

Tracing Past Human Male Movements in Northern/Eastern Africa and Western Eurasia: New Clues from Y-Chromosomal Haplogroups E-M78 and J-M12

Fulvio Cruciani,* Roberta La Fratta,* Beniamino Trombetta,* Piero Santolamazza,* Daniele Sellitto,† Eliane Beraud Colomb,‡ Jean-Michel Dugoujon,§ Federica Crivellaro,*¹ Tamara Benincasa,|| Roberto Pascone,¶ Pedro Moral,# Elizabeth Watson,** Bela Melegh,†† Guido Barbujani,‡‡ Silvia Fuselli,‡‡ Giuseppe Vona,§§ Boris Zagradisnik,||| Guenter Assum,¶¶ Radim Brdicka,## Andrey I. Kozlov,*** Georgi D. Efremov,††† Alfredo Coppa,‡‡‡ Andrea Novelletto,§§§ and Rosaria Scozzari*†

*Dipartimento di Genetica e Biologia Molecolare, Sapienza Università di Roma, Rome, Italy; †Istituto di Biologia e Patologia Molecolari del Consiglio Nazionale delle Ricerche, Rome, Italy; ‡Laboratoire d'Immunologie, Hôpital de Sainte-Marguerite, Marseille, France; §Laboratoire d'Anthropobiologie, FRE 2960 Centre National de la Recherche Scientifique (CNRS), Université Paul Sabatier, Toulouse, France; ||Dipartimento di Biologia Cellulare, Università della Calabria, Rende, Italy; ¶Dipartimento di Scienze Ginecologiche Perinatologia e Puericultura, Sapienza Università di Roma, Rome, Italy; #Departament de Biologia Animal, Universitat de Barcelona, Barcelona, Spain; **The Swedish Museum of Natural History, Stockholm, Sweden; ††Department of Medical Genetics and Child Development, University of Pécs, Pécs, Hungary; ‡‡Dipartimento di Biologia, Università di Ferrara, Ferrara, Italy; §§Dipartimento di Biologia Sperimentale, Università di Cagliari, Cagliari, Italy; |||Laboratory of Medical Genetics, General Hospital Maribor, Maribor, Slovenia; ¶¶Institut für Humangenetik, Universität Ulm, Ulm, Germany; ##Institute for Haematology and Blood Transfusion, Prague, Czech Republic; ***ArctAn C Innovative Laboratory, Moscow, Russia; †††Research Center for Genetic Engineering and Biotechnology, Macedonian Academy of Sciences and Arts, Skopje, Republic of Macedonia; ‡‡‡Dipartimento di Biologia Animale e dell'Uomo, Sapienza Università di Roma, Rome, Italy; and §§§Dipartimento di Biologia, Università "Tor Vergata", Rome, Italy

Detailed population data were obtained on the distribution of novel biallelic markers that finely dissect the human Y-chromosome haplogroup E-M78. Among 6,501 Y chromosomes sampled in 81 human populations worldwide, we found 517 E-M78 chromosomes and assigned them to 10 subhaplogroups. Eleven microsatellite loci were used to further evaluate subhaplogroup internal diversification.

The geographic and quantitative analyses of haplogroup and microsatellite diversity is strongly suggestive of a northeastern African origin of E-M78, with a corridor for bidirectional migrations between northeastern and eastern Africa (at least 2 episodes between 23.9–17.3 ky and 18.0–5.9 ky ago), trans-Mediterranean migrations directly from northern Africa to Europe (mainly in the last 13.0 ky), and flow from northeastern Africa to western Asia between 20.0 and 6.8 ky ago.

A single clade within E-M78 (E-V13) highlights a range expansion in the Bronze Age of southeastern Europe, which is also detected by haplogroup J-M12. Phylogeography pattern of molecular radiation and coalescence estimates for both haplogroups are similar and reveal that the genetic landscape of this region is, to a large extent, the consequence of a recent population growth in situ rather than the result of a mere flow of western Asian migrants in the early Neolithic.

Our results not only provide a refinement of previous evolutionary hypotheses but also well-defined time frames for past human movements both in northern/eastern Africa and western Eurasia.

Introduction

A large number of Y chromosome unique event polymorphisms (UEPs) has been reported in the last 7 years (Shen et al. 2000, 2004; Underhill et al. 2000, 2001; Cruciani et al. 2002, 2004, 2006; The Y Chromosome Consortium 2002; Hammer et al. 2003; Cinnioğlu et al. 2004; Rootsi et al. 2004; Semino et al. 2004; Wilder et al. 2004; Kayser et al. 2006; Mohyuddin et al. 2006; Sengupta et al. 2006; Sims et al. 2007) leading to the identification of hundreds of Y-specific haplogroups. Most of the terminal branches of the present Y-phylogenetic tree show a geographic distribution, which is essentially limited to specific continental or subcontinental areas, mainly as a consequence of the reduced

effective population size of the Y chromosome and/or of the origin of each branch after major peopling episodes (for a review see Jobling and Tyler-Smith 2003). As previously observed (Cruciani et al. 2004), haplogroup E3b1a (E-M78) escapes this rule, being present at high frequencies in a wide area stretching from northern and eastern Africa, Europe, and western Asia (Underhill et al. 2000, 2001; Bosch et al. 2001, 2006; Cruciani et al. 2002, 2004; Semino et al. 2002, 2004; Arredi et al. 2004; Behar et al. 2004; Cinnioğlu et al. 2004; Flores et al. 2004, 2005; Luis et al. 2004; Shen et al. 2004; Alonso et al. 2005; Gonçalves et al. 2005; Marjanovic et al. 2005; Peričić et al. 2005; Sanchez et al. 2005; Wood et al. 2005; Regueiro et al. 2006).

Due to the lack of informative UEPs defining additional nodes internal to this haplogroup, scholars relied upon the information provided by network analysis of fast evolving microsatellites in order to identify putative monophyletic groups of chromosomes within E-M78 (Cruciani et al. 2004; Semino et al. 2004), an approach which had been successfully used in the past for an initial molecular dissection of major unresolved haplogroups (Malaspina et al. 1998, 2000; Scozzari et al. 1999).

¹ Present address: Leverhulme Centre for Human Evolutionary Studies, University of Cambridge, Cambridge, United Kingdom.

Key words: Y-chromosome haplogroups, Y-chromosome phylogeography, human migrations, Bronze Age, European populations, African populations.

E-mail: rosaria.scozzari@uniroma1.it.

Mol. Biol. Evol. 24(6):1300–1311. 2007

doi:10.1093/molbev/msm049

Advance Access publication March 10, 2007

Cruciani et al. (2006) recently reported on the identification of 6 new UEPs within the E-M78 clade, 4 of which seem to be relatively common and informative for evolutionary studies. An evaluation of the correspondence between the subhaplogroups defined by the new UEPs and the E-M78 clusters previously identified by microsatellite network analysis, revealed not only a tight correspondence between the trees generated by the 2 types of markers but also important discrepancies, underlining once more that microsatellite-defined clusters cannot always be considered monophyletic groups of chromosomes (Cruciani et al. 2006).

In the present study, we provide detailed population data on the distribution of E-M78 binary subhaplogroups defined by 10 UEPs (2 of which are here described for the first time) in a sample of 6,501 Y chromosomes belonging to 81 populations mainly from Europe, western Asia, and Africa. In order to obtain estimates of internal diversity and coalescence age of E-M78 subhaplogroups and the associated human migrations and demographic expansions, we also analyzed a set of 11 microsatellites. The same set of microsatellites was also analyzed in a sample of Y chromosomes belonging to the haplogroup J-M12, whose geographic distribution in Europe strictly overlaps that of a single E-M78 subhaplogroup. Our results not only provide a refinement of previous evolutionary hypotheses based on microsatellites alone but also well-defined time frames for different migratory events that led to the dispersal of these haplogroups and subhaplogroups in the Old World.

Subjects and Methods

Subjects

The sample comprised 6,501 unrelated male subjects belonging to 81 populations worldwide. Appropriate informed consent was obtained from all participants. Geographic origin and sample size for each population are reported in table 1 and Supplementary figure 1 (Supplementary Material online).

Molecular Analysis

Samples were obtained from peripheral blood, cultured cells, hair roots, or buccal swabs, and DNA was extracted using appropriate procedures (either phenol-chloroform extraction followed by ethanol precipitation or purification by QIAamp kit from Qiagen, Milan, Italy).

In all, 6,501 Y chromosomes were analyzed for the M78 marker (present study and Cruciani et al. 2002, 2004) by the method of Underhill et al. (2000). Among them, 517 chromosomes carrying the M78-derived T allele were further genotyped for 10 markers defining internal nodes, following a hierarchical approach. Typing methods for 8 of these markers (M148, M224, V12, V13, V19, V22, V27, and V32) were previously described (Underhill et al. 2000, 2001; Cruciani et al. 2006). Two polymorphic markers (V36 and V65) are here reported for the first time. The V36 polymorphism is a T to C transition at position 383 of a 449-bp polymerase chain reaction (PCR) fragment am-

plified using the primers V36 forward (5'-tcctcttccact-tacctcca) and V36 reverse (5'-caaatgcaatcaccatttagg). The V65 polymorphism is a G to T transversion at position 77 of a 349-bp PCR fragment amplified using the primers V65 forward (5'-cctcaacctactaaatgtgaccatg) and V65 reverse (5'-atgccacacaatttccat). Both polymorphisms were genotyped by denaturing high performance liquid chromatography. The M12 polymorphism (Underhill et al. 1997), defining haplogroup J2b (Sengupta et al. 2006), has been analyzed as described in Cruciani et al. (2002).

In all, 483 of the 517 E-M78 subjects were further typed for 4 polymorphic dinucleotide repeats (YCAII and DYS413 duplicated loci) and 7 tetranucleotide repeats (DYS19, DYS391, DYS393, DYS439, DYS460 [formerly A7.1], DYS461 [formerly A7.2], and GATA A10) as previously reported (Cruciani et al. 2004). The same eleven microsatellites were analyzed in a set of 43 European J-M12 chromosomes. The DYS392 microsatellite was analyzed in 101 E-M78 chromosomes using primers reported by Butler et al. (2002) and the method described by Cruciani et al. (2002).

Data Analysis

For each haplogroup, phylogenetic relationships among 11 microsatellite haplotypes were obtained by sequentially performing reduced-median and median-joining procedures (Bandelt et al. 1995, 1999) through the use of the network 4.1 program (Fluxus-engineering.com, <http://www.fluxus-engineering.com/sharenet.htm>). In order to reduce reticulations in the network, microsatellites were weighted proportionally to the inverse of the repeat variance observed in each haplogroup.

To estimate the time to the most recent common ancestor (TMRCA) of haplogroups, we used the 7 tetranucleotide loci and applied the average square distance (ASD) method (Goldstein et al. 1995), where the ancestral haplotype was assumed to be the haplotype carrying the most frequent allele at each microsatellite locus. We employed a microsatellite evolutionarily effective mutation rate (Zhivotovsky et al. 2004). However, because the loci used here and those used by Zhivotovsky et al. (2004) do not overlap completely, we calculated the microsatellite mutation rate as follows: we obtained the mean and standard deviation of the father-to-son mutation rates reported by Gusmão et al. (2005) for the same loci here used, and reduced them by a factor 3.6 (i.e., the discrepancy between the rate estimate obtained from population data and that obtained from father-to-son transmissions [Zhivotovsky et al. 2004]). This resulted in an evolutionarily effective rate $\omega = 7.9 \times 10^{-4}$ (SD = 5.7×10^{-4}), a figure that was also used in recalculating the E-M215 coalescence age (data from Cruciani et al. 2004). Recently, Zhivotovsky et al. (2006) showed that reduced loss of diversity in an expanding population brings the evolutionarily effective rate closer to the germ line rate than in constant-size populations. Thus, in the case of expanding populations, we used a correction of the 7.9×10^{-4} value, that was calculated as follows. With reference to figure 2 in Zhivotovsky et al. (2006), the values of accumulated variance in 200–300 generations for the scenarios of 1) a single rate for exponential

Table 1
Frequencies (%) of the Y-Chromosome E-M78 Subhaplogroups in the 81 Populations Analyzed

Population Number	Region and Population	N	Frequency of Haplogroup (%)						
			E-M78	E-M78*	E-V12*	E-V13	E-V22	E-V32	E-V65
Europe									
1	Northern Portuguese ^a	50	4.00	—	—	4.00	—	—	—
2	Southern Portuguese ^a	49	4.08	—	—	4.08	—	—	—
3	Pasiegos from Cantabria ^a	56	—	—	—	—	—	—	—
4	Asturians ^a	90	10.00	—	—	5.56	4.44	—	—
5	Southern Spaniards ^a	62	3.23	—	—	—	3.23	—	—
6	Spanish Basques ^a	55	—	—	—	—	—	—	—
7	French Basques ^{a,b}	16	6.25	—	6.25	—	—	—	—
8	French ^{a,b}	225	4.44	—	0.44	4.00	—	—	—
9	English ^{a,b}	28	—	—	—	—	—	—	—
10	Danish ^a	35	2.86	—	—	2.86	—	—	—
11	Germans	77	3.90	—	—	3.90	—	—	—
12	Polish ^a	40	2.50	—	—	2.50	—	—	—
13	Czechs	268	4.85	—	—	4.85	—	—	—
14	Slovaks	24	8.33	—	—	8.33	—	—	—
15	Slovenians	104	2.88	—	—	2.88	—	—	—
16	Northern Italians ^{a,b}	94	7.45	—	—	5.32	2.13	—	—
17	Central Italians ^{a,b}	356	7.87	—	0.28	5.34	1.97	—	0.28
18	Southern Italians ^a	141	10.64	—	0.71	8.51	1.42	—	—
19	Sicilians ^{c,d}	153	13.07	—	0.65	7.19	4.58	—	0.65
20	Sardinians ^{a,b,e}	374	3.48	0.27	0.27	1.07	0.80	—	1.07
21	Estonians ^a	74	4.05	—	—	4.05	—	—	—
22	Belarusians	40	—	—	—	—	—	—	—
23	Northern Russians ^{a,b}	82	3.66	—	—	3.66	—	—	—
24	Southern Russians	92	2.17	—	—	2.17	—	—	—
25	Ukrainians	11	9.09	—	—	9.09	—	—	—
26	Moldovians	77	7.79	—	—	7.79	—	—	—
27	Hungarians	106	9.43	—	—	9.43	—	—	—
28	Rumanians ^a	265	7.55	—	—	7.17	0.38	—	—
29	Macedonians	99	18.18	—	—	17.17	1.01	—	—
30	Continental Greeks	147	19.05	—	—	17.69	0.68	—	0.68
31	Greeks from Crete	215	6.51	—	0.93	5.58	—	—	—
32	Greeks from Aegean Islands	71	16.90	—	—	15.49	1.41	—	—
33	Bulgarians ^a	204	16.67	—	0.49	16.18	—	—	—
34	Albanians ^a	96	32.29	—	—	32.29	—	—	—
Northwestern Africa									
35	Moroccan Arabs ^a	55	40.00	3.64	—	—	7.27	—	29.09
36	Asni Berbers	54	3.70	—	—	—	3.70	—	—
37	Bouhria Berbers	67	1.49	—	—	1.49	—	—	—
38	Moyen Atlas Berbers ^a	69	10.14	—	—	—	—	—	10.14
39	Marrakech Berbers ^a	29	6.90	—	3.45	—	3.45	—	—
40	Moroccan Jews	50	12.00	—	2.00	2.00	8.00	—	—
41	Mozabite Berbers ^{a,b}	20	—	—	—	—	—	—	—
Northeastern Africa									
42	Libyan Jews	25	8.00	—	—	4.00	—	—	4.00
43	Libyan Arabs	10	20.00	—	—	—	—	—	20.00
44	Northern Egyptians (Delta) ^a	72	23.61	—	5.56	1.39	13.89	2.78	—
45	Egyptian Berbers	93	6.45	—	2.15	—	—	—	4.30
46	Egyptians from Baharia	41	41.46	—	14.63	2.44	21.95	—	2.44
47	Egyptians from Gurma Oasis	34	17.65	5.88	8.82	—	—	2.94	—
48	Southern Egyptians ^a	79	50.63	—	44.30	1.27	3.80	—	1.27
Eastern Africa									
49	Amhara ^a	34	8.82	—	—	—	—	8.82	—
50	Ethiopian Jews ^a	22	9.09	—	—	—	—	9.09	—
51	Mixed Ethiopians ^a	12	33.33	—	—	—	25.00	8.33	—
52	Borana/Oromo (Kenya/Ethiopia) ^a	32	40.63	—	—	—	—	40.63	—
53	Wolayta ^a	12	16.67	—	—	—	8.33	8.33	—
54	Somali ^a	23	52.17	—	—	—	4.35	47.83	—
55	Nilotic from Kenya ^a	18	11.11	—	—	—	11.11	—	—
56	Bantu from Kenya ^{a,b}	28	3.57	—	—	—	—	3.57	—
57	Western Africa ^{a,b,f}	123	0.81	—	0.81	—	—	—	—
58	Central Africa ^{a,b}	150	0.67	—	0.67	—	—	—	—
59	Southern Africa ^{a,b}	105	—	—	—	—	—	—	—
Western Asia									
60	Istanbul Turkish ^a	35	8.57	—	—	2.86	5.71	—	—
61	Southwestern Turkish ^a	40	2.50	—	—	2.50	—	—	—

Table 1
Continued

Population Number	Region and Population	N	Frequency of Haplogroup (%)						
			E-M78	E-M78*	E-V12*	E-V13	E-V22	E-V32	E-V65
62	Northeastern Turkish ^a	41	—	—	—	—	—	—	—
63	Southeastern Turkish ^a	24	4.17	—	—	4.17	—	—	—
64	Erzurum Turkish ^a	25	4.00	—	4.00	—	—	—	—
65	Central Anatolian ^a	61	6.56	—	1.64	4.92	—	—	—
66	Turkish Cypriots ^a	46	13.04	—	—	10.87	2.17	—	—
67	Sephardi Turkish ^a	19	—	—	—	—	—	—	—
68	Palestinians ^a	29	10.34	—	—	3.45	6.90	—	—
69	Druze Arabs ^a	28	10.71	—	—	10.71	—	—	—
70	Bedouin ^a	28	3.57	—	—	—	3.57	—	—
71	Syrians	100	2.00	—	—	—	2.00	—	—
72	Kurds from Iraq	20	—	—	—	—	—	—	—
73	Arabs from United Arab Emirates ^a	40	2.50	—	—	—	2.50	—	—
74	Omanite ^a	106	0.94	—	—	—	0.94	—	—
75	Adygei ^{a,b}	18	—	—	—	—	—	—	—
76	Azeri ^a	97	2.06	—	—	2.06	—	—	—
77	Southern Asia ^{a,b}	300	1.00	—	—	—	1.00	—	—
78	China ^{a,b}	206	—	—	—	—	—	—	—
79	Eastern Asia ^{a,b}	41	—	—	—	—	—	—	—
80	Oceania ^{a,b}	21	—	—	—	—	—	—	—
81	Central and Southern America Native American ^{a,b}	43	—	—	—	—	—	—	—
	Total	6,501	7.95	0.08	1.00	4.45	1.29	0.54	0.60

^a This sample, or a subset of it, was previously typed for the M78 marker (Cruciani et al. 2004).

^b Sample (or a subset of it) from the Human Genome Diversity Project/CEPH DNA panel (Cann et al. 2002).

^c 43 subjects from Sicily (Trapani) analyzed by Cruciani et al. (2004) are included on the sample.

^d One E-V13 subject also carries the V27 mutation.

^e Two E-V22 subjects also carry the V19 mutation.

^f 106 subjects from Burkina Faso analyzed by Cruciani et al. (2002) are included on the sample.

population growth and 2) growth with 4 distinct consecutive rates were compared with the amount accumulated in constant-size populations. This resulted in evolutionarily effective mutation rates decreased of factors 2.4 and 2.8, respectively (instead of 3.6), that is, 11.9×10^{-4} ($SD = 8.5 \times 10^{-4}$) and 10.2×10^{-4} ($SD = 7.3 \times 10^{-4}$), which were applied to haplogroups E-V13 and J-M12 found in Europe. Confidence intervals (CIs) for the ASD (and TMRCA) were obtained as follows: Mutations on the microsatellite genealogy were simulated using a Poisson process, in which the total number of mutational events was calculated based on branch length and assuming that mutations at each microsatellite were gamma-distributed with mean and standard deviation calculated as above. Each mutation increased or decreased allele length by one step (each with probability 0.5). ASD was then evaluated for the simulated data and the whole process repeated 1,000 times to quote the central 95% of values. This method represents a refinement of that by Thomas et al. (1998) and Scozzari et al. (2001), as it also takes into account heterogeneity of mutation rates across loci. An independent dating method (ρ statistics; Forster et al. 1996; Saillard et al. 2000) was also used to assay how robust the time obtained is to choice of method.

Both dating procedures rely on the appropriate choice of a haplotype to be considered ancestral, which remains an uncontrolled source of uncertainty. We observe that the ρ -based ages are slightly younger than the ASD-based ones (fig. 1). The difference is significant only for the root of the entire haplogroup, this being attributable to the relevant departure from a star-like structure because of repeated

founder effects (Saillard et al. 2000). Only values obtained from ASD are quoted in the text.

Haplogroup diversity and its sampling variance were estimated as in Arlequin 3.0 (Excoffier et al. 2005).

Frequency and variance maps were depicted on a grid of 44×60 lines using the Kriging procedure (Cressie 1991) through the use of the program Surfer 6.0 (Golden Software, Inc., Golden, CO). The map of microsatellite variances was obtained after pooling data from locations with less than 3 observations and assigning the resulting figures to the centroid of the pooled locations. These points are plotted in figure 5.

Results and Discussion

Molecular Dissection of E-M78 Haplogroup

By analyzing a worldwide sample of 6,501 male subjects, we have identified 517 chromosomes belonging to haplogroup E-M78, more than twice the number found in a previous study (Cruciani et al. 2004). These chromosomes have been further analyzed for the biallelic markers M148 (Underhill et al. 2000), M224 (Underhill et al. 2001), V12, V13, V19, V22, V27, V32 (Cruciani et al. 2006), V36, and V65 (present study). Only 2 of the markers analyzed (V13 and V36) were phylogenetically equivalent, leading to the identification of a total of 10 distinct haplogroups/paragroups (fig. 1), with only 5 chromosomes remaining in the paragroup E-M78*. Four subhaplogroups were either rare (1 and 2 subjects for E-V27 and E-V19, respectively) or absent (E-M148 and E-M224) in the global sample, whereas the

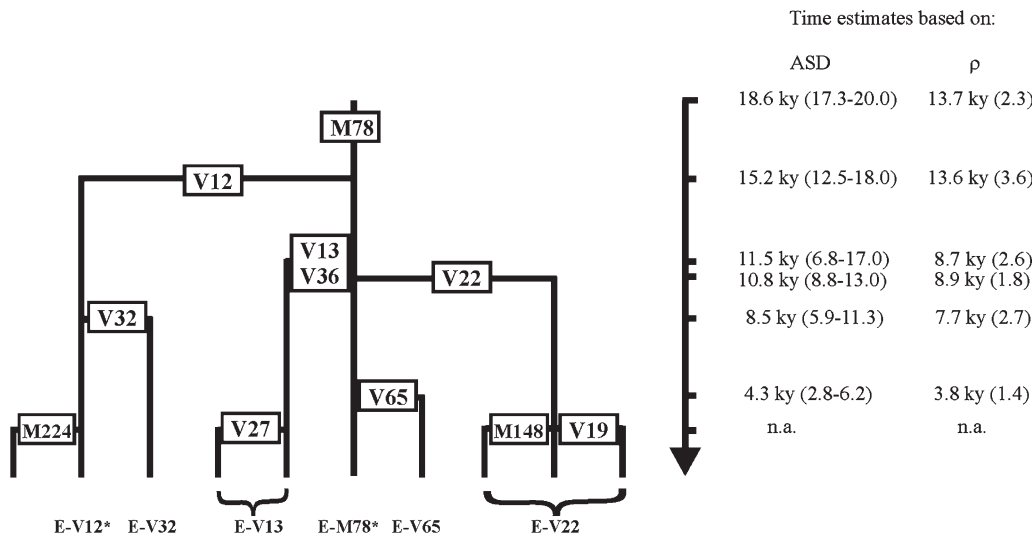


Fig. 1.—Maximum parsimony phylogeny of haplogroup E-M78. Coalescent estimates for the haplogroup E-M78 and major subhaplogroups are shown on the right. The ASD-based estimates are reported with their 95% CIs (in parentheses); the ρ -based estimates are reported with their SD (in parentheses). For haplogroup E-V13, the value obtained on the subset of western Asian samples is reported as this is free from the effect of the population expansion recorded in Europe (see text). Haplogroup nomenclature as cited in the text is reported at the bottom; n.a. —not available (rare haplogroups).

other haplogroups/paragroups were relatively common (table 1 and fig. 2). The E-M78 subhaplogroup identified by the new mutation V65 includes all but 2 of the chromosomes previously included in the cluster β and 1 chromosome from

cluster γ of the E-M78 microsatellite network (Cruciani et al. 2004), once again underlining the strong but not perfect correspondence between microsatellite-defined clusters and UEP-defined haplogroups (Cruciani et al. 2006).

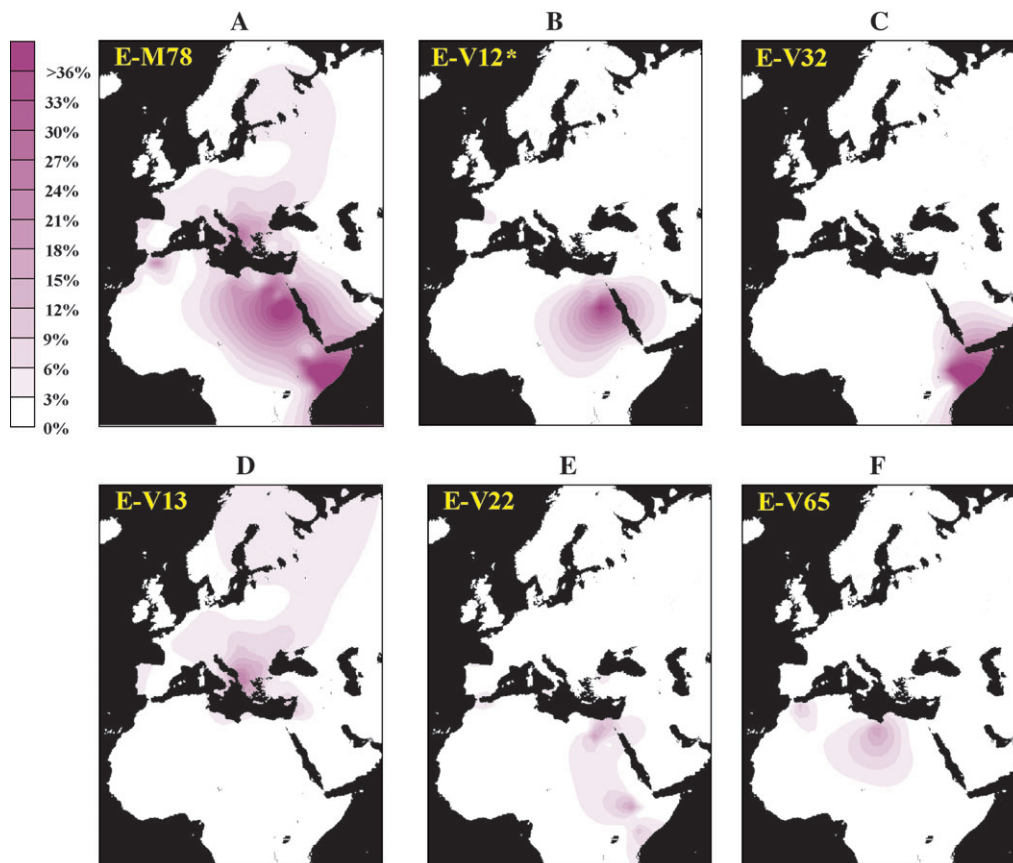


Fig. 2.—Maps of the observed haplogroup/paragroup frequencies. (A) E-M78; (B) E-V12*; (C) E-V32; (D) E-V13; (E) E-V22; and (F) E-V65.

The subdivision of E-M78 in the six common major clades revealed a pronounced geographic structuring (table 1 and fig. 2): Haplogroup E-V65 and the paragroups E-M78* and E-V12* were observed mainly in northern Africa, haplogroup E-V13 was found at high frequencies in Europe, and haplogroup E-V32 was observed at high frequencies only in eastern Africa. The only haplogroup showing a wide geographic distribution was E-V22, relatively common not only in northeastern and eastern Africa but also found in Europe and western Asia, up to southern Asia (table 1, fig. 2).

Locating the Origin of Haplogroup E-M78

An eastern African origin for this haplogroup was hypothesized on the basis of the exclusive presence in that area of a putative ancestral 12-repeat allele at the DYS392 microsatellite, found in association with E-M78 chromosomes (Semino et al. 2004). Northeastern African populations were not represented in that study. In order to test this hypothesis, we analyzed for DYS392, a geographically widespread subset of the E-M78 chromosomes here identified. We observed that the DYS392 12-repeat allele is associated with the majority of the chromosomes belonging to the northeastern African E-V12* (15 out of 18) and to the eastern African E-V32 (21 out of 23), with about half (9 out of 21) of the E-V22 chromosomes (both in eastern and northeastern Africa), with a few of the European E-V13 (2 out of 23), and with some north-African E-V65 (3 out of 16) chromosomes. These findings show that the DYS392 12-repeat allele is common in different regions characterized by high frequencies of E-M78 and suggest that it was most likely generated by multiple mutational events occurring in different UEP-defined subhaplogroups. Thus, the DYS392 allele distribution is not informative to infer the place of origin of E-M78 chromosomes.

An eastern African origin for haplogroup E-M78 was also hypothesized on the basis of the frequency distribution and microsatellite diversity (Cruciani et al. 2004). We may now test this hypothesis by exploiting the new information provided by internal biallelic markers and the extensive re-sampling in which northeastern Africa is covered by a robust group of 90 E-M78 chromosomes. The frequencies of E-M78 in northeastern Africa and eastern Africa are not significantly different (0.25 ± 0.03 and 0.22 ± 0.02 , respectively). As far as the microsatellite diversity is concerned, the highest mean variances across 7 tetranucleotide loci are those observed in eastern Africa and northeastern Africa (0.50 and 0.46, respectively), but an examination of the variances at individual loci reveals that in eastern Africa there is a disproportionate contribution of DYS19 to the mean variance (1.87). This is likely due to a multirepeat deletion associated with the common eastern African E-V32 haplogroup (Cruciani et al. 2006 and supplementary table 1). When this locus is removed from the analysis, we obtain mean variances across 6 loci of 0.41 and 0.27 for northeastern and eastern Africa, respectively. Variances at the 6 individual loci are always higher in the former region, and this difference is statistically significant for the microsatellite locus DYS461 (*F* test for equality of varian-

ces $P < 0.05$). Finally, a greater diversity of E-M78 binary subhaplogroups can be observed in northeastern Africa (0.61 ± 0.04), where all the E-M78 major branches are present, than in eastern Africa (0.30 ± 0.08), where only subhaplogroups E-V22 and E-V32 are found. E-V22 is observed at high frequencies in both northeastern and eastern Africa, with microsatellite variances of 0.46 and 0.35, respectively. The other common eastern African subhaplogroup, E-V32, that represents about 82% of the eastern African E-M78 chromosomes, is a relatively recent terminal branch of E-V12 (8.5 ky, fig. 1), the remaining E-V12 chromosomes being found almost exclusively in northeastern Africa as paralog E-V12*. The haplogroups E-V13 and E-V65 are also found in northeastern Africa. Although an origin for E-V13 outside the region is likely (see below), E-V65 probably originated in situ as inferred on the basis of its nearly exclusive presence and diversity. It is also worth noting that the rare paralog E-M78* has not been observed in eastern Africa; moreover, the 2 northwestern African E-M78* chromosomes are well differentiated from the 2 northeastern African E-M78* chromosomes (supplementary table 1, Supplementary Material online) adding a new argument for a higher haplogroup diversity in northern Africa.

In conclusion, the peripheral geographic distribution of the most derived subhaplogroups with respect to northeastern Africa, as well as the results of quantitative analysis of UEP and microsatellite diversity are strongly suggestive of a northeastern rather than an eastern African origin of E-M78. Northeastern Africa thus seems to be the place from where E-M78 chromosomes started to disperse to other African regions and outside Africa.

A Corridor for Bidirectional Migrations between Northeastern and Eastern Africa

The evolutionary processes that determined the wide dispersal of the E-M78 lineages from northeastern Africa to other regions can now be addressed.

E-M78 belongs to clade E3b (E-M215). On the basis of robust phylogeographic considerations, an eastern African origin has been proposed for E-M215 (Underhill et al. 2001; Cruciani et al. 2004), with a coalescence time of 22.4 ky (95% CI 20.9–23.9 ky; recalculated from Cruciani et al. [2004], see Subjects and Methods). A northeastern African origin for haplogroup E-M78 implies that E-M215 chromosomes were introduced in northeastern Africa from eastern Africa in the Upper Paleolithic, between 23.9 ky ago (the upper bound for E-M215 TMRCA in eastern Africa) and 17.3 ky ago (the lower bound for E-M78 TMRCA here estimated, fig. 1). In turn, the presence of E-M78 chromosomes in eastern Africa can be only explained through a back migration of chromosomes that had acquired the M78 mutation in northeastern Africa. The nested arrangement of haplogroups E-V12 and E-V32 defines an upper and lower bound for this episode, that is, 18.0 ky and 5.9 ky, respectively. These were probably not massive migrations, because the present high frequencies of E-V12 chromosomes in eastern Africa are entirely accounted for by E-V32, which most likely underwent subsequent

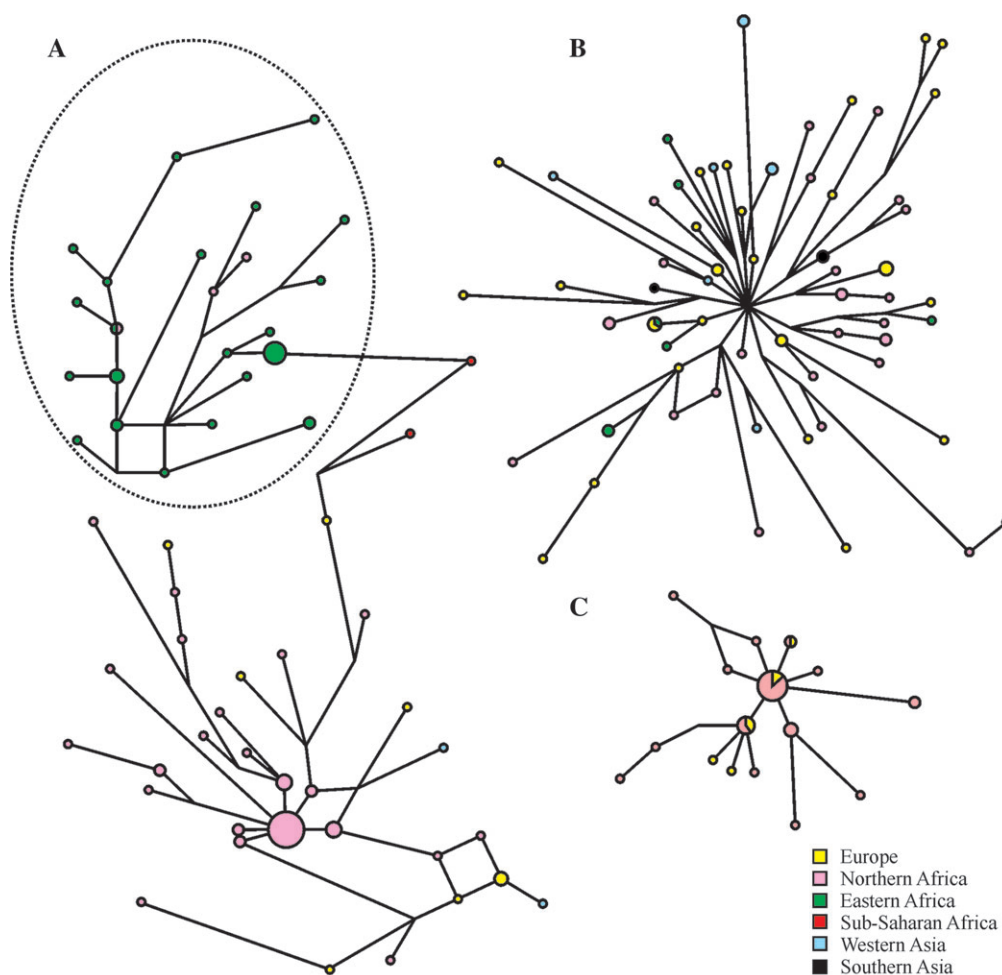


Fig. 3.—Microsatellite networks of haplogroups E-V12 (A); E-V22 (B); and E-V65 (C). In network (A), a dotted circle includes all of the E-V12 chromosomes carrying the V32 mutation. Branch lengths are proportional to the number of one-repeat mutations separating 2 haplotypes. Each circle area is proportional to the frequency of the sampled haplotype.

geographically restricted demographic expansions involving well differentiated molecular types (fig. 3A). Conversely, the absence of E-V12* chromosomes in eastern Africa is compatible with loss by drift. Possible more recent episodes of gene flow are associated with the less common E-V22 subhaplogroup, also present in both northeastern and eastern Africa, but without a clear pattern of microsatellite molecular differentiation (fig. 3B). It is conceivable that the Nile river valley has acted as a genetic corridor for human migrations between northeastern and eastern Africa, a scenario that is also supported by mtDNA analysis both at HV1 (Krings et al. 1999) and entire molecule sequence (Olivieri et al. 2006). There are also other Y-chromosome haplogroups shared by northeastern and eastern African populations, that is, E-M123, J-M267, and K-M70 (Underhill et al. 2000, Cruciani et al. 2004; Luis et al. 2004; Semino et al. 2004; Sanchez et al. 2005). However, unlike E-V12 and E-V22, these haplogroups are also common in western Asia, where they probably originated (Cruciani et al. 2004; Luis et al. 2004; Semino et al. 2004). Thus, it is unclear whether their present geographic distribution in Africa is the consequence of the same evolutionary events that involved the E-M78 chromosomes or

whether they have been introduced independently from western Asia in eastern and northeastern Africa. Only the molecular dissection of haplogroups E-M123, J-M267, and K-M70 along with an extensive sampling of populations from these regions will help in answering this question.

Direct Northern African Contribution to the European Gene Pool

Previous studies on the Y-chromosome phylogeography have revealed that central and western Asia were the main sources of Paleolithic and Neolithic migrations contributing to the peopling of Europe (Underhill et al. 2000; Wells et al. 2001). Only sporadic traces of northern African Y chromosomes were found in the European gene pool, mainly linked to the presence at low frequencies of the E-M81 haplogroup in Mediterranean coastal populations (Bosch et al. 2001; Scozzari et al. 2001; Cruciani et al. 2004; Gonçalves et al. 2005). The molecular dissection of E-M78 contributes to the understanding of the genetic relationships between northern Africa and Europe. Several lines of evidence suggest that E-M78 subhaplogroups E-V12, E-V22, and E-V65 have been involved in trans-Mediterranean

migrations directly from Africa. These haplogroups are common in northern Africa, where they likely originated, and are observed almost exclusively in Mediterranean Europe, as opposed to central and eastern Europe (table 1 and fig. 2). Also, among the Mediterranean populations, they are more common in Iberia and south-central Europe than in the Balkans, the natural entry-point for chromosomes coming from the Levant. Such findings are hardly compatible with a southeastern entry of E-V12, E-V22, and E-V65 haplogroups into Europe. Upper limits for the introduction of each of these haplogroups in Europe are given by their estimated ages (fig. 1), whereas lower bounds should be close to the present times, given the lack of internal geographic structuring (fig. 3A–C; Cruciani et al. 2004; Semino et al. 2004). Considering both these E-M78 sub-haplogroups (present study) and the E-M81 haplogroup (Cruciani et al. 2004), the contribution of northern African lineages to the entire male gene pool of Iberia (barring Pasiegos), continental Italy, and Sicily can be estimated as 5.6%, 3.6%, and 6.6%, respectively. Whether lineages E-M123, J-M267, G-M201, and K-M70, commonly found in both northern Africa and Europe (Bosch et al. 2001; Arredi et al. 2004; Cruciani et al. 2004; Semino et al. 2004), were involved in the same population movements remains to be ascertained due to the poor phylogeographic resolution of these haplogroups.

The Haplogroup E-V13: Migrations and Demographic Expansions in Western Eurasia

Haplogroup E-V13 is the only E-M78 lineage that reaches the highest frequencies out of Africa. In fact, it represents about 85% of the European E-M78 chromosomes with a clinal pattern of frequency distribution from the southern Balkan peninsula (19.6%) to western Europe (2.5%). The same haplogroup is also present at lower frequencies in Anatolia (3.8%), the Near East (2.0%), and the Caucasus (1.8%). In Africa, haplogroup E-V13 is rare, being observed only in northern Africa at a low frequency (0.9%). The European E-V13 microsatellite haplotypes are related to each other to form a nearly perfect star-like network (fig. 4A), a likely consequence of a rapid demographic expansion (Jobling et al. 2004). The TMRCA of the European E-V13 chromosomes turns out to be 4.0–4.7 ky (under 2 different demographic expansion scenarios, see Subjects and Methods; 95% CI 3.5–4.6 ky and 4.1–5.3 ky, respectively). On the other hand, when only E-V13 chromosomes from western Asia are considered, the resulting network (fig. 4B) does not show such a star-like shape, and a much earlier TMRCA of 11.5 ky (95% CI 6.8–17.0; fig. 1) is obtained. These results open the possibility of recognizing time windows for 1) population movements from the E-M78 homeland in northeastern Africa to Eurasia and 2) population movements from western Asia into Europe and later within Europe.

The low E-V13 frequency (0.9%) and microsatellite variance (0.13) in northern Africa do not support an antiquity greater than in western Asia. Thus, the most parsimonious and plausible scenario is that E-V13 originated in western Asia about 11 ky ago, and its presence in northern Africa is the result of a more recent introgression. Under this hypothesis, E-V13 chromosomes sampled in western Asia and their coalescence estimate detect a likely Paleo-

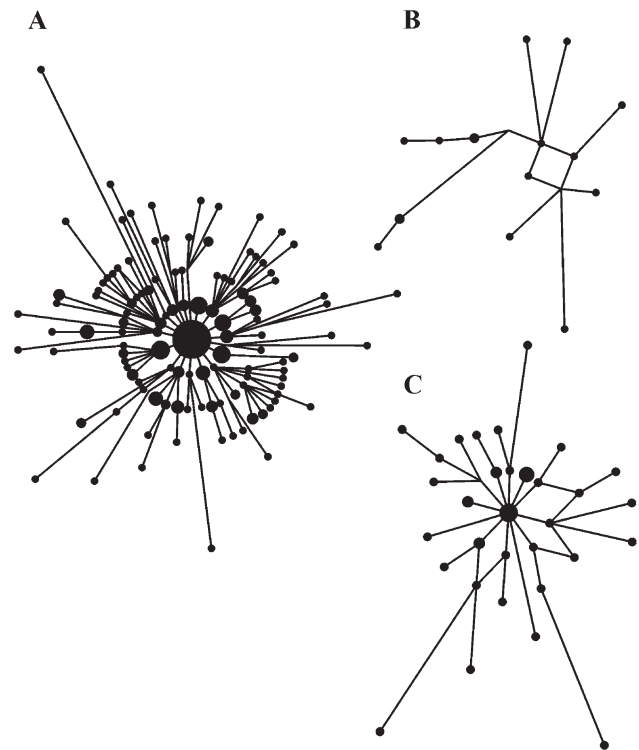


Fig. 4.—Microsatellite networks of haplogroups E-V13 in Europe (A), E-V13 in western Asia (B), and J-M12 in Europe (C).

lithic exit out of Africa of E-M78 chromosomes devoid of the V13 mutation, which later occurred somewhere in the Near East/Anatolia. The refinement of location for the source area of such movements and associated chronologies here attained may be relevant to controversies on the spread of cultures (and languages) between Africa and Asia in the corresponding time frames (Bellwood 2004; Ehret et al. 2004, and references therein).

As to a western Asia–Europe connection, our data suggest that western Asians carrying E-V13 may have reached the Balkans anytime after 17.0 ky ago, but expanded into Europe not earlier than 5.3 ky ago. Accordingly, the allele frequency peak is located in Europe, whereas the distribution of microsatellite allele variance shows a maximum in western Asia (fig. 5). Based on previously published data (Scozzari et al. 2001; Di Giacomo et al. 2004; Semino et al. 2004; Marjanovic et al. 2005), we observed that another haplogroup, J-M12, shows a frequency distribution within Europe similar to that observed for E-V13. In order to evaluate whether the present distribution of these 2 haplogroups can be the consequence of the same expansion/dispersal microevolutionary event, we first compared the 2 frequency distributions in Europe (J-M12 frequencies obtained from both published and new data; supplementary table 2, Supplementary Material online). We observed a high and statistically significant correspondence between the frequencies of the 2 haplogroups ($r = 0.84$, 95% CI 0.70–0.92). A similar result ($r = 0.85$, 95% CI 0.70–0.93) was obtained when the series was enlarged with the J-M12 data from Bosnia, Croatia, and Serbia (Marjanovic et al. 2005) matched with the frequencies of E-M78 cluster α (Peričić et al. 2005) as a proxy for haplogroup E-V13

(Cruciani et al. 2006). We then constructed a microsatellite network of 43 European J-M12 chromosomes (supplementary table 3, Supplementary Material online) and found a clear star-like structure (fig. 4C), a further feature shared with E-V13. This similarity was mirrored by a unimodal distribution of haplotype pairwise differences for both haplogroups (not shown). Finally, we used tetranucleotide microsatellite data in order to obtain a coalescence estimate for the J-M12 haplogroup in Europe. By taking into consideration 2 different demographic expansion models (see Subjects and Methods), we obtained TMRCA estimates very close to those of E-V13, that is, 4.1 ky (95% CI 2.8–5.4 ky) and 4.7 ky (95% CI 3.3–6.4 ky), respectively. Thus, the congruence between frequency distributions, shape of the networks, pairwise haplotypic differences, and coalescent estimates points to a single evolutionary event at the basis of the distribution of haplogroups E-V13 and J-M12 within Europe, a finding never appreciated before. These 2 haplogroups account for more than one-fourth of the chromosomes currently found in the southern Balkans, underlining the strong demographic impact of the expansion in the area.

Either environmental or cultural transitions are usually considered to be at the basis of dramatic changes of the size of human populations (Jobling et al. 2004). At least 4 major demographic events have been envisioned for this geographic area, that is, the post-Last Glacial Maximum expansion (about 20 kya) (Taberlet et al. 1998; Hewitt 2000), the Younger Dryas–Holocene reexpansion (about 12 kya), the population growth associated with the introduction of agricultural practices (about 8 kya) (Ammerman and Cavalli-Sforza, 1984), and the development of Bronze technology (about 5 kya) (Childe 1957; Piggott 1965; Renfrew 1979; Kristiansen 1998). Though large, the CI for the coalescence of both haplogroups E-V13 and J-M12 in Europe exclude the expansions following the Last Glacial Maximum or the Younger Dryas. Our estimated coalescence age of about 4.5 ky for haplogroups E-V13 and J-M12 in Europe (and their CIs) would also exclude a demographic expansion associated with the introduction of agriculture from Anatolia and would place this event at the beginning of the Balkan Bronze Age, a period that saw strong demographic changes as clearly testified from archeological records (Childe 1957; Piggott 1965; Kristiansen 1998). The arrangement of E-V13 (fig. 2D) and J-M12 (not shown) frequency surfaces appears to fit the expectations for a range expansion in an already populated territory (Klopfstein et al. 2006). Moreover, similarly to the results reported by Peričić et al. (2005) for E-M78 network α , the dispersion of E-V13 and J-M12 haplogroups seems to have mainly followed the river waterways connecting the southern Balkans to north-central Europe, a route that had already hastened by a factor 4–6 the spread of the Neolithic to the rest of the continent (Tringham 2000; Davison et al. 2006). This axis also served as a major route for the following millennia, enabling cultural and material (and possibly genetic) exchanges to and from central Europe (Childe 1957; Piggott 1965; Kristiansen 1998). Thus, the present work discloses a further level of complexity in the interpretation of the genetic landscape of southeastern Europe, this being to a large extent the consequence of a recent population increase in situ rather than the result of a

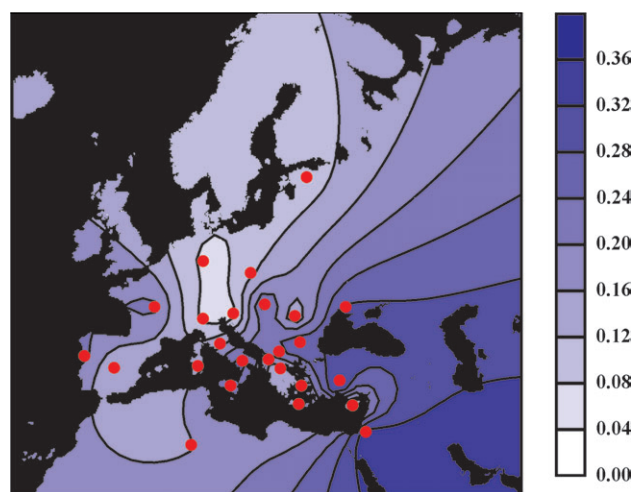


Fig. 5.—Mean variance spatial distribution of the Y-chromosome haplogroup E-V13 after pooling data from locations with <3 observations (see Subjects and Methods).

mere flow of western Asian migrants in the early Neolithic. Indeed, Y-chromosomal data from regions to the north (Kasperavičiūtė et al. 2004), northwest (Luca et al. 2007), and west (Di Giacomo et al. 2004) to the Balkans show signatures of demographic events that match archeologically documented changes in the population size in the 1st millennium BC.

Concluding Remarks

The buildup of the present day male-specific Y-chromosome (MSY) diversity can be viewed as an increase of complexity due to the repeated addition of new variation to the preexisting background by 2 main mechanisms: immigration of differentiated MSY copies from outer regions and accumulation of novel MSY variants generated by new mutations in loco. The question is whether a DNA polymorphism, which is able to mark a specific episode indeed exists and is known. Recently, Sengupta et al. (2006) pointed out that combining high resolved phylogenetic hierarchy, haplogroup internal diversification, geography, and expansion time estimates can lead to the appropriate diachronic partition of the MSY pool. The DNA content of the MSY ensures that abundant diversity exists to proceed a long way in this process of phylogeographic refinement eventually leading to a level of resolution for human history comparable with, or even greater, than that achieved by mitochondrial DNA (Torrioni et al. 2006).

Supplementary Material

Supplementary figure 1 and tables 1–3 are available at *Molecular Biology and Evolution* online (<http://www.mbe.oxfordjournals.org/>).

Acknowledgments

We are grateful to all the donors for providing DNA samples and to the people that contributed to the sample

collection. In particular, we thank João Lavinha (for the Portuguese samples); Farha El Chennawi, Anne Cambon-Thomsen, M.S. Issad, Eric Crubézy, Abdellatif Baali, Mohammed Cherkaoui, and Mohammed Melhaoui for their help in the collection of the Moroccan, Algerian, and Egyptian Berbers samples; and the National Laboratory for the Genetics of Israeli Populations. The useful comments and suggestions of 2 anonymous reviewers are gratefully acknowledged. This research received support from Grandi Progetti Ateneo, Sapienza Università di Roma (to R.S.), and the Italian Ministry of the University (Progetti di Ricerca di Interesse Nazionale 2005 to R.S. and Fondo Integrativo Speciale Ricerca 1999 to G.B. and R.S.). Russian samples were collected in the frame of an Italian-Russian scientific-technological project (3.RB3). The sampling of the Berbers was made within the framework of the Inserm ((Réseau Nord/Sud)) N°490NS1 (Mozabite Berbers), “The Origin of Man, Language and Languages”, EURO-CORES Programme and benefited from funding by the Région Midi-Pyrénées (Toulouse, France), the CNRS, and the E.C. Sixth Framework Programme under Contract ERASCT-2003-980409.

Literature Cited

- Alonso S, Flores C, Cabrera V, Alonso A, Martín P, Albarrán C, Izagirre N, de la Rúa C, García O. 2005. The place of the Basques in the European Y-chromosome diversity landscape. *Eur J Hum Genet.* 13:1293–1302.
- Ammerman AJ, Cavalli-Sforza LL. 1984. *The neolithic transition and the genetics of populations in Europe.* Princeton: Princeton University Press.
- 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 H-J, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol.* 16:37–48.
- Bandelt H-J, Forster P, Sykes BC, Richards MB. 1995. Mitochondrial portraits of human populations using median networks. *Genetics.* 141:743–753.
- Behar DM, Garrigan D, Kaplan ME, Mobasher Z, Rosengarten D, Karafet TM, Quintana-Murci L, Ostrer H, Skorecki K, Hammer MF. 2004. Contrasting patterns of Y chromosome variation in Ashkenazi Jewish and host non-Jewish European populations. *Hum Genet.* 114:354–365.
- Bellwood P. 2004. The origins of Afroasiatic. *Science* 306:1681.
- 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.
- Bosch E, Calafell F, González-Neira A, et al. (13 co-authors). 2006. Paternal and maternal lineages in the Balkans show a homogeneous landscape over linguistic barriers, except for the isolated Aromuns. *Ann Hum Genet.* 70:459–487.
- Butler JM, Schoske R, Vallone PM, Kline MC, Redd AJ, Hammer MF. 2002. A novel multiplex for simultaneous amplification of 20 Y chromosome STR markers. *Forensic Sci Int.* 129:10–24.
- Cann HM, de Toma C, Cazes L, et al. (41 co-authors). 2002. A human genome diversity cell line panel. *Science.* 296:261–262.
- Childe VG. 1957. *The dawn of European civilization.* London: Routledge and Kegan Paul.
- Cinnioglu C, King R, Kivisild T, et al. (15 co-authors). 2004. Excavating Y-chromosome haplotype strata in Anatolia. *Hum Genet.* 114:127–148.
- Cressie NAC. 1991. *Statistics for spatial data.* New York: John Wiley and Sons Inc.
- Cruciani F, La Fratta R, Santolamazza P, et al. (19 co-authors). 2004. Phylogeographic analysis of haplogroup E3b (E-M215) Y chromosomes reveals multiple migratory events within and out of Africa. *Am J Hum Genet.* 74:1014–1022.
- Cruciani F, La Fratta R, Torroni A, Underhill PA, Scozzari R. 2006. Molecular dissection of the Y chromosome haplogroup E-M78 (E3b1a): a posteriori evaluation of a microsatellite-network-based approach through six new biallelic markers. *Hum Mutat.* 27:831–832.
- Cruciani F, Santolamazza P, Shen P, et al. (16 co-authors). 2002. A back migration from Asia to sub-Saharan Africa is supported by high-resolution analysis of human Y-chromosome haplotypes. *Am J Hum Genet.* 70:1197–1214.
- Davison K, Dolukhanov P, Sarson GR, Shukurov A. 2006. The role of waterways in the spread of the Neolithic. *J Archaeol Sci.* 33:641–652.
- Di Giacomo F, Luca F, Popa LO, et al. (27 co-authors). 2004. Y chromosomal haplogroup J as a signature of the post-neolithic colonization of Europe. *Hum Genet.* 115:357–371.
- Ehret C, Keita SOY, Newman P. 2004. The origins of Afroasiatic. *Science.* 306:1680–1681.
- Excoffier L, Laval G, Schneider S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol Bioinform Online.* 1:47–50.
- Flores C, Maca-Meyer N, González AM, Oefner PJ, Shen P, Pérez 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, Larruga JM, Cabrera VM, Karadsheh N, González AM. 2005. Isolates in a corridor of migrations: a high-resolution analysis of Y-chromosome variation in Jordan. *J Hum Genet.* 50:435–441.
- Forster P, Harding R, Torroni A, Bandelt H-J. 1996. Origin and evolution of Native American mtDNA variation: a reappraisal. *Am J Hum Genet.* 59:935–945.
- Goldstein DB, Ruiz Linares A, Cavalli-Sforza LL, Feldman MW. 1995. Genetic absolute dating based on microsatellites and the origin of modern humans. *Proc Natl Acad Sci USA.* 92:6723–6727.
- Gonçalves 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 Açores record elements of Sephardim and Berber ancestry. *Ann Hum Genet.* 69:443–454.
- Gusmão L, Sánchez-Diz P, Calafell F, et al. (42 co-authors). 2005. Mutation rates at Y chromosome specific microsatellites. *Hum Mutat.* 26:520–528.
- Hammer MF, Blackmer F, Garrigan D, Nachman MW, Wilder JA. 2003. Human population structure and its effects on sampling Y chromosome sequence variation. *Genetics.* 164:1495–1509.
- Hewitt G. 2000. The genetic legacy of the Quaternary ice ages. *Nature.* 405:907–913.
- Jobling MA, Hurler ME, Tyler-Smith C. 2004. *Human evolutionary genetics.* New York: Garland Science.
- Jobling MA, Tyler-Smith C. 2003. The human Y chromosome: an evolutionary marker comes of age. *Nat Rev Genet.* 4:598–612.
- Kasperavičiūtė D, Kučinskas V, Stoneking M. 2004. Y chromosome and mitochondrial DNA variation in Lithuanians. *Ann Hum Genet.* 68:438–452.

- Kayser M, Brauer S, Cordaux R, et al. (15 co-authors). 2006. Melanesian and Asian origins of Polynesians: mtDNA and Y chromosome gradients across the Pacific. *Mol Biol Evol.* 23:2234–2244.
- Klopfstein S, Currat M, Excoffier L. 2006. The fate of mutations surfing on the wave of a range expansion. *Mol Biol Evol.* 23:482–490.
- Krings M, Salem AEH, Bauer K, et al. (13 co-authors). 1999. mtDNA analysis of Nile River Valley populations: a genetic corridor or a barrier to migration? *Am J Hum Genet.* 64:1166–1176.
- Kristiansen K. 1998. *Europe before history*. Cambridge: Cambridge University Press.
- Luca F, Di Giacomo F, Benincasa T, Popa LO, Banyko J, Kracmarova A, Malaspina P, Novelletto A, Brdicka R. 2007. Y-chromosomal variation in the Czech Republic. *Am J Phys Anthropol.* 132:132–139.
- Luis JR, Rowold DJ, Regueiro M, Caeiro B, Cinnioglu C, Roseman C, Underhill PA, Cavalli-Sforza LL, Herrera RJ. 2004. The Levant versus the Horn of Africa: evidence for bidirectional corridors of human migrations. *Am J Hum Genet.* 74:532–544.
- Malaspina P, Cruciani F, Ciminelli BM, et al. (24 co-authors). 1998. Network analyses of Y-chromosomal types in Europe, northern Africa, and western Asia reveal specific patterns of geographic distribution. *Am J Hum Genet.* 63:847–860.
- Malaspina P, Cruciani F, Santolamazza P, et al. (24 co-authors). 2000. Patterns of male-specific inter-population divergence in Europe, West Asia and North Africa. *Ann Hum Genet.* 64:395–412.
- Marjanovic D, Fornarino S, Montagna S, et al. (14 co-authors). 2005. The peopling of modern Bosnia-Herzegovina: Y-chromosome haplogroups in the three main ethnic groups. *Ann Hum Genet.* 69:757–763.
- Mohyuddin A, Ayub Q, Underhill PA, Tyler-Smith C, Mehdi SQ. 2006. Detection of novel Y SNPs provides further insights into Y chromosomal variation in Pakistan. *J Hum Genet.* 51:375–378.
- Olivieri A, Achilli A, Pala M, et al. (15 co-authors). 2006. The mtDNA legacy of the Levantine early Upper Palaeolithic in Africa. *Science.* 314:1767–1770.
- Peričić M, Barac Lauc L, Martinović Klarić I, et al. (18 co-authors). 2005. High-resolution phylogenetic analysis of southeastern Europe traces major episodes of paternal gene flow among Slavic populations. *Mol Biol Evol.* 22:1964–1975.
- Piggott S. 1965. *Ancient Europe from the beginnings of agriculture to classical antiquity*. Edinburgh, UK: Edinburgh University Press.
- Regueiro M, Cadenas AM, Gayden T, Underhill PA, Herrera RJ. 2006. Iran: tricontinental nexus for Y-chromosome driven migration. *Hum Hered.* 61:132–143.
- Renfrew C. 1979. *Before civilization. The radiocarbon revolution and prehistoric Europe*. Cambridge: Cambridge University Press.
- Rootsi S, Magri C, Kivisild T, et al. (45 co-authors). 2004. Phylogeography of Y-chromosome haplogroup I reveals distinct domains of prehistoric gene flow in Europe. *Am J Hum Genet.* 75:128–137.
- Saillard J, Forster P, Lynnerup N, Bandelt H-J, Nørby S. 2000. mtDNA variation among Greenland Eskimos: the edge of the Beringian expansion. *Am J Hum Genet.* 67:718–726.
- Sanchez JJ, Hallenberg C, Børsting C, Hernandez A, Morling N. 2005. High frequencies of Y chromosome lineages characterized by E3b1, DYS19-11, DYS392-12 in Somali males. *Eur J Hum Genet.* 13:856–866.
- Scozzari R, Cruciani F, Pangrazio A, et al. (17 co-authors). 2001. Human Y-chromosome variation in the western Mediterranean area: implications for the peopling of the region. *Hum Immunol.* 62:871–884.
- Scozzari R, Cruciani F, Santolamazza P, et al. (17 co-authors). 1999. Combined use of biallelic and microsatellite Y-chromosome polymorphisms to infer affinities among African populations. *Am J Hum Genet.* 65:829–846.
- Semino O, Magri C, Benuzzi G, et al. (16 co-authors). 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.* 74:1023–1034.
- Semino O, Santachiara-Benerecetti AS, Falaschi F, Cavalli-Sforza LL, Underhill PA. 2002. Ethiopians and Khoisan share the deepest clades of the human Y-chromosome phylogeny. *Am J Hum Genet.* 70:265–268.
- Sengupta S, Zhivotovsky LA, King R, et al. (15 co-authors). 2006. 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.* 78:202–221.
- Shen P, Lavi T, Kivisild T, et al. (11 co-authors). 2004. Reconstruction of patrilineages and matrilineages of Samaritans and other Israeli populations from Y-chromosome and mitochondrial DNA sequence variation. *Hum Mutat.* 24:248–260.
- Shen P, Wang F, Underhill PA, et al. (13 co-authors). 2000. Population genetic implications from sequence variation in four Y chromosome genes. *Proc Natl Acad Sci USA.* 97:7354–7359.
- Sims LM, Garvey D, Ballantyne J. 2007. Sub-populations within the major European and African derived haplogroups R1b3 and E3a are differentiated by previously phylogenetically undefined Y-SNPs. *Hum Mutat.* 28:97.
- Taberlet P, Fumagalli L, Wust-Saucy A-G, Cosson J-F. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Mol Ecol.* 7:453–464.
- The Y Chromosome Consortium. 2002. A nomenclature system for the tree of human Y-chromosomal binary haplogroups. *Genome Res.* 12:339–348.
- Thomas MG, Skorecki K, Ben-Ami H, Parfitt T, Bradman N, Goldstein DB. 1998. Origins of Old Testament priests. *Nature.* 394:138–140.
- Torroni A, Achilli A, Macaulay V, Richards M, Bandelt H-J. 2006. Harvesting the fruit of the human mtDNA tree. *Trends Genet.* 22:339–345.
- Tringham R. 2000. Southeastern Europe in the transition to agriculture in Europe: bridge, buffer, or mosaic. In: Price TD, editor. *Europe's first farmers*. Cambridge: Cambridge University Press. p. 19–56.
- Underhill PA, Jin L, Lin AA, Mehdi SQ, Jenkins T, Vollrath D, Davis RW, Cavalli-Sforza LL, Oefner PJ. 1997. Detection of numerous Y chromosome biallelic polymorphisms by denaturing high-performance liquid chromatography. *Genome Res.* 7:996–1005.
- Underhill PA, Passarino G, Lin AA, Shen P, Mirazón Lahr M, Foley RA, Oefner PJ, Cavalli-Sforza LL. 2001. The phylogeography of Y chromosome binary haplotypes and the origins of modern human populations. *Ann Hum Genet.* 65:43–62.
- Underhill PA, Shen P, Lin AA, et al. (21 co-authors). 2000. Y chromosome sequence variation and the history of human populations. *Nat Genet.* 26:358–361.
- Wells RS, Yuldashva N, Ruzibakiev R, et al. (27 co-authors). 2001. The Eurasian heartland: a continental perspective on Y-chromosome diversity. *Proc Natl Acad Sci USA.* 98:10244–10249.
- Wilder JA, Kingan SB, Mobasher Z, Pilkington MM, Hammer MF. 2004. Global patterns of human mitochondrial DNA and Y-chromosome structure are not influenced by higher migration rates of females versus males. *Nat Genet.* 36:1122–1125.

- Wood ET, Stover DA, Ehret C, et al. (11 co-authors). 2005. Contrasting patterns of Y chromosome and mtDNA variation in Africa: evidence for sex-biased demographic processes. *Eur J Hum Genet.* 13:867–876.
- Zhivotovsky LA, Underhill PA, Cinniöglu C, et al. (17 co-authors). 2004. The effective mutation rate at Y chromosome short tandem repeats, with application to human population-divergence time. *Am J Hum Genet.* 74:50–61.
- Zhivotovsky LA, Underhill PA, Feldman MW. 2006. Difference between evolutionarily effective and germ-line mutation rate due to stochastically varying haplogroup size. *Mol Biol Evol.* 23:2268–2270.

Sarah Tishkoff, Associate Editor

Accepted March 4, 2007