNP and STR profile of the two major Albanian ethnic groups (Tosks and Ghegs) and of two minorities (Gabels and Ghegs). The Tosk profile is largely consistent with the variability expected in a panmictic population of southern Balkan origin. Weak signatures of gene flow with Roma groups, probably mediated by the Gabels or the Jevgs, have been identified in the presence of H1-M52 lineages (2.5%). The Gheg profile closely resembles the Tosk one for haplogroup composition and level of H1 introgression (0.6%). However, haplotype diversity values and the skewed E1b1b1-M35 and I-M170 haplogroup frequencies suggest a higher degree of reproductive isolation than in Tosks, a condition that might also explain the partially marginal position of the Ghegs in Y-SNP-based MDS plots (Fig. 3). The Gabel profile is typical of European Roma tribes and shows high affinity to Vlax subgroups. The 12-locus haplotype diversity is among the lowest so far observed in Europe and reflects the gypsy tradition of endogamous marriages. As for other gypsy groups most of E1b1b1-M35, I-M170, R1-M173 and J2-M172 lineages could be related to admixture events occurred in an early phase of the history of Romani populations in Europe, but a more recent gene flow from non-Roma Albanians cannot be excluded.

The Y profile of the Jevg minority can be confidently ascribed to a Roma ancestry. It differs from the Gabel's features for showing a higher diversity value, as a consequence of a lower ratio between the Roma (H1-M52 chromosomes) and the non-Roma component (E1b1b1-M35, I-M170, R1-M173, J2-M172 chromosomes). A larger genetic contribution from host European populations justify the left-oriented shift of Jevgs on the MDS plot and makes their genetic profile much more similar to gypsy groups with a long migration history as Portuguese, Spanish and Lithuanian Roma. The exact matchings observed with North African samples at three E1b1b1 haplotypes is a genetic link too weak to claim a recent Egyptian ancestry of Jevg people. Evidence of identity by state among haplotypes belonging to M35 sub-lineages (see the network of Fig. 2a) calls for a more resolved genealogy of Albanian E chromosomes before one can assign those haplotypes to the same genealogy.

Conclusions

The Powerplex Y system hardly ensures an adequate probability of exclusion to suspects belonging to Albanian populations. The generally high degree of endogamy, the reduced effective population size and the exchange of male lineages which occurred between major and minor population groups decrease unacceptably the discrimination capacity of this set of loci even if the STR profile is supported by SNP typing. Therefore, great care should be taken when analysing a Powerplex® Y profile match against Albanian databases. In crime-scene items, autosomal STRs are indeed the markers of choice also for very close communities, confirmed by our Identifiler analysis (233 individuals, 90 Ghegs, 64 Tosks, 49 Gabels and 30 Jevgs) that yielded high values of forensic parameters in all groups

(results available upon request). However, there are a number of cases (as sex-related crimes) where male–female admixed samples need to be investigated, and male-specific markers are the only reliable information if the mixture is strongly skewed toward the female component.

The Jevgs, the Albanian group self-assigned to the wider Balkan Egyptian community, turned out to have a very close genetic affinity with European Roma tribes, particularly with the Albanian Roma (Gabels). Despite the relatively small sample size, our results can reject the hypothesis of a North African origin for at least 85% of Jevg chromosomes (lineages H1, I*, J2, R1) and suspend the judgement on the remaining 15% (E1b1b1 chromosomes) to future analyses on M35 downstream SNPs.

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