Population Genetic Structure and Colonization Sequence of *Drosophila subobscura* in the Canaries and Madeira Atlantic Islands as Inferred by Autosomal, Sex-Linked and mtDNA Traits

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The genetic structure in Atlantic islands and continental populations of *Drosophila subobscura* has been studied using autosomal and sex-linked allozymes and mitochondrial DNA (mtDNA) haplotypes. From the data it is deduced that whereas the Canary Islands have long been isolated, the neighboring island of Madeira has been subjected to continuous migration from the mainland. In addition, sex-linked allozymes and mtDNA data show a large divergence between the geologically younger western islands of the Canarian Archipelago and the older central ones, finding strong founder effects in the former. Divergence rates of sex-linked and mitochondrial genes relative to autosomic loci several times higher than expected under neutrality have been explained by differential migration between sexes. The Canarian Archipelago colonization fits in well with a stepping-stone model of a directional east-west migration that parallels the geological origin of these islands.

Drosophila subobscura is a Paleartic member of the *obscura* subgroup with a wide geographical distribution in the Old World from Finland to Morocco and from Western Asia to the Atlantic archipelagos of the Canaries, Madeira, and the Azores. From the information gathered on this species, the high degree of differentiation of the Canary Islands population stands out. At the chromosomal level, Prevosti (1971, 1974) observed that the polymorphism in these islands is different from that in adjacent continental areas, suggesting a long isolation and an ancient polymorphism for them. Enzymatic studies revealed a Canarian population with large genetic distances from other southwestern range populations, including those from the nearby archipelagos of Madeira and the Azores (Larruga et al. 1983). More recently analyses of molecular variation on mitochondrial DNA (mtDNA) (Afonso et al. 1990) and in genomic sequences that include the *rp49* gene (ribosomal protein 49) (Rozas and Aguadé 1990, 1991) also corroborated that the Canarian population has long been isolated from the mainland. Of the above, studies only used samples from one Canarian population, Raices, assuming that all the archipelago populations are identical; however, the different ages of each island make this assumption doubtful. In fact, some published results at chromosomal (Prevosti 1971) and enzymatic (Cabrera et al. 1980) levels have found some heterogeneity among populations.

In the present article we analyze autosomal, sex-linked, and maternal inherited traits in samples of the different islands of the Canarian Archipelago and other nearby geographically related insular and continental populations to determine the influence that founder effects, migration, and selection have had on the different trait distributions. In addition, we have tried to correlate the possible colonization sequence of the Canary Islands with their geological history.

Material and Methods

Samples

Samples from the following 12 populations have been analyzed: La Gomera, Gran Canaria, El Hierro, La Palma, and Tenerife (Canary Islands); Poiso and Ribeiro (Madeira); Agadir, Asni, and Marrakech (Morocco); Barcelona and Escorial (Spain). The two samples from Madeira were pooled for allozyme data and the three samples from Morocco were pooled for the mtDNA restriction analyses.

Mean sample sizes for enzymatic loci were 71 ± 14 and for mtDNA restriction analyses were 36 ± 4. The locations of the samples are shown in Figure 1.

Chromosomal Analysis

For chromosomal analysis, previous published data for the following populations

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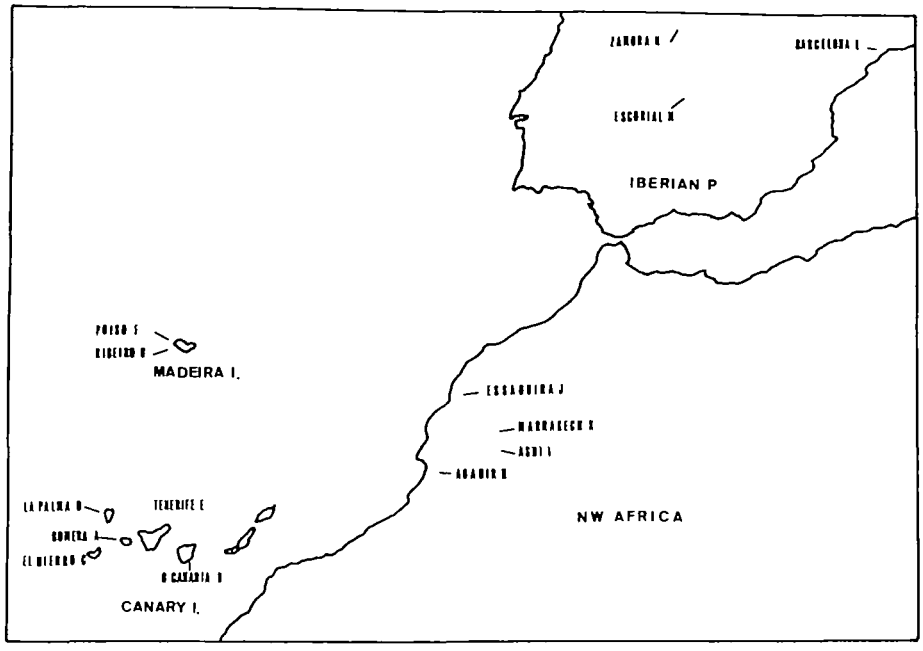

Figure 1. Geographic origin of the *Drosophila subobscura* samples.

were used: La Gomera, Gran Canaria, El Hierro, La Palma (Canary Islands); Agadir, Asni, and Essaouira (Morocco; from Prevosti 1974); Tenerife (Canary Islands; from Padrón 1986); Poiso and Ribeiro (Madeira; from Larruga et al. 1983); Barcelona (Spain; from Prevosti et al. 1984); Zamora (Spain; from Frutos 1972).

Enzymatic Analysis

Four sex-linked enzymatic loci—diaphorase (*Dia-4*), glucose-6-phosphate dehydrogenase (*G-6-pdh*), hydroxybutyrate dehydrogenase (*Hbdh*), and 6-phosphogluconate dehydrogenase (*6-Pgdh*)—and eleven autosomal loci—alcohol dehyrogenase (*Adh*), aldehyde oxidase (*Ao*), amylase (*Amy*), esterase (*Est-7* and *Est-8*), glutamate oxaloacetate (*Got-2*), hexokinase (*Hk-1*), leucine aminopeptidase (*Lap-4*), malate dehydrogenase (*Mdh*), peptidase (*Pep-1*), and xanthine dehydrogenase (*Xdh*)—were analyzed. References for the electrophoretic and staining methods are in Cabrera et al. (1980) and Larruga et al. (1983).

Mitochondrial DNA Analysis

Mitochondrial DNA was extracted as in Larruga et al. (1993). Enzymatic digestion by restriction enzymes, electrophoresis, mapping, and nomenclature is as described by Afonso et al. (1990). Eleven restriction enzymes were used in the present study. Two of them (*Hae*III and *Hpa*II) recognize 4 bp sequences. The other nine (*Bam*HI, *Eco*RI, *Eco*RV, *Hind*III, *Pst*I, *Pvu*II, *Sac*I, *Xba*I, and *Xho*I) recognize 6 bp sequences.

Statistical Analysis

The genetic structure of the populations was inferred for chromosomal and enzymatic data by Wright's F_{ST} statistics (Wright 1978) using the BIOSYS-1 package (Swofford and Selander 1981) and for mtDNA data by the N_{ST} of Lynch and Crease (1990) using the HAPLO program written by the same authors.

The DISPAN program (version 1.1; Ota 1993) was used to estimate, for chromosomal and enzymatic data, the standard genetic distances between populations (Nei 1972) and to construct UPGMA trees (Sneath and Sokal 1973) with bootstrap tests (Felsenstein 1985). For mtDNA data the average number of nucleotide substitutions per site within (p) and between populations (d) (Nei and Miller 1990) and the UPGMA tree was obtained using the RESTSITE program (version 1.2; Miller 1991). Standard errors of branching points of the UPGMA tree were calculated following the method of Nei et al. (1985).

Geographical patterns of genetic variation in the studied populations were examined by the general correlation method of Mantel (Sokal 1979) using option 7 of the GENEPOP program (version 1.2; Raymond and Rousset 1995) in order to test different modes of dispersal among islands as in Finston and Peck (1995).

Table 1. Standard genetic distances (Nei 1972) between populations estimated from chromosomal data

	Populations*										
	B	C	D	E	F	G	H	I	J	L	N
A	0.000	0.024	0.002	0.001	0.101	0.091	0.378	0.374	0.321	0.381	0.332
B		0.017	0.005	0.004	0.092	0.083	0.345	0.338	0.286	0.381	0.330
C			0.009	0.021	0.111	0.104	0.384	0.355	0.300	0.416	0.363
D				0.002	0.105	0.097	0.415	0.398	0.342	0.396	0.345
E					0.083	0.076	0.379	0.367	0.311	0.360	0.318
F						0.000	0.384	0.407	0.329	0.275	0.247
G							0.409	0.418	0.341	0.267	0.246
H								0.014	0.010	0.392	0.346
I									0.000	0.367	0.332
J										0.285	0.274
L											0.067

* Population letters correspond to letters in Figure 1.

Results

Chromosomal Population Structure

From the inversion polymorphism data a matrix of standard Nei (1972) distances was calculated (Table 1) and from this a UPGMA dendogram showing the chromosomal relatedness among populations was constructed (Figure 2a). As previously commented (Prevosti 1974), insular populations from the Canaries and the nearby island of Madeira cluster first and are well differentiated from continental European and North African populations, though some chromosomal orders as A_{2+6} and $A_{2+3+5+7}$ are only shared among some Canarian and Moroccan samples (Prevosti 1974; Cabrera et al. 1983; Padrón 1986).

On average, 88% of the total variance of rearrangement frequencies between populations (F_{ST} = 0.254) was due to genetic differences among geographic regions.

There is a significant positive correlation between geographical and chromosomal distances for all the studied populations when the Mantel test is applied (r = +.589; P = .00), but this does not hold when only the Canarian populations are analyzed (r = −.012; P = .48).

Figure 2. Relationships (UPGMAs) among the populations analyzed based on (a) inversions, (b) autosomal loci, (c) sex-linked loci, and (d) mtDNA data. Numbers on trees indicate the percentage of bootstrapped trees supporting each node and bars show the standard errors of branching points.

Enzymatic Population Structure

Although the mean number of alleles per locus and the mean heterozygosities for the 15 polymorphic enzymatic loci studied are very similar in all populations (Table 2), particular alleles and allele frequencies of six loci clearly differentiate populations. The most common allele for *Amy*, *Est-8*, and *Pep-1* in the Canaries is not the same as in the other sampled populations, which is in accordance with previous studies of this area (Larruga et al. 1983). Furthermore the Canarian populations do not behave as a homogeneous cluster for sexual loci. Two islands, El Hierro and La Palma, shared some exclusive alleles as *G-6-pdh*^{99} and have the most common allele for *Dia-4* and *6-Pgdh* different from those of the rest of the Canarian samples and from any of the studied populations. Genetic distances between populations for autosomal and sex-linked loci are shown in Table 3. UPGMA dendograms relating populations for each set of data are depicted in Figure 2b,c. The most outstanding difference between both trees is the great differentiation of El Hierro and La Palma from all the populations for sex-linked loci, while for autosomal loci they closely cluster with the other Canarian samples, this cluster being significantly different from the rest of the insular and continental populations.

The different level of enzymatic differentiation for sex-linked and autosomal loci is also reflected by using the F_{ST} statistic. A partition of the total variance among populations for sex-linked (22%) and for autosomal (13%) loci into differences within and among areas are 19% and 3% for the former, but 3% and 10% for the latter. This difference is mainly due (92%) to the commented heterogeneity among the Canary Island samples.

Although less significant than with chromosomal data there is also a positive correlation between geographic and enzymatic distances ($r = +.340$; $P = .04$) for all the populations studied. Furthermore, for these traits a significant positive correlation also exists for the Canarian populations when only autosomal loci are analyzed ($r = +.836$; $P = .02$).

Mitochondrial DNA Population Structure

As in previous studies (Afonso et al. 1990) of the 11 restriction enzymes used in this work only *Xho*I did not cut the mtDNA molecule in any of the strains studied here, three (*Bam*HI, *Pvu*II and *Xba*I) produced the same pattern in all populations,

Table 2. Allelic frequencies of six enzymatic loci showing differences among populations

Locus	Populations[a] A	B	C	D	E	F[b]	H	I	K	L	M
Dia-4											
(*N*)	40	35	56	63	148	59	42	15	43	36	22
0.92	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000
0.96	0.063	0.043	0.696	0.627	0.020	0.000	0.012	0.066	0.035	0.014	0.045
1.00	0.937	0.957	0.295	0.373	0.963	1.000	0.940	0.867	0.919	0.944	0.909
1.02	0.000	0.000	0.009	0.000	0.017	0.000	0.048	0.066	0.035	0.042	0.045
Obs. het.	0.125	0.086	0.464	0.429	0.074	0.000	0.119	0.200	0.163	0.111	0.182
G-6-pdh											
(*N*)	41	35	56	63	70	59	53	28	78	36	22
0.97	0.049	0.000	0.000	0.000	0.036	0.000	0.085	0.089	0.045	0.000	0.000
0.99	0.000	0.000	0.080	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.00	0.890	0.971	0.893	0 992	0.928	0.966	0.906	0.911	0.949	0.958	1.000
1.02	0.061	0.029	0.027	0.000	0.036	0.034	0.009	0.000	0.006	0.042	0.000
Obs. het.	0 171	0.057	0.179	0.016	0.143	0.068	0.189	0.107	0.103	0.083	0.000
6-Pgdh											
(*N*)	41	35	55	63	57	59	42	11	44	36	33
0.92	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000	0.000	0.014	0.000
0.96	0.293	0.386	0.527	0.651	0.298	0.220	0.107	0.091	0.136	0.083	0.106
1.00	0.695	0.528	0.473	0.349	0.702	0.763	0.857	0 909	0.830	0.903	0.879
1.03	0.012	0.086	0.000	0.000	0.000	0.017	0.012	0.000	0.034	0.000	0.015
Obs. het.	0.293	0.429	0 473	0.476	0.386	0.373	0 286	0.182	0 273	0.139	0.182
Amy											
(*N*)	32	59	112	73	54	105	63	92	67	31	31
0 96	0.531	0.415	0.375	0.596	0.361	0.029	0.079	0.158	0.164	0 048	0.081
0 98	0.313	0.551	0.513	0.377	0.472	0.857	0.778	0.717	0.739	0.677	0.742
1.00	0.156	0.034	0.112	0.027	0.167	0.114	0.143	0.114	0.090	0.242	0.113
1.02	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.007	0.032	0.065
Obs. het.	0.594	0.593	0.571	0.534	0.556	0.219	0.413	0.424	0.433	0.387	0.387
Est-8											
(*N*)	47	60	114	70	59	139	50	49	49	36	21
0.94	0.000	0.000	0 000	0.000	0.017	0.004	0 030	0.031	0.031	0.069	0.024
0.97	0 032	0.033	0.000	0.021	0.076	0.799	0.410	0.541	0.469	0.583	0 833
1.00	0 936	0.958	0.965	0 929	0.864	0.194	0.460	0.347	0 480	0 222	0 119
1 03	0.032	0.008	0.035	0.050	0.042	0.004	0.060	0.000	0.000	0.125	0.024
1.05	0.000	0.000	0.000	0.000	0 000	0.000	0.040	0.082	0.020	0 000	0 000
Obs. het.	0.106	0.083	0.070	0.129	0.203	0.266	0.460	0.490	0.429	0.583	0.238
Pep-1											
(*N*)	47	95	152	75	646	119	45	58	57	36	24
0.92	0.000	0.000	0.000	0.000	0.000	0.000	0 000	0.009	0.000	0.000	0.000
0.94	0.128	0.289	0.099	0.113	0.185	0.849	0.600	0.629	0.649	0.500	0.583
0.97	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000
1.00	0 840	0.700	0.891	0.873	0.790	0 151	0.378	0.328	0.333	0.486	0.417
1.01	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000
1.03	0.032	0.011	0.010	0.013	0.017	0.000	0.022	0.034	0.018	0.014	0.000
1.05	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000
Obs. het.	0.298	0.495	0.171	0.253	0.359	0.235	0.356	0.517	0.491	0 472	0 417
Mean het.	0.276	0.233	0.264	0.290	0.273	0.231	0.254	0.285	0.270	0.273	0.241
(± SE)	0.049	0.049	0.052	0.049	0.049	0.048	0.043	0.044	0.042	0.042	0.038
Mean	3.0	2 7	2.8	2.7	3.3	2.7	3.3	3.1	3.3	3.2	2.9

Mean number of alleles per locus and mean heterozygosities for the 15 loci studied at the bottom.

[a] Population letters correspond to letters in Figure 1.

[b] Madeira pooled data.

and the rest were polymorphic in some of them. The restriction patterns found, following the Afonso et al. (1990) nomenclature, are shown in Figure 3. A total of 29 composite haplotypes have been detected (Table 4), 7 of which (numbers from 27 to 33) were not found previously (Afonso et al. 1990; Rozas et al. 1990). Excluding the Tenerife sample, 14 haplotypes have been detected for the rest of the Canarian populations with 13 in all the other populations studied. Since the sample number for these two subsets is similar, this result reinforces the high polymorphism attributed to the Canary Islands based on the study of only one population (Raices; Afonso et al. 1990). Furthermore, the number of endemic haplotypes found in the Canaries (16) is twice that of those observed in the rest of the studied areas (8). Also, a great heterogeneity in the frequency distribution of the haplotypes in the Canarian Archipelago is detected. Two islands, La Gomera and Gran Canaria, have the most frequent haplotype (two, similar to Tenerife and different from the rest of the populations studied as was previously reported, but the other two islands, El Hierro and La Palma, share their most common haplotype (three) with the continental and Madeira Island populations.

The mtDNA variability within populations has been measured by using the π statistic (Nei and Li 1979). Results on the diagonal of Table 5 show that whereas three of the Canarian populations (La Gomera, Gran Canaria, and Tenerife) have the highest levels of nucleotide diversity of all populations, the other two (El Hierro and La Palma) show the lowest ones. The total nucleotide diversity (π_T) for the mtDNA of this species is 0.0083, the same value (0.0081) obtained from a different set of populations by Afonso et al. (1990). The estimates of mtDNA divergence (δ) between populations are shown in Table 5 and a dendogram relating populations is presented in Figure 2d. The only significant branching is between two clusters, one grouping La Gomera, Gran Canaria, and Tenerife, and the other grouping the rest of the populations including El Hierro and La Palma.

When the N_{ST} statistic is applied, an important level of the total differentiation among populations (60%) is detected. The distribution of this heterogeneity within (34%) and among (26%) geographic areas resembles the population structure obtained with sex-linked loci. Again, most of the value (87.6%) of this differentiation is due to the Canary Islands samples.

In contrast with previous traits, there is a lack of correlation between geographical and mtDNA genetic distances either for all the samples ($r = -.126$; $P = .28$) or for the Canarian populations ($r = +.139$; $P = .27$).

Discussion

Different results have been obtained with the different markers used in this study. At the chromosomal level, the similarity between the Canarian and Madeira Islands and the difference with continental populations was explained supposing that isolated populations maintain primitive features in their chromosomal polymorphism due to the nonrecurrence of rearrangements, isolation, and the difficulty of establishing in one population the gene arrangements originated in another area because of a lack of coadaptation with the gene pool of the recipient population (Prevosti et al. 1975). This well-differentiated chromosomal population structure between islands and mainland is in contrast with the similarity found in allozyme types and frequencies between Madeira and the continental populations studied here and

Table 3. Standard genetic distances (Nei 1972) between populations estimated for autosomic loci (above the diagonal) and sex-linked loci (below the diagonal)

	Populations[a]										
	A	B	C	D	E	F[b]	H	I	K	L	M
A	—	0.012	0.011	0.001	0.005	0.220	0.113	0.132	0.114	0.139	0.158
B	0.002	—	0.021	0.025	0.001	0.154	0.060	0.078	0.061	0.091	0.113
C	0.156	0.150	—	0.014	0.017	0.236	0.124	0.150	0.127	0.153	0.161
D	0.148	0.127	0.003	—	0.025	0.252	0.139	0.149	0.139	0.167	0.174
E	0.003	0.003	0.172	0.161	—	0.157	0.064	0.088	0.069	0.086	0.112
F[b]	0.001	0.010	0.197	0.189	0.000	—	0.021	0.016	0.019	0.024	0.013
H	0.006	0.026	0.206	0.212	0.006	0.003	—	0.000	0.000	0.000	0.015
I	0.006	0.031	0.182	0.195	0.008	0.005	0.000	—	0.000	0.000	0.004
K	0.003	0.020	0.189	0.192	0.004	0.001	0.000	0.000	—	0.003	0.016
L	0.023	0.045	0.241	0.247	0.019	0.009	0.009	0.007	0.005	—	0.002
M	0.011	0.029	0.198	0.203	0.009	0.002	0.001	0.000	0.000	0.000	—

[a] Population letters correspond to letters in figure 1.

[b] Madeira pooled data.

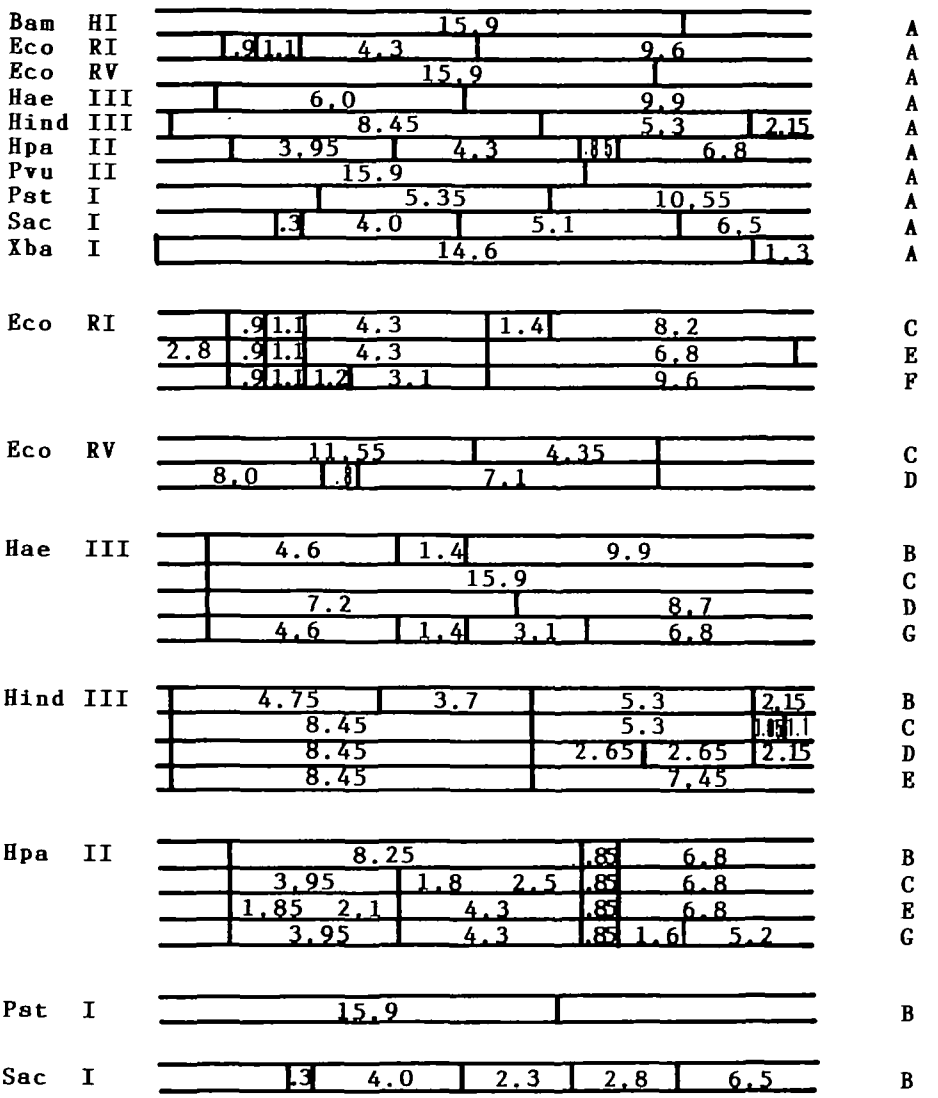

Figure 3. Mitochondrial DNA cleavage maps from the 10 enzymes that cut the molecule at least once. The 10 restriction enzymes producing the fragments indicated are listed on the left side; the capital letters designating the respective restriction morphs are listed on the right.

previously (Larruga et al. 1983), and resembles the same enzymatic similarity, in spite of chromosomal differentiation, found by Charlesworth et al. (1979) between British and European populations. Even among continental populations there are strong gene arrangement clines (Krimbas and Loukas 1980) that contrast with the homogeneity of the majority of the allozymic frequencies (Pinsker and Sperlich 1979; Saura et al. 1973), except those associated with closely linked gene arrangements (Cabrera et al. 1983; Loukas et al. 1979; Zouros et al. 1974). This discrepancy could be explained under the following assumptions: (1) gene arrangements have an adaptive role; (2) there is allozymic polymorphism within inversions due to recurrent gene mutation; (3) there is transfer of genetic information between gene arrangements due to recombination and/or gene conversion; and (4) enough gene flow exists to keep the allelic similarity among populations. There are clues that support all these requirements. About the adaptive role of the chromosomal inversion polymorphism are the repetition in the recently colonized New World, of the clines that this species shows in the Old World (Prevosti et al. 1988), and the effect that inversions have on patterns of chromosomal puffing activity (Latorre et al. 1988).

There is direct evidence in this species of genetic transfer between different chromosomal gene arrangements, either by double crossover or gene conversion in the *rp49* region (Rozas and Aguadé 1994). Important levels of gene flow are not only evident by the already noted allozymic homogeneity but are reinforced by the fact that continental populations show relatively little heterogeneity for mtDNA polymorphism (Afonso et al. 1990; Larruga et al. 1993; Latorre et al. 1992), despite the fact that this marker, owing to its clonal and maternal inheritance, is more sensitive to detect founder effects and population subdivision. It is the high similarity of the Madeira populations at enzymatic and mtDNA levels with the continental ones, in spite of their chromosomal differences, that strongly supports that migration from the mainland has played an important role in its genetic structure.

A completely different situation holds for the Canary Islands. It is clear that this is an old population, long isolated from the mainland as has been repeatedly demonstrated by previous studies at chromosomal (Prevosti 1971), enzymatic (Cabrera et al. 1980; Larruga et al. 1983), and molecular levels, including mtDNA (Afonso et

Table 4. Haplotype frequencies in the samples studied

Haplotypes[b]	Populations[a] A	B	C	D	E	F	G	H[c]	L	M
1 AAABAAAAAAB	4	—	—	—	1	—	—	—	—	—
2 AAABAAAAABB	14	13	1	—	45	—	—	—	—	—
3 AAAAAAAAACA	5	6	40	46	2	15	29	21	15	11
4 ACABAAAAABB	—	—	—	—	2	—	—	—	—	—
5 ACABAAAAACB	—	—	—	—	1	—	—	—	—	—
6 AAAAAAAAAAB	—	2	—	—	1	—	—	—	—	—
7 AAABAAAAAAA	—	—	—	—	3	—	—	1	—	—
8 AAABAAAAACA	—	2	—	—	3	—	2	3	—	—
9 AAABAAAAABA	—	—	—	—	2	—	—	—	—	—
10 AAABAABAABB	—	—	—	—	1	—	—	—	—	—
11 AAABAAAAACB	—	—	—	—	1	—	—	—	—	—
12 AAABBAAAABB	—	—	—	—	1	—	—	—	—	—
13 AAAAAAAAAAA	—	4	—	—	1	5	12	8	15	4
14 AACAAAAAAAA	—	—	—	—	—	—	1	—	—	—
15 AAAAAAAAADA	—	—	—	—	—	—	1	—	—	—
16 AAAABAAAACA	—	—	1	—	—	—	1	—	—	—
17 AAADAAAAAAA	—	—	—	—	—	—	1	—	—	—
18 AADAAAAAAAA	—	—	—	—	—	—	—	—	—	1
20 AAAAAAAAAAC	—	—	—	—	—	—	—	—	1	—
23 AAACAAAAACA	—	—	—	—	—	—	—	1	—	—
25 AAAEAAAAACA	—	—	—	—	—	—	—	—	1	—
26 AAAAAAAAACE	—	—	—	—	—	—	—	—	1	—
27 AEAAAAAAACA	—	—	—	1	—	—	—	—	—	—
28 AAAAAAAAACG	—	—	—	1	—	—	—	—	—	—
29 AFABAAAAABB	2	—	—	—	—	—	—	—	—	—
30 AAAAAAAAABA	—	1	—	—	—	—	—	—	—	—
31 AAAAAAAAABB	—	1	—	—	—	—	—	—	—	—
32 AAAAAAAAACB	—	1	—	—	—	—	—	—	—	—
33 AAABAAAAAGB	—	1	—	—	—	—	—	—	—	—
Total no. in sample	25	31	42	48	64	20	47	34	33	16

[a] Population letters correspond to letters in Figure 1.

[b] Haplotype designations correspond to the restriction patterns for *Bam*HI, *Eco*RI, *Eco*RV, *Hind*III, *Pst*I, *Pvu*II, *Sac*I, *Xba*I, *Xho*I, *Hae*III and *Hpa*II, in that order.

[c] Morocco pooled data.

al. 1990; Latorre et al. 1986) and nuclear single-copy genes (Rozas and Aguadé 1991). In addition, here we demonstrate that the Canarian populations are highly heterogeneous. This was not detected in previous chromosomic (Prevosti 1971) and autosomal enzymatic studies (Cabrera et al. 1980) because no important differences among islands exist for these markers as confirmed here (Figure 2a,b). But when sex-linked loci and mtDNA polymorphism are taken into account, two islands (El Hierro and La Palma) diverge from the remaining three (Figure 2c,d). The fact that these two discrepant islands are the youngest geologically, with origins of 0.75 (Ancochea et al. 1994) and 1.6 million years (Fuster et al. 1993), respectively, whereas the central ones have origin estimates of up to 16 million years for Gran Canaria (Abdel-Monem et al. 1971), 12 million years for La Gomera (Cantagrel et al. 1984), and 11 million years for Tenerife (Ancochea et al. 1990), points to differences among islands due to more recent and strong founder events in El Hierro and La Palma.

That populations from central islands are older is supported with mtDNA data by the abundance of endemic haplotypes (Table 4) and their levels of nucelotide diversity (Table 5), which are the highest of the sampled populations including the continental ones. In contrast El Hierro and La Palma show a low number of endemic haplotypes and the lowest levels of nucleotide diversity (Tables 4 and 5). On the other hand, strong founder effects are also evident by the fact that the Canary Islands endemic but frequent haplotype 2 (Table 4) is absent in the sample of La Palma and is very rare in El Hierro. Furthermore, the cosmopolitan haplotype 13 was not found in these islands (Table 4). Founder effects are also detectable with sex-linked allozymes since the most common allele for some loci (*Dia-4* and *Pgdh*) in El Hierro and La Palma are different from all other populations. In addition, it seems that some direct migration between these two islands has taken place since they exclusively shared the rare allele $G6pdh^{99}$. It is expected that sex-linked loci and mitochondrial genes are about one-third and three-fourths more sensitive to founder effects than autosomal allozymes under the supposition of neutrality for these genetic markers and equal migration rates for both sexes. It is possible to have a relative divergence rate between pairs of traits in the Canary Islands using k values as proposed by Crease et al. (1990). The rate of sex-linked and of mitochondrial to autosomal loci are $k = 6.5$ and $k = 23.9$, respectively. Both values, much higher than those expected under neutrality, could be explained by differences in migration between sexes, males having a greater mobility than females.

There are several hints favoring this explanation, if we assume that colonization of the new islands was mainly due to passive transport driven by the northwest-southeast dominant winds. In studies of dispersal capacity of *D. subobscura* it was detected that the wind had a stronger dragging effect on males than on females (Serra et al. 1987). This is in agreement with less activity in females than in males (Inglesfield and Begon 1983), with less-exposed habitat preferences for females (Cabrera et al. 1985) and with the field observation that females have a greater tendency than males to be trapped on the ground (Prevosti, personal communication).

Though significant correlation among allozymic and geographical distances exists for all the populations studied, and it is near to the significance for the Canarian Archipelago ($r = +.564$; $P = .07$), a step-

Table 5. Nucleotide diversity (on the diagonal) and DNA divergence (above the diagonal) multiplied by 10^2

	Populations[a] A	B	C	D	E	F	G	H[b]	L	M
A	0.625	0.035	0.918	0.979	0.006	0.822	0.774	0.744	0.730	0.787
B		0.800	0.525	0.570	0.117	0.438	0.405	0.389	0.368	0.411
C			0.093	0.000	1.193	0.016	0.027	0.023	0.083	0.028
D				0.033	1.263	0.021	0.034	0.030	0.091	0.033
E					0.407	1.082	1.025	0.990	0.969	1.041
F						0.156	0.000	0.000	0.015	0.000
G							0.267	0.000	0.009	0.000
H[b]								0.263	0.018	0.000
L									0.275	0.003
M										0.277

[a] Population letters correspond to letters in Figure 1.

[b] Morocco pooled data.

ping-stone model with directional east-west migration seems more appropriate to explain the successive colonization of the islands from an hypothetical African population. A Mantel test under this assumption effectively gives a stronger and now significant correlation ($r = -.606$; $P = .03$). In the same way, application of both models to the mtDNA data again shows higher correlation with the stepping-stone model ($r = -.485$; $P = .09$) than with the isolation by distance one ($r = +.139$; $P = .27$). In conclusion, in spite of the strong founder effects detected for the sexual loci and the mtDNA, the direction of colonization parallels the geological origin of the Canary Islands.

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