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Mitochondrial DNA: a tool for populational genetics studies

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Summary Mitochondria are cellular organelles that have the function of the oxidative phosphorilation and the formation of ATP. In humans, the mtDNA is a double-stranded, circular, covalent closed molecule of 16.5 kb. The mtDNA is inherited as a haploid from the mother and heteroplasmy has been found rarely. From a populational perspective, it could be considered as a system of small, sexually isolated demes, or clonal lineages, with an evolutionary rate 5 to 10 times faster than the nuclear genome. All these characteristics make this molecule ideal for evolutionary studies. We present two applications of this molecule in genetical studies. One of these is referred to the Balearic Islands populations, Majorca, Minorca, Ibiza, and Chuetas. The other example is the populational dynamics of the different mitochondrial haplotypes in *Drosophila subobscura*. We also discuss the importance of nuclear markers to complete these studies as well as the study of the Y chromosome to compensate the bias produced by the study of only the mtDNA.

Key words Mitochondria · Mitochondrial DNA · Balearic Islands populations · Mitochondrial haplotypes · *Drosophila subobscura*

General characteristics of mitochondria and mitochondrial DNA

Choloroplasts and mitochondria are both eukaryotic intracellular organelles; the first are only present in plants and the second are ubiquitous, in plants, fungi and animals. Because the mitochondrion is a cellular organelle that has the function of the oxidative phosphorilation and the formation of ATP, they seem to have a life of their own in the cell. These organelles contain DNA that codes for proteins specific for themselves, and they also replicate by themselves. The idea that chloroplasts and mitochondria arose from bacteria-like cells that were assimilated by eukaryotic cells is called the endosymbiosis theory [24]. In this theory, a proto-eukaryotic cell takes in other cells to their mutual advantage. The cells that are taken in eventually become organelles. The modern techniques of sequencing have helped to prove this theory. The analyses of rRNA sequences of these organelles and those of eukaryotes and modern prokaryotes show that the organelle genomes are different from the nuclear genomes of their eukaryotic host cells and closely resemble eubacterial genomes. In addition, chloroplast and mitochondrial genomes differ from each other and must be derived from different eubacteria. This conclusion means that at least two independent endosymbiotic events took place throughout the evolution of these organelles, one involving a cyanobacterium-like cell that gave rise to chloroplasts and the other involving a purple photosynthetic bacterium that gave rise to mitochondria [38].

Nuclear and mitochondrial genomes are functionally interdependent [13]. Most mitochondrial proteins are encoded by nuclear genes. Only 2 rRNAs (the only ones in the mitochondrial ribosome), 20 to 35 tRNAs (all those needed for translation in the mitochondrion), and 13 proteins (subunits of the mitochondrial complexes involved in the respiratory chain and the synthesis of ATP) are known to be encoded by the mitochondrial DNA (mtDNA). The remaining subunits of those complexes (the around 90 proteins required for the mitochondrial ribosomes assemblage, 20 different aminoacyl synthetases, and all the enzymes needed to replicate and transcribe the mtDNA) are encoded by genes in the nucleus. All mitochondrially encoded proteins form components of metabolic pathways or enzyme complexes whose remaining constituents are nuclear encoded [7]. In this situation, we have the mitochondrial complexes ATP synthetase, the cytochrome c oxydase, and the NADH dehydrogenase b-c_1 that have subunits encoded by both nuclear and mitochondrial genes. The mtDNA has different genome sizes; some examples are: Metazoan: *Ascaris suum*, 14.5 kb; *Drosophila subobscura*, 15.8 kb. Yeast: *Saccharomyces cerevisiae*, 78 kb. Fungus: *Neurospora crassa*, 60 kb. Plants: *Zea mays*, 570 kb,

Muskmelon, 2500 kb. In humans, the mtDNA is a double-stranded, circular, covalent closed molecule of 16,569 bp that has been completely sequenced [1]. Mammalian mtDNA is inherited as a haploid from the mother [16] and heteroplasmy has been found rarely [15]. In this way, from a populational perspective, it could be considered as a system of small, sexually isolated demes, or clonal lineages. The evolutionary rate of the mtDNA is 5 to 10 times faster than the nuclear genome [5], mainly because the mitochondria do not have repair enzymes for errors in the replication, nor for the damages of the DNA [9]. In this way, the mtDNA has a high level of transitions and transversions, as well as a high incidency of small length mutations [8]. In humans, mtDNA high mutation rate (2–4% per million years, [40]; see also [17]) leads to a high degree of variability between individuals. A high ratio of transitional to transversional substitution in primate mtDNA has been found [6]. This ratio was close to 20, much higher than the ratio of approximately 1 in nuclear DNA. This observation was made in coding sequences and the control region. This region, except for the central part, appears to be subjected to very weak selective constraints, and so, it has evolved very rapidly [39]. Tamura and Nei [36] have studied the substitution pattern in the control region of 95 human sequences. The conservative central portion was excluded from the analysis and they found an average transition/transversion ratio of 15.7, the transitional rate between pyrimidines (C and T) being higher than that between purines (A and G).

The rate of nucleotide substitution does not correlate with the rate of structural changes in the genome of organelles. In mammals, the mtDNA evolves very rapidly in terms of nucleotide substitutions, but the spatial arrangement of genes and the size of the genome are fairly constant among species. In contrast, the mitochondrial genome of plants undergoes frequent structural changes, but the rate of nucleotide substitutions is extremely low. The lack of correlation between the rates of nucleotide substitution and the rates of structural evolution suggests that the two processes occur independently [23].

In relation with the recombination of this molecule, the data found up to now indicate the extreme rarity (and possibly the absence) of intergenomic or reciprocal mtDNA recombination in human cells. Nevertheless, Thyagarajan et al. [37] have recently shown that extracts of mitochondria from human cells contain enzymes that catalyze an homologous recombination in plasmids. In this way, these authors present an important discrepancy, as mentioned by Howell [18]. If mitochondria have the enzymatic machinery to carry out homologous recombination in vitro between DNA plasmids, then why has recombination in situ between mtDNA molecules not been detected? As Howell [18] indicates, the lack of recombination may be a mechanism that has evolved to slow Muller's ratchet in mammalian mitochondrial genomes. Because, although recombination is almost a universal genetic process, natural selection will act against recombination if the advantages of the maintenance of sets of coadapted genes outweigh those gained through recombination [26]. Surely, the homologous-recombination activity detected by Thyagarajan et al. [37] may be part of a topoisomerase/resolvase complex that functions to separate daughter monomers at the termination of replicacion, and then to introduce superhelical turns into these monomers [9]. More data indicate that a resolvase function may be important not only for proper replication and partitioning of human mtDNA, but also to control the segregation [18].

Populational genetics studies

The first human population studies based on mtDNA were performed by restriction enzyme analyses (RFLPs) [10, 27], and they revealed differences between the four great ethnic groups (Caucasian, Amerindian, African, and Asian). Differences in mtDNA patterns have also been shown in communities with a different geographic origin within the same ethnic group [3, 35]. In the Balearic Islands, we have studied the genetic structure of these populations through restriction fragment polymorphism (RFLP) analysis of their mtDNA by using 5 restriction enzymes that survey approximately 1.2% of the mitochondrial genome for sequence variation [31]. Due to the geographical location in the Western Mediterranean Sea, the Balearic Islands have been settled by many peoples coming from different areas of the Mediterranean. Present-day Balearic inhabitants are a reflection of these events in the genetic pool. Previous studies with haematic polymorphisms [28] suggested that the genetical differentiation of the Ibizan population, with respect to Majorca, Minorca and other Spanish and European populations, should be mainly due to its Carthaginian origin inbreeding by the small size of population and/or periodical bottleneck and isolation from other Balearic populations. For some markers, Ibiza seems to be closer to North African populations [30]. This trend is also confirmed at the level of mtDNA, where the number of haplotypes found was low in comparison to other populations studied (Table 1). The genetic homogeneity is higher in Majorca than in Minorca and Ibiza. This result is due to the high frequency of haplotype 1 in this population. In Minorca, with the most heterogeneous population, the most frequent haplotype is also 1, although there are other haplotypes in polymorphic frequencies as well as a new one, 150 (8%). Ibiza had only four haplotypes, with haplotype 1 also having the highest frequency.

The genetic diversity between populations is about seven fold higher for Majorca–Ibiza and Minorca–Ibiza than for Majorca–Minorca, which indicates a genetic differentiation of Ibiza with respect to Majorca and Minorca. Nevertheless, the analyses of genetical heterogeneity indicated that, globally, there had never been any geographic heterogeneity among the

Table 1 Frequencies (in percentage) and haplotypic diversities of the different haplotypes detected in the Balearic Islands

Types of mtDNA	Haplotype	*Majorca*		*Minorca*		*Ibiza*		*Chuetas*	
		N	%	N	%	N	%	N	%
1	2-1-1-1-1	44	84.6	28	56.0	25	50.0	39	72.2
2	3-1-1-1-3	0	–	0	–	5	10.0	0	–
6	2-1-2-1-1	2	3.8	7	14.0	0	–	7	13.0
7	3-1-1-1-1	1	1.9	0	–	0	–	0	–
8	1-1-1-1-1	0	–	3	6.0	0	–	0	–
11	2-2-3-1-5	0	–	0	–	1	2.0	0	–
18	2-3-1-4-9	3	5.8	0	–	19	38.0	4	7.4
22	2-3-1-1-9	1	1.9	0	–	0	–	0	–
24	2-1-1-4-2	0	–	1	2.0	0	–	0	–
39	2-1-4-1-1	0	–	0	–	0	–	1	1.8
56	2-1-1-1-6	0	–	1	2.0	0	–	0	–
57	2-3-1-4-13	1	1.9	6	12.0	0	–	2	3.7
59	2-1-1-1-20	0	–	0	–	0	–	1	1.8
150	2-1-2-1-6	0	–	4	8.0	0	–	0	–
Total		52	100	50	100	50	100	54	100
h		0.284		0.655		0.607		0.445	

h = haplotypic diversity.
N = number of individuals.

three populations [25]. This lack of population subdivision could be due to the frequency of haplotype 1, the highest in the three populations. It, therefore, may not be able to counteract the power of haplotype 18, with a high frequency in Ibiza (38%), only 6% in Majorca but absent in Minorca.

Considering the different haplotypes, the most characteristic in Majorca are Caucasians (mainly haplotype 1 and also haplotypes 6 and 18), although the presence of haplotype 7 suggests an Arabian/African genetic contribution to this population. Haplotype 7 could have been introduced into Majorca by the Arabians, who lived in the islands for around five hundred years. This haplotype is not as frequent in Majorca (1.9%) as it is in Arabian communities (4.8%) [31] but it is in the same order as in other Caucasian groups such as the Romans (1.1%) [4] and Askenazi Jews (1.3%) [31].

Haplotype 22 has only been found in Majorca and in Iraqi and Israelian Jews. The presence in Majorca of a Jewish community could explain the existence of this haplotype. This haplotype is the ancestor of haplotypes 18 and 57. Sartoris et al. [33] suggested that haplotype 18 could represent a Mediterranean ancestral type because of its absence or very low frequency in other geographical groups studied. Therefore, this mtDNA lineage could be considered a Mediterranean one. These three haplotypes (22, 18 and 57) have been found in Majorca.

Minorca is also represented by Caucasian haplotypes. Interestingly, haplotype 6 (frequent in Jewish communities) is also present in this population. Haplotype 24 also seems to be Caucasian and has the same frequency (2%) in Minorca and in the Caucasian groups studied [19]. The mtDNA Mediterranean lineage is represented by haplotype 57, in a frequency (12%) similar to that found in Turkish Jews (12.5%) and much higher than that found in the other Mediterranean groups studied (0–3.4%). Haplotype 56 is infrequent, and its frequency in Minorca is similar to that found in Sicily (1.1%) and in the central Italian peninsula (1%). Haplotype 150 is derived from this haplotype and is found in Minorca at a polymorphic frequency. In this way, this mtDNA lineage is present in Minorca at a relatively high frequency. It is possible that haplotype 56 be an ancient one, introduced into Minorca by the first settlements about 4000BC, and this soon led to haplotype 150 (Fig. 1).

The mtDNA analysis of the Chueta community (Majorcan Jews) has shown a reduced number of Caucasian haplotypes (Table 1), being particularly interesting the presence of haplotypes 6, 39 and 57, typical of the Jewish populations. This corroborates the Jewish origin of this community.

In Ibiza, the exceptional fact is the high frequency of haplotype 18 (38%). This is considered an ancient Mediterranean mtDNA type found at a maximum frequency of 12% in the center and south of the Italian peninsula and Sicily. We can say that, in Ibiza, two mtDNA lineages are present, one represented by haplotype 1 and the other by haplotype 18. Haplotype 11 is a Caucasian one but not very frequent in the Mediterranean groups studied, as in the case of Ibiza. Haplotype 2 is an African genotype, suitable for the study of the mixture between Negroid and Caucasian populations [34]. In Ibiza, the frequency of this haplotype (10%) is the highest observed in non-African populations, even higher than that observed in Sicily (3.3%), which is a population that is supposed to have African maternal ancestors [34]. Unfortunately, there are not any mtDNA polymorphism data about North African populations, with which the data obtained in Ibiza could be compared. However, the results of analysis

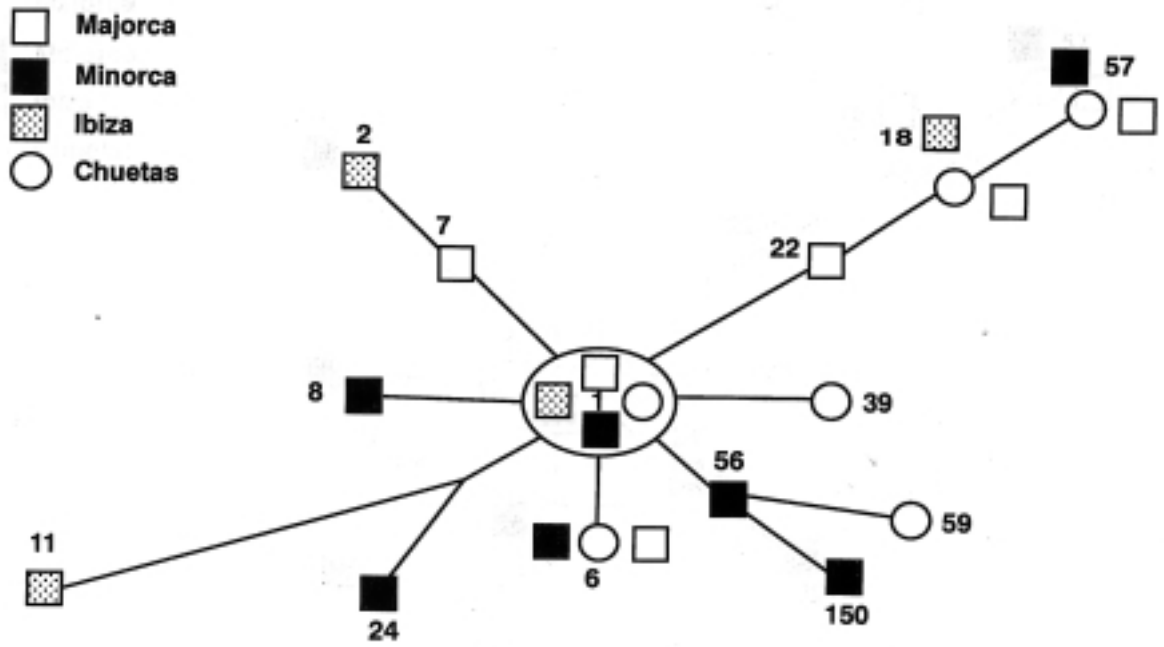

Fig. 1 Maximum parsimony tree which relates all the haplotypes found in the Balearic Islands. The derivation of haplotype 150 from 56 was tested by a maximum likelihood analysis

of nuclear gene frequencies in North African populations indicate that one third of African ancestry is present in North Africans [14]. On the other hand, Arabs have typical African nuclear genes at high frequencies [29]. So, it is reasonable to think that African markers could have arrived to Ibiza and Majorca by direct introduction of African slaves by Phoenicians or Romans and/or indirectly through Arab migrations [25].

As it can be seen, the study of the Balearic islands by means of the RFLPs constitutes a good example of the usefulness of this molecule in human populational studies. But we need to study these populations in depth. In this way, the sequentiation of some fragments, such as the control region can cast more light to this research. Moreover, other approaches, with nuclear markers, such as the variation of STRs, could complete these studies. Interestingly, in Balearic populations we have found high concordances between some nuclear markers (blood groups, red cell enzymes, serum proteins) and the mtDNA RFLPs. But, in other cases, the results are not so concordant. In this line of research, an approximation of several markers to elucidate populational studies has been carried out for instance by Jorde et al. [20]. These authors tried to test hypotheses about the origin of modern humans; for this purpose, they used mtDNA sequences, 30 nuclear restriction-site polymorphisms and 30 STR in 243 Africans, Asians and Europeans. Although the results with mtDNA are consistent with an African origin of modern humans, the results do not show such a clear distinction when using the nuclear markers. That is, nuclear and mtDNA data present discordant pictures of human population affinites. For this reason, they conclude that these results undermine genetic evidence for an African origin of modern humans.

As mtDNA is maternally inherited, all the populational inferences are made in relation with the evolution of the maternal lineages. To complete these studies, nowadays the laboratories (including ours) are also studying the markers in the Y chromosome. In this way, we will have information from the two progenitors.

Apart from the human populational studies, the use of mtDNA in other organisms and aspects is very important. This is the case, for instance, of Juan et al. [21], who studied the phylogeny of darkling beetles of the Canary Islands.

In the last few years, we have dedicated a lot of effort to understand the populational dynamics of different mitochondrial haplotypes in *Drosophila subobscura*. Previous studies on the distribution of mtDNA haplotypes in Old World populations of *Drosophila subobscura* have shown the presence of two widespread and equally frequent haplotypes, and a set of sporadic haplotypes generally never present in more than one single locality [22]. The same pattern of distribution has been detected in the New World colonizing populations of *Drosophila subobscura* [32]. Nowadays, one of the most important challenges we have is to know the genetical forces which are maintaining these haplotypes in nature.

An approach to solve this problem is the study of the mtDNA–nuclearDNA genetical interactions. Nuclear–cytoplasmic interactions, especially in plants, are also manifested at the phenotypic level, and there is increasing experimental evidence in *Drosophila* that forces maintaining

Table 2 Estimates of four measurements of cytonuclear disequilibria, their standard errors and χ^2 tests (1 degree of freedom) for *Drosophila subobscura* populations of Majorca and Minorca

Locus	Disequilibrium	D_M^{AA}	D_M^{Aa}	D_M^{aa}	D_M^{A}
*ACPH-1**	Estimator	0.0220	–0.0176	–0.0044	0.0132
	Standard error	0.0133	0.0118	0.0088	0.0094
	χ^2 (1)	2.67	2.15	0.24	1.85
*ACPH-2**	Estimator	–0.0102	0.0129	–0.0027	–0.0037
	Standard error	0.0078	0.0073	0.0027	0.0054
	χ^2 (1)	2.28	4.85*	0.64	0.70
*ADH**	Estimator	–0.0040	0.0037	0.0003	–0.0021
	Standard error	0.0041	0.0037	0.0018	0.0027
	χ^2 (1)	1.04	1.11	0.03	0.75
*FUM**	Estimator	0.0043	–0.0046	0.0003	0.0020
	Standard error	0.0043	0.0039	0.0018	0.0025
	χ^2 (1)	0.91	1.24	0.03	0.53
*IDH**	Estimator	0.0068	–0.0044	–0.0024	0.0046
	Standard error	0.0073	0.0066	0.0032	0.0043
	χ^2 (1)	0.83	0.42	0.53	0.97
*MDH-1**	Estimator	–0.0143	0.0125	0.0018	–0.0081
	Standard error	0.0099	0.0093	0.0041	0.0062
	χ^2 (1)	2.17	1.86	0.21	1.90
*PGM-1**	Estimator	–0.0136	0.0110	0.0026	–0.0081
	Standard error	0.0100	0.0095	0.0038	0.0061
	χ^2 (1)	1.89	1.37	0.48	1.94

D_M^{AA}, D_M^{Aa}, D_M^{aa} are genotypic disequilibria. D_M^{A} is the allelic disequilibrium.

mtDNA variability are mediated through specific nuclear–cytoplasmic interactions [11]. Such varied interactions between products of nuclear and mitochondrial genotypes could provide many opportunities for epistatic interactions on fitness and hence for cytonuclear disequilibria [2, 12]. Because of maternal and clonal inheritance, the effective population size for mitochondrial genes is only approximately one-quarter that for nuclear genes. Thus, for equivalent mutation rates and selection pressures, the variation at equilibrium for mitochondrial genes within populations is expected to be lower, and the divergences between populations higher, than in the case of nuclear genes [22].

To test the relationship between the mtDNA haplotypes and nuclear markers, a set of cytonuclear disequilibria was made following the methodology of Asmussen et al. [2], with the most relevant haplotypes (I and II) and some nuclear markers, such as the allozymes. The results are indicated in Table 2. With the exception of *ACPH-2**, cytonuclear disequilibria were not detected. This is congruent with an absence of mtDNA haplotype-nuclear allozyme fitness interactions. In the absence of such interactions, the disequilibria parameter, D, will decrease rapidly to 0 unless the recombination (r) is close to 0. In our case, we reasonably assumed the cytonuclear recombination was r = 0.5. Unless fitness interactions are extremely strong, D is quickly going to be negligible. Only some transient disequilibria might appear as a consequence of genetic hitchhiking, or with adaptive markers, such as inversions. In our case, the disequilibria associated with *ACPH-2** could represent a transient disequilibrium established at the moment the samples were taken or simply a statistical artefact.

Due to the adaptive value of the inversions, experiments in population cages (i.e., a new environment different from that of nature) might generate genetic hitchhiking on different and neutral competing haplotypes. The detection of these associations could help us to understand the dynamics of mtDNA polymorphisms in natural populations of a given species.

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