

A Signal, from Human mtDNA, of Postglacial Recolonization in Europe

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Mitochondrial HVS-I sequences from 10,365 subjects belonging to 56 populations/geographical regions of western Eurasia and northern Africa were first surveyed for the presence of the T→C transition at nucleotide position 16298, a mutation which has previously been shown to characterize haplogroup V mtDNAs. All mtDNAs with this mutation were then screened for a number of diagnostic RFLP sites, revealing two major subsets of mtDNAs. One is haplogroup V proper, and the other has been termed "pre*V," since it predates V phylogenetically. The rather uncommon pre*V tends to be scattered throughout Europe (and northwestern Africa), whereas V attains two peaks of frequency: one situated in southwestern Europe and one in the Saami of northern Scandinavia. Geographical distributions and ages support the scenario that pre*V originated in Europe before the Last Glacial Maximum (LGM), whereas the more recently derived haplogroup V arose in a southwestern European refugium soon after the LGM. The arrival of V in eastern/central Europe, however, occurred much later, possibly with (post-)Neolithic contacts. The distribution of haplogroup V mtDNAs in modern European populations would thus, at least in part, reflect the pattern of postglacial human recolonization from that refugium, affecting even the Saami. Overall, the present study shows that the dissection of mtDNA variation into small and well-defined evolutionary units is an essential step in the identification of spatial frequency patterns. Mass screening of a few markers identified using complete mtDNA sequences promises to be an efficient strategy for inferring features of human prehistory.

Introduction

In the distant past, the human species was undoubtedly subject to ecological constraints similar to those of other species (Willis and Whittaker 2000). Humans, though highly adaptable to different environments, could not (for example) have lived on the vast glaciers that covered most of northern Europe during the last glaciation.

Northern Europe was gradually recolonized from refugia after the Last Glacial Maximum (LGM), ~20,000 years ago (Housley et al. 1997). The two major refugia were in southwestern France/Cantabria (Atlantic and western Mediterranean zone) and Ukraine/Central Russian Plain (Periglacial zone), but other minor refugia could have existed in between (Dolukhanov 2000). The way in which the western European refugia were interconnected after the LGM and fueled different expansion routes towards the north is, however, rather unclear. The timing of and interaction with the recolonization coming from the eastern refugia also require examination.

It is possible that the wealth of new genetic data from present-day European populations can shed some light on these questions. In particular, Semino et al. (2000)

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have highlighted two Y-chromosome markers, mutations M170 and M17, that may have expanded, after the LGM, from (southern) central and eastern European refugia in the northern Balkans and Ukraine, respectively. A distribution compatible with an expansion from an eastern European refugium after the LGM has also been observed, for another Y-chromosome mutation (SRY₁₀₈₃₁A), by Malaspina et al. (2000). In an earlier study (Torrioni et al. 1998), it was suggested that the geographical distribution of a mtDNA marker, haplogroup V, resembles that of the second principal component of nuclear markers (Cavalli-Sforza et al. 1994) and testifies to the recolonization from western European refugia (see, however, Izagirre and de la Rúa [1999] and Simoni et al. [2000]). In the meantime, more mtDNA data (e.g., Richards et al. 2000) and additional mtDNA coding-region information (Macaulay et al. 1999) have become available, so that the variation of haplogroup V can be accurately dissected, and the geographical extent and timing of the western recolonization can now be reconsidered with more precision.

Subjects and Methods

Ethnic affiliations/geographical origins of the 10,365 subjects from 56 populations (fig. 1), whose HVS-I sequences were surveyed for the presence of 16298C, are listed in table 1. The large majority of these sequences encompassed nucleotide positions (np) 16050–16370,

and, at minimum, they included np 16090–16365. Sequences harboring 16298C together with control-region motifs of haplogroups other than V—for example, C, T, U5, and Z (Torrioni et al. 1993, 1996; Macaulay et al. 1999; Schurr et al. 1999)—were excluded. With the exception of 529 sequences that were extracted from the literature (table 1), all samples were collected with appropriate informed consent and were available for additional DNA typing. In addition to the HVS-I sequencing, ~65% of these 9,836 samples have also been screened for haplogroup-diagnostic RFLP markers. In general, the remainder were not screened because they harbored an HVS-I motif that allowed them to be assigned unequivocally to a specific haplogroup (e.g., 16069T-16126C signals haplogroup J). The RFLP analysis described below was also performed on mtDNAs that lacked 16298C but harbored 16256T, as suggested by the observation of this HVS-I motif in one Basque mtDNA belonging to V (–4577 *Nla*III). This observation raises the possibility that some other small subset of V mtDNAs lacking 16298C may have been overlooked in our screening procedure.

RFLP Screening

Candidate V mtDNAs underwent additional RFLP testing. As a technical prerequisite to combining the data and DNA samples acquired in the course of numerous years by a rather large number of different European laboratories, candidate V mtDNAs were screened for the

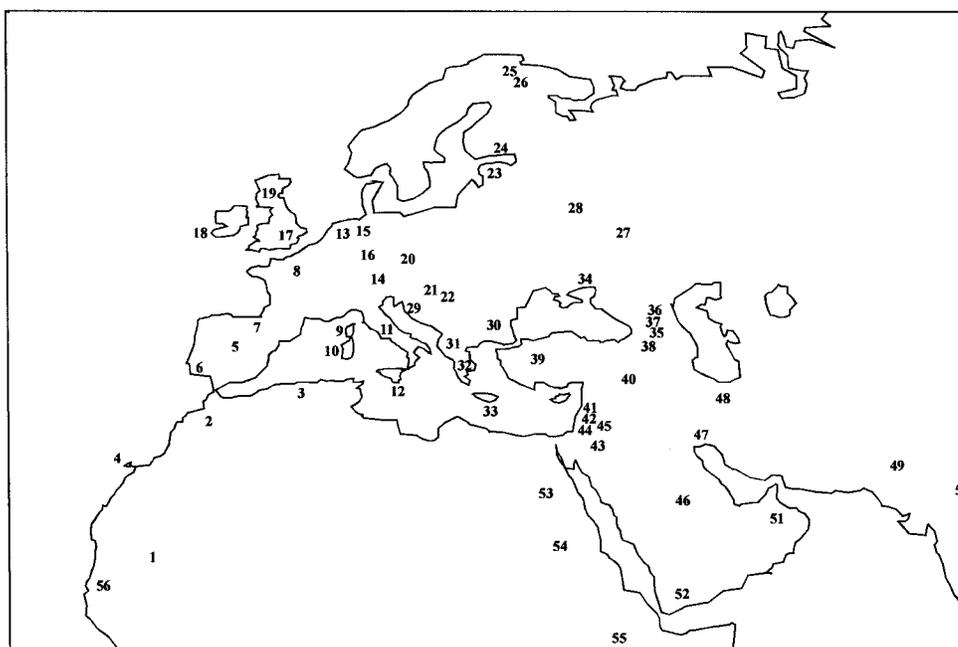


Figure 1 Geographical locations of the 56 populations included in the present study. The correspondence between numerical codes and populations is given in table 1.

Table 1**Distribution of V and Pre*V mtDNAs**

ID NUMBER AND REGION/POPULATION (CODE)	SAMPLE SIZE	V		PRE*V	
		No. in Sample	Estimated Frequency ^a (%)	No. in Sample	Estimated Frequency ^a (%)
1. Mauritania (MAU)	30	0	0 (0–9.2)	1	3.3 (.8–16.7)
2. Morocco (MOR)	92	1	1.1 (.3–5.8)	4	4.3 (1.8–10.6)
3. Algeria (ALG)	49	3	6.1 (2.2–16.5)	1	2.0 (.5–10.6)
4. Canary Islands (CAN)	300	6	2.0 (.9–4.3)	0	0 (0–1.0)
5. Spain (SPA)	293	6	2.0 (1.0–4.4)	8	2.7 (1.4–5.3)
6. Portugal (POR)	54	2	3.7 (1.1–12.5)	0	0 (0–5.3)
7. Basque Country (BSQ)	97	12	12.4 (7.3–20.4)	0	0 (0–3.0)
8. France (FRA)	56	4	7.1 (2.9–17.0)	0	0 (0–5.1)
9. Corsica (COR)	56	0	0 (0–5.1)	0	0 (0–5.1)
10. North Sardinia (SAR)	133	8	6.0 (3.1–11.4)	1	.8 (.2–4.1)
11. Continental Italy (ITA)	240	4	1.7 (.7–4.2)	2	.8 (.3–3.0)
12. Sicily (SIC)	634	14	2.2 (1.3–3.7)	16	2.5 (1.6–4.1)
13. Netherlands (NET)	21	1	4.8 (1.1–22.8)	0	0 (0–12.7)
14. Bavaria (GER)	33	1	3.0 (.7–15.3)	1	3.0 (.7–15.3)
15. Germany-Lower Saxony (GER)	632	17	2.7 (1.7–4.3)	9	1.4 (.8–2.7)
16. Germany-mixed (GER) ^b	157	7	4.5 (2.2–8.9)	1	.6 (.2–3.5)
17. England (ENG)	293	7	2.4 (1.2–4.8)	3	1.0 (.4–3.0)
18. Western Ireland (EIR)	88	5	5.7 (2.5–12.6)	1	1.1 (.3–6.1)
19. Scotland (SCO)	733	25	3.4 (2.3–5.0)	1	.1 (0–.8)
20. Czech Republic (CZE)	89	4	4.5 (1.8–11.0)	1	1.1 (.3–6.0)
21. Hungary (HUN)	194	6	3.1 (1.5–6.6)	0	0 (0–1.5)
22. Csángó (CSA)	68	0	0 (0–4.2)	3	4.4 (1.6–12.2)
23. Estonia (EST)	148	1	.7 (.2–3.7)	0	0 (0–2.0)
24. Finns (FIN)	236	6	2.5 (1.2–5.4)	0	0 (0–1.3)
25. Skolt Saami (SSA)	50	26	52.0 (38.5–65.2)	0	0 (0–5.7)
26. Inari Saami (ISA) ^c	127	9	7.1 (3.8–12.9)	0	0 (0–2.3)
27. Erza Mordvins (ERZ)	58	2	3.4 (1.1–11.7)	0	0 (0–5.0)
28. Russia (RUS)	144	5	3.5 (1.5–7.9)	2	1.4 (.4–4.9)
29. Croatian Islands (CRO)	447	25	5.6 (3.8–8.1)	5	1.1 (.5–2.6)
30. Bulgaria (BUL)	81	0	0 (0–3.6)	1	1.2 (.3–6.6)
31. Albania (ALB)	199	1	.5 (.1–2.8)	3	1.5 (.5–4.3)
32. Mainland Greece (GRE)	208	3	1.4 (.5–4.1)	1	.5 (.1–2.6)
33. Crete (CRE)	206	0	0 (0–1.4)	2	1.0 (.3–3.4)
34. Adygei (ADY)	50	0	0 (0–5.7)	0	0 (0–5.7)
35. Georgia (GEO)	137	1	.7 (.2–4.0)	0	0 (0–2.1)
36. North Ossetia (NOS)	106	0	0 (0–2.8)	4	3.8 (1.5–9.3)
37. South Ossetia (SOS)	201	0	0 (0–1.5)	0	0 (0–1.5)
38. Armenia (ARM)	192	0	0 (0–1.5)	0	0 (0–1.5)
39. Turkey (TUR) ^d	606	1	.2 (0–.9)	2	.3 (.1–1.2)
40. Kurds (KUR)	53	0	0 (0–5.4)	0	0 (0–5.4)
41. Lebanon (LEB)	171	0	0 (0–1.7)	0	0 (0–1.7)
42. Druze (DRU)	45	0	0 (0–6.3)	0	0 (0–6.3)
43. Jordan (JOR)	150	0	0 (0–2.0)	0	0 (0–2.0)
44. Palestinians (PAL)	117	0	0 (0–2.5)	0	0 (0–2.5)
45. Syria (SYR)	36	1	2.8 (.7–14.2)	0	0 (0–7.8)
46. Saudi Arabia (SAB)	194	0	0 (0–1.5)	0	0 (0–1.5)
47. Iraq (IRQ)	110	0	0 (0–2.7)	0	0 (0–2.7)
48. Iran (IRN)	91	0	0 (0–3.2)	0	0 (0–3.2)
49. Pakistan (PAK)	100	0	0 (0–2.9)	0	0 (0–2.9)
50. India (IND)	1,002	0	0 (0–.3)	0	0 (0–.3)
51. Oman/U.A. Emirates (OAE)	57	0	0 (0–5.0)	0	0 (0–5.0)
52. Yemen (YEM)	43	0	0 (0–6.6)	0	0 (0–6.6)
53. Egypt (EGY) ^e	68	0	0 (0–4.2)	0	0 (0–4.2)
54. Nubia (NUB) ^e	80	0	0 (0–3.6)	0	0 (0–3.6)
55. Ethiopia (ETH)	270	0	0 (0–1.1)	0	0 (0–1.1)
56. Senegal (SEN) ^f	240	0	0 (0–1.2)	0	0 (0–1.2)
Total	10,365	214		73	

^a The point estimate of frequency is the most probable value given a uniform prior. The most central 95% credible region that includes the most probable value is shown in parentheses.

^b Includes 67 sequences from Hofmann et al. (1997).

^c Inari Saami mtDNAs were analyzed only by RFLP; HVS-I sequences are not available.

^d Includes 74 sequences from Calafell et al. (1996) and Comas et al. (1996).

^e From Krings et al. (1999).

^f From Graven et al. (1995) and Rando et al. (1998).

presence/absence of the *MseI* site at np 16297, a site that must be absent in the samples harboring 16298C. This allowed the identification of a number of trivial mistakes in the identification codes of DNA samples and in the aliquoting of DNAs, as well as other misassignments (Bandelt et al., in press). This preliminary step was followed by screening of the following RFLP sites: *NlaIII* at np 4577, the absence of which characterizes haplogroup V (Torrioni et al. 1996); *MseI* at np 15904, the presence of which is typical of haplogroup V (Macaulay et al. 1999); *AluI* at np 7025 the absence of which distinguishes haplogroup H (Torrioni et al. 1994); and *MseI* at np 14766, the absence of which is shared by the sister haplogroups H and V and thus defines the larger haplogroup HV (Torrioni et al. 1998; Macaulay et al. 1999). The status at nps 72 and 73 in HVS-II was also checked in all samples, either through use of *SphI* (which cuts when 72C-73A is present) and *Alw44I* (which cuts when 73G is present) (Morelli et al. 2000) or by HVS-II sequencing. 72C-73A is characteristic of haplogroup V (Torrioni et al. 1998; Macaulay et al. 1999), whereas 72T-73A identifies haplogroup H (Torrioni et al. 1996; Wilkinson-Herbots et al. 1996). Four mtDNAs with 16298C in HVS-I (one Italian, one Irish, one German, and one Scot) were found to lack the diagnostic haplogroup V RFLP markers. Moreover, they also lacked the *AluI* site at np 7025 and showed 72T-73A in HVS-II; hence, they belonged to haplogroup H and were excluded.

Phylogenetic Analysis and Ages

A reduced median network (Bandelt et al. 1995) of the haplotypes (defined by their HVS-I sequence plus additional RFLP information) was manually constructed and checked by the program Network 2.0d (Shareware Phylogenetic Network Software Web site). To estimate the time to the most recent common ancestor (MRCA) of subsets of mtDNAs, we used the HVS-I sequence diversity accumulated on top of the assumed ancestral sequence type within the region covered by nps 16090–16365. We used a calibration of the rate of transitional mutations in this range ($\mu = 4.96 \times 10^{-5}$ transitions per year; Forster et al. 1996), in conjunction with the average number of transitions on the reconstructed phylogeny from the ancestral type to each sample (ρ) (Morral et al. 1994; Forster et al. 1996; Thomson et al. 2000), to obtain an unbiased estimate, T , of the time to the MRCA ($T = \rho/\mu$). To summarize the uncertainty in this point estimate, we followed the approach of Saillard et al. (2000). In brief, we first used information on the relative mutability of sites (Hasegawa et al. 1993), to remove all reticulation in the reduced median network. We estimated a (multifurcating) genealogy directly from the resolved phylogeny, assigning a length to each branch

that was proportional to the number of mutations that occurred on that branch. We then evaluated the variance, $(\Delta\rho)^2$, of ρ on this genealogy, over repeated runs of the mutation process. We use $\Delta T = \Delta\rho/\mu$ as the standard error of T (in the form $T \pm \Delta T$). An approximate 95% confidence interval is given by $T - 2\Delta T$ to $T + 2\Delta T$. This procedure does not take into account any uncertainty in the mutation rate or in the reconstructed genealogy. We applied this approach principally to clades in the phylogeny but also to subsets of mtDNAs that are not manifestly clades, under the hypothesis that each subset may be derived from a single founder and, hence, may represent clades in the genealogy. Intrapopulation diversities of haplogroup V were computed as $1 - \sum_i x_i^2$, where x_i is the relative frequency of the i th V haplotype (defined in the region covered by nps 16090–16365) within the set of V sequences from the respective population.

Frequency-Map Construction

Maps were obtained using Surfer, version 6 (Golden Software, Inc.), with the Kriging procedure (Delfiner 1976). A 20×12 grid was used, and estimates at each grid node were obtained by consideration of the entire data set. The advantages and disadvantages of this procedure are carefully discussed in section 1.14 of the book by Cavalli-Sforza et al. (1994).

Results

RFLP Screening

The survey of 10,365 HVS-I sequences identified 291 candidate V mtDNAs (with 16298C or 16256T). The RFLP screening revealed that they all belonged to haplogroup HV (–14766 *MseI*), but only 210 turned out to be regular members of haplogroup V in having –4577 *NlaIII* and +15904 *MseI* (15904T). Four mtDNAs (one Italian, one Irish, one German, and one Scot) showing +4577 *NlaIII*, –7025 *AluI*, and 72T-73A belonged to haplogroup H. Four Sardinian mtDNAs had –4577 *NlaIII* and –15904 *MseI*; however, sequence analysis of the region around np 15900 showed that they bore the mutation 15905C in addition to 15904T, so that the *MseI* site generated by the mutation at 15904 has experienced a subsequent loss because of the mutation at 15905. Thus, the total number of V mtDNAs is 214 (table 1). Results of HVS-II analyses showed that these mtDNAs generally harbored 72C-73A, but a number of exceptions were observed: 2 with 73G and 17 with 72T (fig. 2).

The remaining 73 mtDNAs all had +4577 *NlaIII* but were polymorphic at the other V site: 13 bore +15904 *MseI*, whereas 60 had –15904 *MseI* and thus were indistinguishable from the root of haplogroup HV when coding-region RFLP markers were used. This observa-

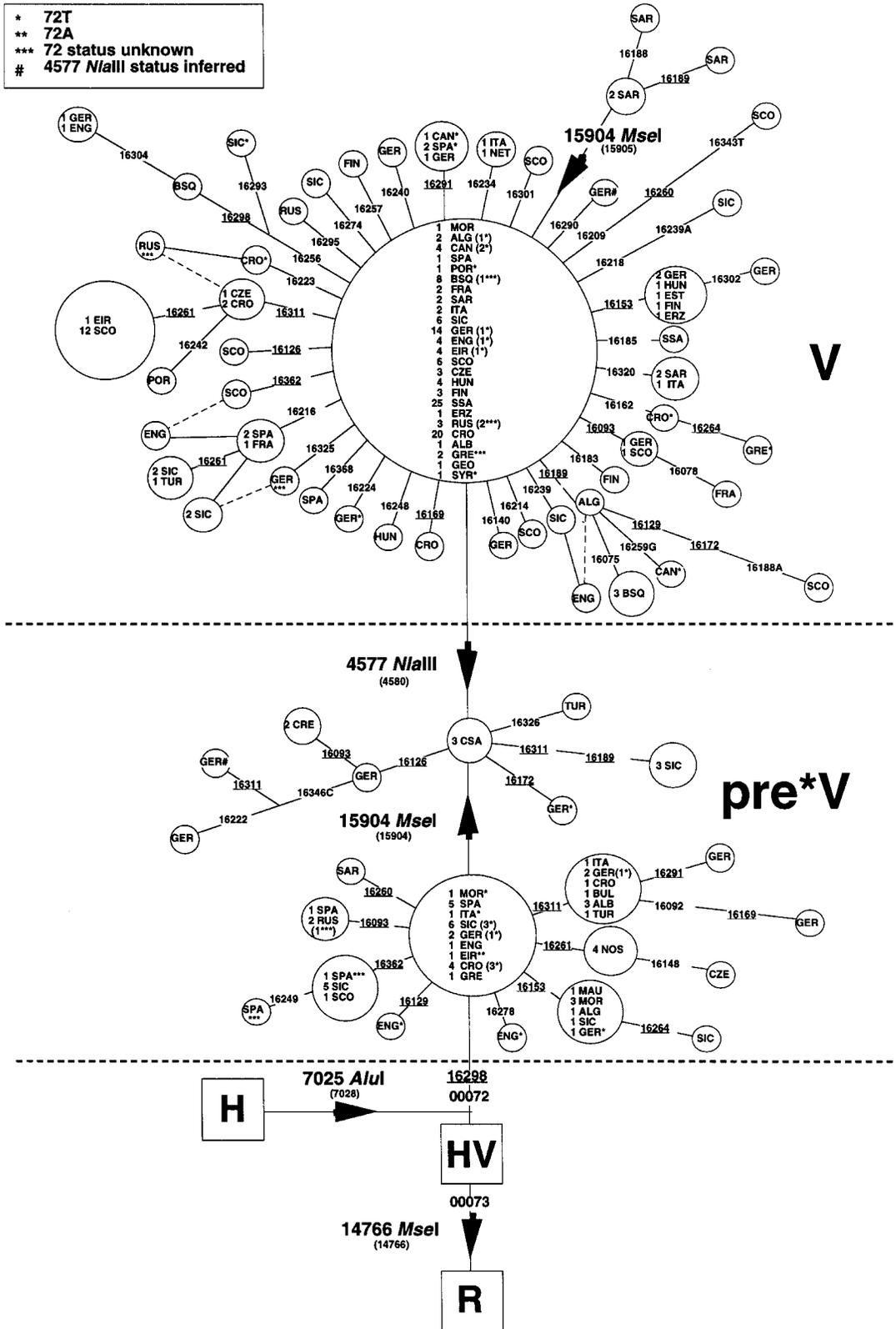


Figure 2 Network of pre*V and V mtDNAs. Areas of circles are proportional to the number of sampled individuals, and population codes correspond to those in table 1. The listed nucleotide positions are those differing from the Cambridge Reference Sequence (Anderson et al. 1981). Mutations are transitions (T→C, A→G), unless the base change is specified explicitly. Recurrent mutations are underlined, and arrows point to the presence of the restriction site. Squares indicate the phylogenetic position of the roots of the nested haplogroups H, HV, and R (Macaulay et al. 1999).

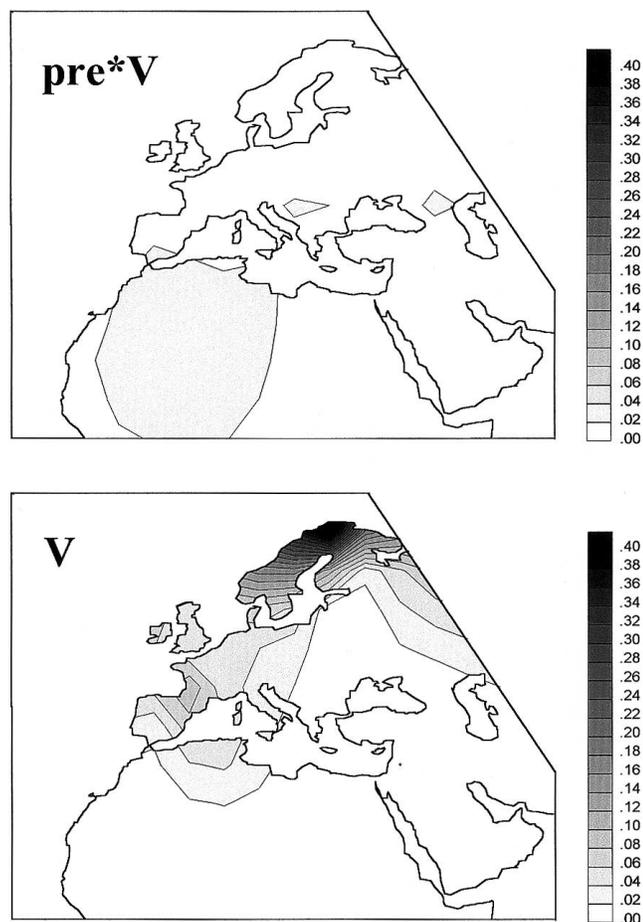


Figure 3 Spatial frequency distributions. Distributions of pre*V (top) and V (bottom) mtDNAs are shown.

tion reveals the temporal order of mutations leading to V (first, the gain of the 15904 *MseI* site; then, the loss of the 4577 *NlaIII* site), and identifies a slightly larger haplogroup, pre-V, which is solely defined by the two control-region mutations 16298C and 72C. Haplogroup pre-V encompasses the previously defined V plus additional mtDNAs which we refer to as “pre*V” (fig. 2). Pre*V as a paraphyletic grouping comprises at least two major subsets separated by 15904 *MseI*. Also, in the case of pre*V, considerable variation at np 72 was observed in 15 subjects characterized by 72T and in 1 subject with 72A (fig. 2). Full details of the HVS-I sequence and the 72-73 status of all pre-V mtDNAs are available at V.M.’s Web site.

It should be emphasized that published complete sequences of pre-V mtDNAs (Ingman et al. 2000; Finnilä et al. 2001) confirm that the four transitions at nps 72, 16298, 15904, and 4580 are the only mutations that separate the roots of V and HV. The ultimate level of

resolution has thus been reached for this part of the phylogeny.

Population Distribution

Table 1 reports the frequencies of pre*V and V in the 56 populations studied. Pre*V mtDNAs are generally rare and apparently scattered throughout Europe, although they are less uncommon in the Mediterranean area (including northwestern Africa). In contrast, V mtDNAs are more frequent and tend to be restricted to western, central, and northern Europe, with the highest population frequencies in the Skolt Saami (52%) and the Basques (12%). The difference in the frequency patterns of pre*V and V is strikingly clear in the geographical frequency maps (fig. 3).

Relatively recent founder events in specific populations, however, could have strongly affected the observed population frequencies. To address this issue, the diversity of V mtDNAs was measured in the six populations (Sicilians, Scots, Germans, Basques, Croatians, and Saami) in which >10 individual V mtDNAs were found (each population sample contributing >5% to the total V data set) (table 2). Diversity values <.5 were observed in the Saami (.074), the Croatians (.349), and the Basques (.486). The exclusion of the Saami from the frequency map had a major impact on the overall distribution of V, by completely erasing the frequency peak centered in Scandinavia (fig. 4A). The exclusion of the Croatians did not have any major impact (fig. 4B), and the exclusion of the Basques lowered, but did not erase, the frequency peak centered in southwestern Europe (fig. 4C).

Time Depths

Age estimates were calculated for the different subsets of pre-V. The two subsets of pre*V, with and without the *MseI* site at np 15904, gave age estimates of 26,400

Table 2

Diversity of Haplogroup V in Samples Contributing >5% to the Total Set of V Sequences

Sample ^a	Haplogroup V Diversity
SSA	.074
CRO	.349
BSQ	.486
GER	.666
SCO	.701
SIC	.755

^a Region/population codes for samples are defined in table 1.

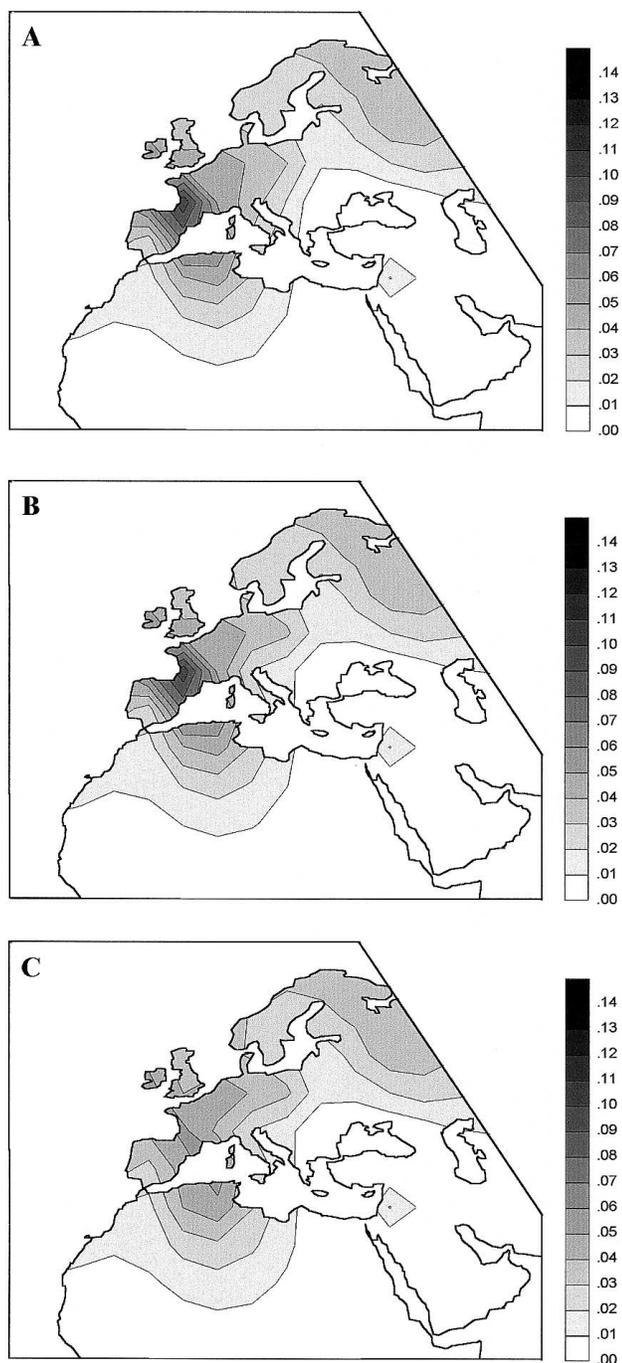


Figure 4 Spatial frequency distributions of V mtDNAs, excluding possible outlier populations. A, without Saami; B, without Saami and Croatians; C, without Saami, Croatians, and Basques.

and 14,800 years, respectively, when treated as potential clades, albeit with rather large standard errors (table 3).

For V, a somewhat younger time depth of $11,200 \pm 2,700$ years was estimated when all populations were included. This value, however, progressively increased when the potential outlier populations—first the Saami,

then the Croatians, and, finally, the Basques—were excluded, eventually reaching $14,600 \pm 3,600$ years. Moreover, there is a contrast in V diversities between west and east. We dissected the total sample along a NW-SE axis, so that northwest Africa, Iberia, France, Italy, the Netherlands, and the British Isles (a total of 98 V mtDNAs) were on the western side, and the rest of Europe (excluding the Saami because of their extreme V homogeneity), the Caucasus, and the Near East (a total of 81 V mtDNAs) were on the eastern side. The V age in the west is then $16,300 \pm 4,800$ years, and that in the east is $8,500 \pm 2,300$ years.

Discussion

The geographical distribution of haplogroup V is much more clear-cut than that of the pooled pre-V distribution considered in our earlier study (Torroni et al. 1998). Except for rare, isolated occurrences, V is virtually absent in the southern Balkans, Turkey, the Caucasus, and the Near East. Attested migrations from the north(west) into these regions in historic times are certainly sufficient to explain these few “erratics” (Richards et al. 2000, p. 1269). With regard to age and frequency, there is a clear cline from west to east (fig. 4); the age for V in the west ($\sim 16,000$ years) is almost twice that in the east, indicating the direction of settlement. We interpret this pattern in the following way: the older age reflects the onset of the recolonization of Europe from western refugia (Housley et al. 1997), whereas later founder events are responsible for the limited occurrences and reduced diversity of V mtDNAs in the east.

In contrast, the distribution of pre*V—that is, the non-V mtDNAs of the more ancient haplogroup, pre-V—suggests that these rather rare mtDNAs must have been present in more than one refuge area. The estimated age and the conspicuous, though scattered, occurrence of potential pre*V clades in the Mediterranean suggests that pre-V could have originated before the LGM (like its larger sister haplogroup H), perhaps in eastern Europe, whence it spread along an east-west axis with Gravettian contacts.

The present study demonstrates the potential of a phylogeographic approach based on the screening of markers that are now being identified from complete mtDNA sequences. A haplogroup-focused sequencing strategy (Finnilä et al. 2000, 2001) allows the systematic detection of numerous mutations that define sub-haplogroups (i.e., clades of the phylogeny), which may have interesting geographical distributions. In the same way that we have assayed several thousand samples for a small set of specific mutations (viz., those relevant for assessment of V and pre*V status), one can perform a mass screening of a few sites characteristic of other clades of the mtDNA phylogeny (Rich-

Table 3**Age Estimates of Different Subsets of Haplogroup Pre-V**

Set of Sequences Considered	No. of Subjects	ρ^a	$T (\pm \Delta T)^b$ (years)
Pre-V (all populations)	278	175/278 = .629	12,700 ($\pm 2,400$)
V (all populations)	205	114/205 = .556	11,200 ($\pm 2,700$)
V (without SSA)	179	113/179 = .631	12,700 ($\pm 3,100$)
V (without SSA and CRO)	154	108/154 = .701	14,200 ($\pm 3,400$)
V (without SSA, CRO, and BSQ)	142	103/142 = .725	14,600 ($\pm 3,600$)
V (western)	98	79/98 = .806	16,300 ($\pm 4,800$)
V (eastern, without Saami)	81	34/81 = .420	8,500 ($\pm 2,300$)
Pre*V (+15904MseI; all populations) ^c	13	17/13 = 1.308	26,400 ($\pm 11,100$)
Pre*V (-15904MseI; all populations) ^c	60	44/60 = .733	14,800 ($\pm 5,700$)

^a The average number of transitions between nps 16090 and 16365 from the (common HVS-I) ancestral sequence type, which differs from the CRS by only one transition (at np 16298).

^b Estimate of the time to the most recent common ancestor of each cluster, with standard errors calculated from an estimate of the genealogy, in the manner of Saillard et al. (2000). A mutation rate estimate of 1/20,180 transitions per year between nps 16090 and 16365 (Forster et al. 1996) was employed.

^c Age estimated under the assumption that the set of sequences, which do not manifestly form a clade in the phylogeny, nevertheless diverged from a single ancestor.

ards and Macaulay 2001). Inasmuch as there is evidence that $\geq 3/4$ of the present-day European genes are descended from indigenous Paleolithic ancestors (Richards et al. 2000; Semino et al. 2000), a full understanding of the genetic landscape in Europe requires the (partial) reconstruction and distinction of the gene pools in the various glacial refugia.

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Electronic-Database Information

The URLs for data in this article are as follows:

Author's Web site, <http://www.stats.ox.ac.uk/~macaulay/preV2001/index.html> (for HVS-I data)
Shareware Phylogenetic Network Software, <http://www.fluxus-engineering.com/sharenet.htm> (for Network 2.0d)

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