

## Human Mitochondrial DNA Types in Two Israeli Populations—A Comparative Study at the DNA Level

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### SUMMARY

Variations in human mtDNA restriction endonuclease fragment patterns were investigated in a sample number of 81 Israelis—Jews and Arabs—using total blood cell DNA. Eight new morphs were observed using five enzymes: *HpaI*, *BamHI*, *HaeII*, *MspI*, and *AvaII*. Of the 18 different combinations of fragment patterns (mtDNA types), only three were shared by both groups, but with striking frequency differences. The Arab sample disclosed “African” characteristics and was found to be slightly more polymorphic than the Israeli sample. One of the new types filled a “missing link” originally postulated in a phylogeny of mtDNA human types.

### INTRODUCTION

Extensive genetic data on the distribution of “classical” genetic markers such as blood groups, enzymes, and serum proteins, and including the immunoglobulins and the HLA systems, have been accumulated since the 1950s on different population groups in Israel [1–3].

Most of these data have been used in attempts to evaluate the ethnic affinities between the various Jewish communities and to identify the possible elements of a common Mediterranean origin for the diverse groups [4–6].

Among the more specific issues dealt with were the amount of admixture of the Jewish communities with local populations of the same geographical re-

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gions, as well as an evaluation of the extent of genetic heterogeneity within the separate communities.

In recent years, methods of distinguishing among individuals on the basis of differences in fragment patterns in mtDNA produced by restriction enzymes have been introduced to human population studies of mtDNA [7–10, 11]. Some of these studies have indicated the existence of significant differences in frequencies of mtDNA between racial groups and have also suggested that shared mtDNA polymorphisms may indicate group affinities [10] within major ethnic groups.

The purpose of this investigation is to introduce this new tool to human genetic studies of the diverse ethnic communities now gathered in Israel, and it is further hoped that polymorphisms in mtDNA will yield an independent measure of the degree of relatedness between different communities. In addition, it seemed of particular interest to compare mtDNA markers with conventional blood groups and with enzyme polymorphisms studied before, also considering that mtDNA is transmitted maternally [12].

#### MATERIALS AND METHODS

##### *Populations*

Two distinct groups constituted the population sample: 41 were Arabs picked at random from several villages in central and northern Israel and 40 were randomly picked Israeli-born Jews, comprising 35 of Ashkenazi origin, three Sephardi, two Yemenite, and one Iraqi.

##### *Blood Samples*

Blood samples of the Israeli Jews were collected in Stanford, California, and the buffy coats from samples of 20–30 ml whole blood collected in ACD from the Israeli Arabs were shipped to the Department of Genetics at Stanford University.

##### *DNA Isolation*

Total human DNA was extracted from the buffy coats, following the methods of Kan et al. [13], including an overnight dialysis against 10 mM Tris HCl (pH 7.5/1 mM EDTA following the first phenol extraction).

##### *Restriction Analysis*

Two micrograms of total DNA were used for each digestion. The conditions and buffers used were those recommended by the manufacturer. Samples were digested with a fivefold excess of enzyme for 5–6 hrs and then heated at 65°C for 10 min to inactivate the enzyme. A dye marker of bromocresol purple was added along with glycerol to a final concentration of 10% (v/v). The digested fragments were separated on horizontal agarose slab gels ranging from .8% to 1.8% depending on the expected size of the restriction fragments. One-kilobase (kb) ladder was used as a size marker.

Gels were run overnight at 1.5 V/cm; the fragments were then transferred (Southern 1975) to a Zetabind filter (AMF Cuno, Meriden, Conn.). Hybridization was in  $5 \times$  SSPE ( $1 \times$  SSPE = 0.18 M NaCl, 10 mM  $\text{NaH}_2\text{PO}_4$ , 1 mM  $\text{Na}_2$  EDTA, pH 7.0) at 42°C for 48 hrs using mtDNA purified from human tissue culture cells [12] as a probe, nick-translated to a specific activity of  $> 1 \times 10^8$  cpm/mg. Filters were washed for 2 hrs in  $2 \times$  SSPE 0.1% SDS, and fragments were visualized after overnight autoradiography.

## RESULTS

*Bam*HI

In 80 out of 81 individuals, the common cleavage pattern or morph 1 [8] has been observed. This morph has been described [10, 12] and corresponds to the published mtDNA sequence [14] with a single *Bam*HI restriction site at base pair (bp) 14,258. Morph 2, which has two fragments of approximately 14.4 and 2.1 kb, was found in only one Israeli of Sephardi Jewish origin. The two populations thus do not differ significantly from each other with respect to this enzyme or from previous human samples tested (see table 1).

*Hpa*I

Two different cleavage patterns were detected in the Israeli Jewish group when their DNA was digested with the *Hpa*I restriction endonuclease. These were originally described as morph 2 and morph 3 [7] and differ by the presence of one additional site in the 9.8-kb fragment, generating thereby four bands of 7.7, 4.3, 2.4, and 2.1 kb. This *Hpa*I-3 morph can be most likely compared with the 12 variant individuals reported recently by Cann et al. [9] in which the location of the variable site was specified to bp 3,592.

All five individuals with the *Hpa*I-3 morph in our sample belong to the Israeli Arabs and come from different locations in Israel. The Israeli Jews of diverse origins display the same single morph and are thus compatible with frequencies published for Caucasians (table 1).

*Hae*II

With the exception of two individuals (table 1), the Israeli Arab population is monomorphic for morph 1. In contrast, the Israeli Jewish group is polymorphic, having four different morphs as follows: 57 exhibit morph 1; 37 exhibit morph 2, and the rare morphs 3 and 4 are represented by one individual each. Those exhibiting morph 2 in this group are all of Ashkenazi origin in whom, if regarded separately, morph 2 is exhibited at a frequency of 44%. Additionally, the rare morph 4, found originally in a person from Taiwan [8], was encountered in a Yemenite Jewish woman. Seven different patterns have previously been described for this enzyme, with morph 1 being the most common in all populations investigated and frequencies varying between 76% and 100%.

In this sample, a new pattern, morph 8, was found in one Arab, generated by the loss of the 4.1-kb base and the appearance of 3.4-kb and 0.7-kb bands (see fig. 1). Double digestion with *Hae*II/*Sst*I revealed that the new *Hae*II site occurs at about 9,700. This new type can be added to the phylogeny of the seven earlier morphs (fig. 2 of Johnson et al. [8]) and can be derived, as can all other six, from morph 1 by loss of a single *Hae*II site. This gain of a new site can be postulated to be a transition from A→G at bp 9,689—a potential *Hae*II site.

TABLE 1

NO. INDIVIDUALS AND FREQUENCIES OF ALL MORPHS LISTED BY ENZYME FOR THE TWO ISRAELI GROUPS COMPARED WITH FREQUENCIES FOR THREE WORLD POPULATIONS [7, 8]

MORPH	ISRAELI JEWS (NO. = 40)		ISRAELI ARABS (NO. = 41)		CAUCASIANS (NO. = 50)	ORIENTAL (NO. = 46)	AFRICAN (BANTU) (NO. = 40)
	No.	%	No.	%	%	%	%
<i>Bam</i> HI - 1	39	97.5	41	100	86.0	100	100
- 2	1	2.5	0	0	8.0	0	0
- 3	0	0	0	0	6.0	0	0
<i>Hae</i> II - 1	23	57.5	39	95.1	76.0	80.4	97.5
- 2	15	37.5	1	2.4	14.0	13.0	2.5
- 3	1	2.5	0	0	6.0	0	0
- 4	1	2.5	0	0	0	2.2	0
- 8	0	0	1	2.4	0	0	0
<i>Hpa</i> I* - 2	38	100	35	87.5	98.1	81.3	25.0
- 3	0	0	5	12.5	0	0	70.8
<i>Ava</i> II* - 1	29	76.3	27	69.2	74.0	95.7	40.0
- 2	0	0	0	0	4.0	0	12.5
- 3	...	0	2	5.1	2.0	0	37.5
- 4	0	0	0	0	0	0	0
- 5	5	13.1	8	20.5	8.0	0	5
- 9	1	2.6	0	0	6.0	0	0
-12	0	0	1	2.6	0	0	0
-13	1	2.6	1	2.6	0	0	0
-14	1	2.6	0	0	0	0	0
-15	1	2.6	0	0	0	0	0
<i>Msp</i> I - 1	40	100	35	89.7	92.0	97.8	87.5
- 2	0	0	0	0	0	0	12.5
- 4	0	0	1	2.6	8.0	2.2	0
- 6	0	0	1	2.6	0	0	0
- 7	0	0	1	2.6	0	0	0
- 8	0	0	1	2.6	0	0	0

\* The morph was not identified in all individuals.

## MspI

Twenty-three *Msp*I sites are found in the published human mtDNA sequence [13], and all fragments above 0.3 kb can be resolved [8] by separation on 1.8% agarose gels. In the Israeli Jews, only one pattern was found—morph 1—the most common pattern encountered in all populations, except the Africans. However, three additional morphs that had not been observed previously were detected in four individuals belonging to the Arab-Israeli group (table 1).

One person exhibited morph 4, which occurs in about eight Caucasians and in 2.7 Orientals. This morph has a new fragment of approximately 2.7 kb, resulting from the fusion of the 2.21-kb and 0.523-kb bands due to a site loss.

A new pattern—*Msp*I morph 6 (fig. 2)—is due to a site loss at 8,112 bp that results in the fusion of two adjacent fragments into a fragment of 1.15 kb.

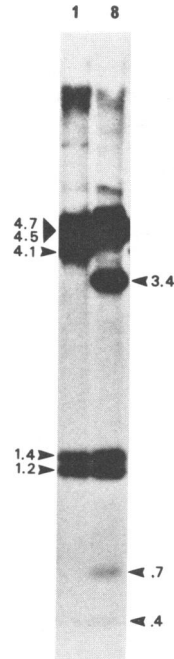


FIG. 1.—Autoradiogram of a new *HaeII* morph (no. 8) found in this study and compared with the common morph 1. Morphs are denoted by numbers at top of lanes; fragment sizes are designated in kilobases.

Morph 7 is the result of a site gain between bp 13,712 and 15,925. Double digestion with *Msp* and *Bam*HI indicates the presence of a new site at bp 15,510 causing the 2.2-kb fragment to be split into two new fragments of 1.8 + 0.4 kb. Several potential sites exist within 50 bp of this site—two would create an *Msp*I site by a transition of A→G, while a third potential site would require a transversion from C→G to create a new *Msp*I site.

The third new pattern, morph 8, is due to the loss of the 1.2-kb band and the appearance of another band of about 0.95 (fig. 2). Presumably, the 1.2-kb fragment is split into two new bands of 0.95 and 0.25 kb, although the latter is too small to resolve in this assay. Double digests with *Msp*I and *Hpa*I indicate that the new site occurs at  $\approx$  bp 13,070. A C→G transversion at bp 13,119 could explain this new *Mpa*I site. Another possibility is a G→A transition at bp 13,059; still, most likely is a C→A transversion at bp 13,100, a variable site that has been also reported by Cann et al. to occur in one individual [9]. A phylogeny of our *Msp*I morphs shows that all of them can be interconverted by single site changes from morph 1 (see fig. 3 of Johnson et al. [8]).

#### *Ava*II

This enzyme was found to be the most polymorphic in our samples, albeit with different distribution of “old” and “new” types.

From among the eight different morphs encountered in these populations,

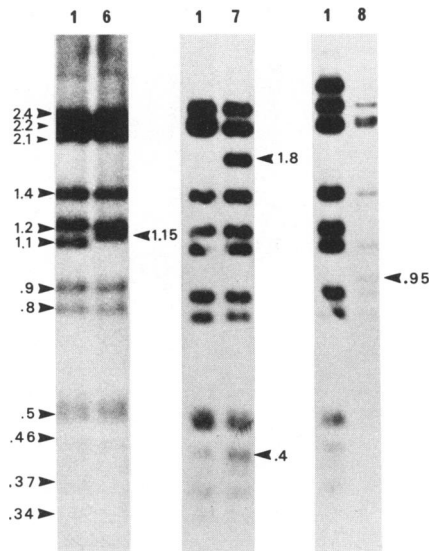


FIG. 2.—*MspI* autoradiogram depicting the three new morphs aligned individually against morph 1 for comparison (see text for details).

only three were shared by both. One of these, morph 1, was found to be most common with frequencies, respectively, of 76% and 69% (table 1). The second more common type shared is morph 5—with 13% occurrence in the Jewish group and 20% occurrence among the Arabs. All other morphs are rather rare, being represented by only one individual.

Morph 12 is characterized by a site loss (at bp 12,629) resulting in a band of 10.5 kb due to the fusion of the 9.8 and 0.738 fragments. Another band of 3.8 kb is due to the fusion of the 3.0 and 0.8 fragments, attributed to another site loss at bp 16,390. Interestingly, only this second loss in morph 12 has been encountered previously (see fig. 1 in Cann et al. [9]). Morph 13 is further derived from morph 12 by additional loss of a site (bp 13,367) causing fusion of the 10.5 band with that of 3.0, yielding 13.5-kb fragment (fig. 3). Both morphs originate from the central morph 1 (fig. 4 of Johnson et al. [8]). Morph 13 can be compared with that found in four individuals exhibiting the same variable site [9].

Unfortunately, there is no ethnic identification for "variant" individuals reported by Cann et al. to complete the comparison with our findings for the *HpaI*-3, *MspI*-8, and *AvaII*-12 and 13 morphs.

Morph 14 is formed by a new site gain where the 4.4-kb fragment of morph 5 is split into two fragments of 3.9 and 0.5 kb. The new site in morph 14 was found by double digestion of *AvaII* and *SstI* to occur at  $\approx$  bp 12,130. This morph can be derived from morph 5, described by Johnson et al. [8].

Morph 15 is also due to a site gain in the 9,853-bp fragment generating two new fragments of 8.4 and 1.5 kb (see fig. 3). Double digests with *SstI* and *AvaII*

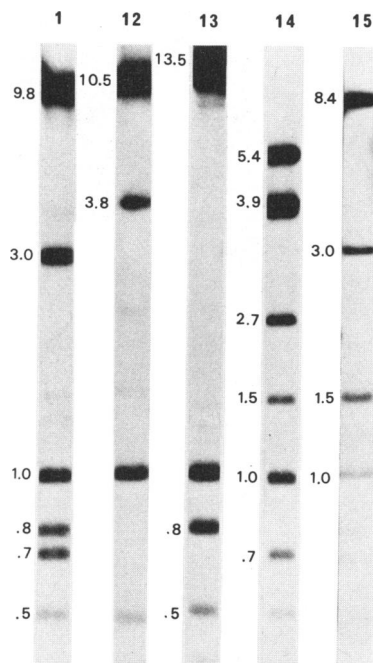


FIG. 3.—Autoradiogram of *AvaII*'s four new morphs compared with morph 1, which corresponds to the published mtDNA sequence [13].

cut the 8.4-kb fragment into two fragments of 5.3 and 3.1 kb. This implies that the new site occurs at bp 4,280. Numerous potential sites were found in the published sequence that could account for these two new *AvaII* sites by single base pair mutation.

#### DISCUSSION

This study has focused on a comparison of mtDNA polymorphisms in two population groups living in Israel. The samples are communities that differ in their ethnic religious affiliations but both represent populations that emerged as unique peoples, perhaps 5,000–10,000 years ago. Both belong to the same major ethnic division (Caucasians), but in view of the paucity of the data, no attempt was made to use these preliminary data for the discussion of evolutionary trees and divergence times.

Of the five enzymes used in this study, four proved particularly useful and contributed to the mtDNA variability encountered. *BamHI*, which generally generates the least morphs, was the least informative. The numbers of individuals forming the samples are rather small, yet both populations differ significantly from the existing Caucasian data in the fragment patterns resulting from the three restriction enzymes *HpaI*, *MspI*, and *HaeII*.

*HpaI* yielded only two morphs (2 and 3). Previously, morph 3 was found only

in African populations [7]. In our study it was confined to the Arab community. This finding is consistent with earlier studies reporting the presence of typical "African" markers such as Fy (a-b-), Rh<sub>0</sub>, and Sutter among Arab communities in Israel [15, 16].

The other enzymes also show a slight tendency toward "African" characteristics of the Arab population (especially when compared with the Jewish one), such as the lower *Ava*II morph 1 frequency, the existence of types 3 and 5, and the lowered frequency of morph 1 of the *Msp*I enzyme.

The same trend is apparent from the combination of the restriction endonuclease morphs observed for each individual (table 2). Altogether, 18 distinct mtDNA types were found, eight of which have been reported earlier [8] and 10 that have not. Of these 18 types, only three are shared by both Israeli groups and, even then, with striking differences in frequencies.

Type 1 (2-1-1-1-1), which was found to be the most common type in all major groups, was also the most frequent in our two communities, although less among the Israeli Jews (38% vs. 56.4%).

Type 6 (2-1-2-22), on the other hand, was rather frequent among the Israeli Jews (36%)—more common than in all major populations, due to the frequency of *Hae*II morph 2.

In spite of the fact that the Israeli Jewish sample was composed of representatives of the Ashkenazi, Sephardi, and Oriental divisions, the Arab sample seems slightly more polymorphic, perhaps reflecting the contribution of more numerous ancestral females.

In figure 4, the 10 new mtDNA types have been added to the suggested phylogeny of the 35 types already described [8]. On the basis of relating the types through single site changes, more than half of the new patterns radiate directly from the central and most common type 1 (2-1-1-1-1), two derive from types belonging to the Caucasian branch, while one type (no. 43) derives from a branch that forms a distinct African lineage. In order to relate another new type (no. 36) by minimal number of mutations, a missing intermediate was postulated. Also, of particular interest was our discovery of a new type (no. 39) that forms one of the missing intermediates originally postulated to exist between types 1 and 27.

In spite of the very small numbers in each group, these data demonstrate the possible existence of group-specific mtDNA fragment patterns and that certain types may be unique to certain groups. Even in a relatively short evolutionary time new types appear and may remain unique in populations that are relatively isolated one from the other. This differentiation process is probably faster with mtDNA than with nuclear genes that are believed to have a lower mutation rate.

Obviously, larger sample sizes and representation of members of different families from more Jewish and Arab communities are needed for confirmation of these speculations for assessment of the extent of heterogeneity within the isolated communities and of the degree of similarity among the various historical and geographical subdivisions.



TABLE 2  
 FREQUENCIES OF "NEW" AND "OLD" mtDNA TYPES IN THE TWO ISRAELI GROUPS COMPARED WITH THOSE FOUND IN THREE WORLD POPULATIONS

	ENZYME MORPH	ISRAELI JEWS		ISRAELI ARABS		CAUCASIAN %	ORIENTAL %	AFRICAN (BANTU) %
		No.	%	No.	%			
"Old" type:*								
1	(2-1-1-1)	15	38.0	22	56.4	58.0	69.6	25.0
2	(3-1-1-1)	0	0	1	2.6	0	0	32.5
6	(2-1-2-1)	14	36.0	1	2.6	12.0	2.2	0
7	(3-1-1-1)	0	0	1	2.6	0	0	10.0
11	(2-2-3-1-5)	1	2.6	0	0	3.0	0	0
17	(2-1-1-9)	1	2.6	0	0	2.0	0	0
22	(2-1-1-5)	4	10.3	6	15.4	2.0	0	0
31	(3-1-1-5)	0	0	2	5.1	0	0	2.5
"New" type:								
36	(2-1-1-13)	1	2.6	0	0	0	0	0
37	(2-1-1-14)	1	2.6	0	0	0	0	0
38	(2-1-1-15)	1	2.6	0	0	0	0	0
39	(2-1-4-1)	1	2.6	0	0	0	0	0
40	(2-1-6-1)	0	0	1	2.6	0	0	0
41	(2-1-7-1)	0	0	1	2.6	0	0	0
42	(2-1-8-1)	0	0	1	2.6	0	0	0
43	(3-1-1-12)	0	0	1	2.6	0	0	0
44	(2-1-1-4-3)	0	0	1	2.6	0	0	0
45	(2-1-8-1-1)	0	0	1	2.6	0	0	0

\* The type no. corresponds to those encountered previously [8]. The enzyme morphs are listed in parentheses in the order: *HpaI*, *BamHI*, *HaeII*, *MspI*, and *AvaII*.

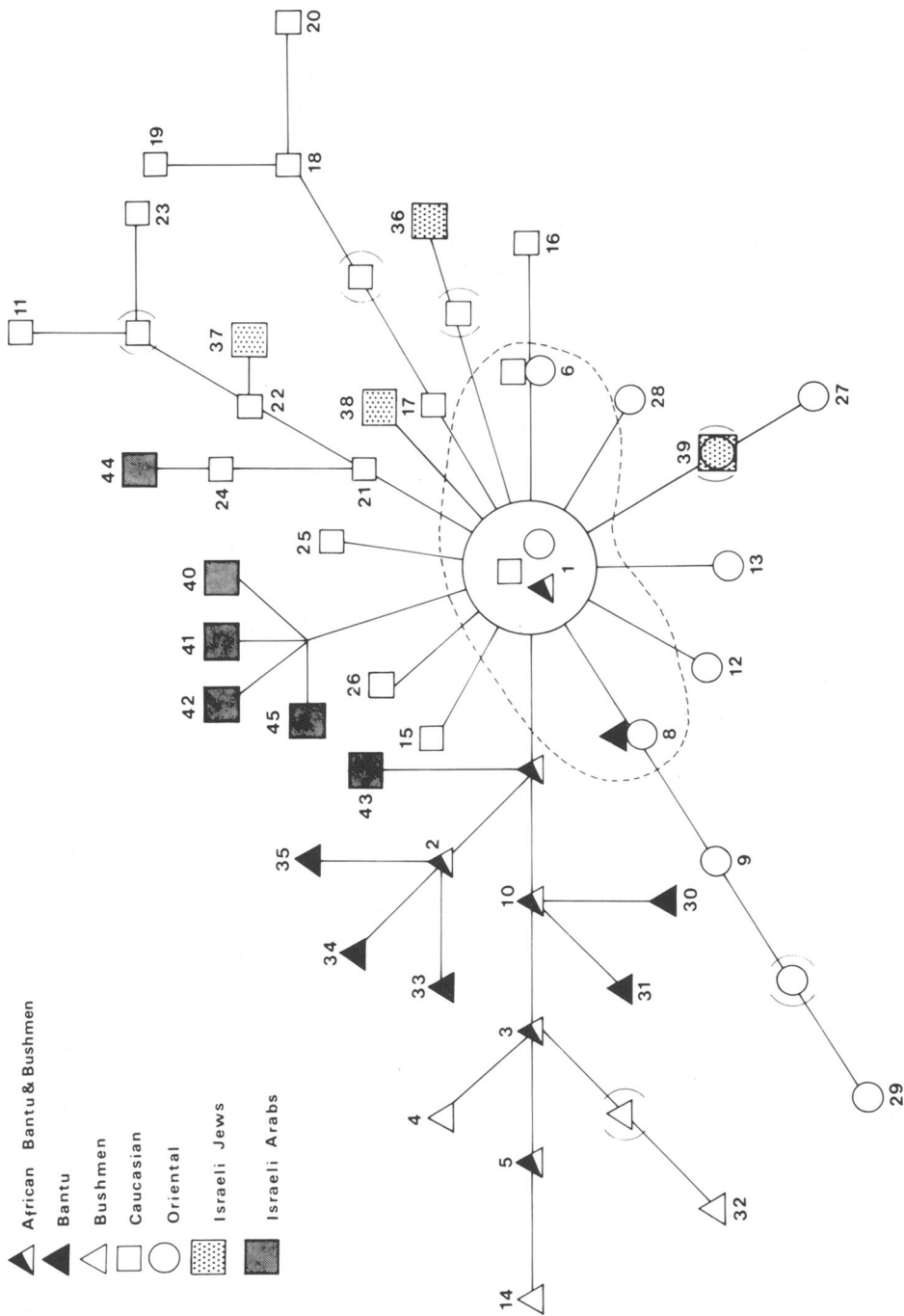


FIG. 4.—Phylogeny of mtDNA types including the 10 new types observed in this study (for details see text and [8])

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