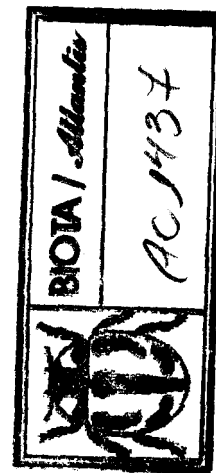


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ENZYMATIC POLYMORPHISM IN *DROSOPHILA SUBOBSCURA* POPULATIONS FROM THE CANARY ISLANDS

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Numerous studies of biochemical polymorphism in natural populations of *Drosophila* have demonstrated great similarity of the amount and pattern of genetic variation throughout the continuous distribution area of the species. Differences in allelic frequencies and/or in average heterozygosity are more frequent when isolated populations are compared (Stone et al., 1968; Prakach et al., 1969; Ayala et al., 1971; Johnson, 1971; Steiner et al., 1976; Prakash, 1977). We have undertaken a genetic analysis, using gel electrophoresis, of several island populations of *Drosophila subobscura*, a species distributed from Finland to Morocco, and from Western Asia to the Macaronesian Islands (the Canaries, Madeira and the Azores).

Whereas chromosomal polymorphism is geographically differentiated in *D. subobscura* (Sperlich and Feuerbach, 1969; Lakovaara and Saura, 1971; Saura et al., 1973; Zouros et al., 1974; Marinković et al., 1978). For the Canary Island and Madeira populations, Prevosti (1971, 1972) found that the degree of chromosomal polymorphism is much lower than in the nearby populations of Europe and North Africa. Furthermore, for a variety of reasons, he reached the conclusion that this reduced chromosomal polymorphism is a relict phenomenon corresponding to a less evolved stage than that present on the mainland. Therefore it seems probable that these island populations are genetically isolated from the continental ones.

In this first research report we have studied the genetic variation of 47 enzymatic loci in 10 natural populations of *Drosophila subobscura* collected in differ-

ent islands of the archipelago within their forests of conifers and laurisilva made up of a relict flora from the Tertiary with clearly differentiated ecological characteristics. Our purpose is to estimate the levels of enzymatic polymorphism of these isolated populations, compare them with the chromosomal polymorphism found in the Canaries by other authors, and determine whether or not there is some type of correlation between phenotypic and environmental differences.

MATERIAL AND METHODS

Samples from the following Canary Islands populations have been studied (Fig. 1): 1. El Pinar, El Hierro, 600 m altitude, in *Pinus canariensis* forest; 2. Jinarna, El Hierro; 800 m, in endemic laurel forest; 3. La Cumbrecita, La Palma; 1,500 m, in *P. canariensis* forest; 4. Los Tiles, La Palma; 800 m, in endemic laurel forest; 5. El Cedro, La Gomera; 1,000 m, in endemic laurel forest; 6. Ucanca, Tenerife; 2,200 m, in an artificial forest of *P. insignis*; 7. Las Raices, Tenerife; 900 m, in *P. canariensis* forest; 8. Llano los Viejos, Tenerife; 700 m, in endemic laurel forest; 9. Tamadaba, Gran Canaria; 1,200 m, in *P. canariensis* forest; and 10. Moya, Gran Canaria; 500 m, in a small patch of laurel forest.

No *D. subobscura* were found on the islands of Fuerteventura or Lanzarote, most probably due to their drier climate and absence of forests.

Flies were collected with fermenting banana bait, from February to March 1977. The males captured were immediately used for electrophoretic analyses. Each female was placed in an individual

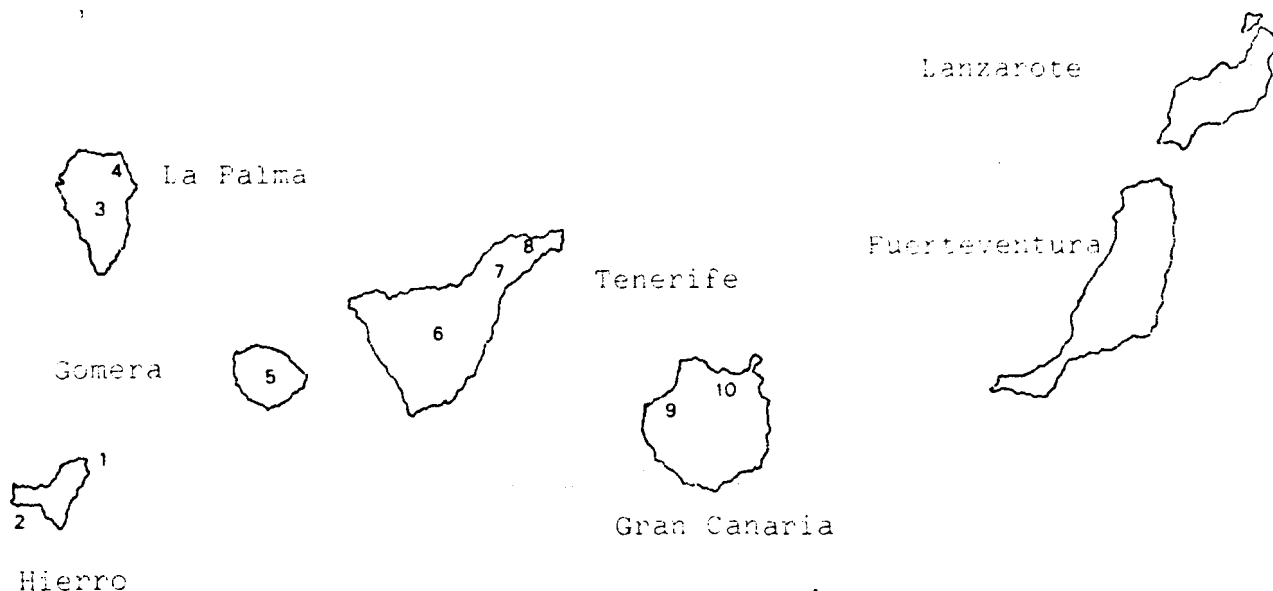


FIG. 1. Canary Islands populations studied. See the localities' names in the text.

culture for five days and then transferred into fresh vials three consecutive times in order to obtain abundant offspring. One F_1 larva, pupa or adult from each female was later assayed.

The nomenclature for the gene loci coding for enzymes and their alleles are those described by Hubby and Lewontin (1966) and Ayala et al. (1972).

Sample preparation and techniques for gel electrophoresis are those described by Ayala et al. (1972) employing only Sigma starch. The following enzymes were assayed and generally the staining methods were those described in the references: Acid phosphatase (*Acph-1*) (Shaw and Prasad, 1970); Adenylate kinase, two loci (*Ak-1* and 2), Aldolase (*Ald*), Catalase (*Cal*), Hexokinase, three loci (*Hk-1*, 2 and #), Leucine aminopeptidase, four loci (*Lap-4*, 5, 6 and 7), Phosphoglucomutase, two loci (*Pgm-1* and 2), 6-Phosphogluconate dehydrogenase (*6-Pgdh*), and Phosphohexose isomerase (*Phi*), according to Brewer (1970); Alcohol dehydrogenase (*Adh*), Alkaline phosphatase, seven loci (*Aph-1*, 2, 3, 4, 5, 6 and 7), Esterase, five loci (*Est-1*, 4, 7 and 10), Glyceraldehyde-3-phosphate dehydrogenase (*G3pdh*), α -Glycerophosphate dehydrogenase (α -*Gpdh*), Isocitrate dehydrogenase (*Idh*), Malate dehydrogenase (*Mdh-2*), Malic en-

zyme, two loci (*Me-1* and #), Octanol dehydrogenase (*Odh*), Tetrazolium oxidase (*To*), and Triosephosphate isomerase (*Tpi-2*), according to Ayala et al. (1972); Aldehyde oxidase, two loci (*Ao-1* and 2), and Xanthine dehydrogenase (*Xdh*), according to Ayala et al. (1974a); Glutamate oxaloacetate transaminase, two loci (*Got-1* and 2) (Grell, 1976); Hydroxybutyrate dehydrogenase (*Hbdh*), Fumerase, two loci (*Fum-1* and 2) and Glucose-6-phosphate dehydrogenase (*G6pdh*), according to Ayala et al. (19748). Minor modifications are as follows: *Aph-1*, 2, 3, 5 and 6 were assayed in third instar larvae; *Aph-4* and # in adult; 10 g NaCl/100 ml buffer was added to the staining solution and 500 mg of $MgCl_2$ was added to the electrophoresis buffer. *Est-5* was assayed in third instar larvae or early pupae, *Est-1*, 4, 7 and 10 were assayed in adults. The agar overlay method (Brewer, 1970) was used to stain *G3pdh* and *Ak-1* and 2. *Lap-4* was assayed in late pupae; *Lap-5*, 6 and 7 were assayed in adults. 0.1% Triton X100 (Sigma) was added to the homogenates and 0.1% bovine albumin to homogenates and gel for *Got-1* and 2 detection.

Lakovaara and Saura (1971), and Saur et al. (1973) in Finland, Zouros et al. (1974) in Greece, Marinković et al. (1978) in Yugoslavia and Pinsker et al. (1978) in

TABLE 1. Allelic frequencies at 25 polymorphic gene loci for enzymes in ten natural populations of *D. subobscura* from the Canary Islands.

Gene		Locality										Mean heterozygosity
Chrom.	Allele	1	2	3	4	5	6	7	8	9	10	
<i>Acph-1</i> O	Sample	66	76	148	120	114	148	130	202	110	132	0.098 ± 0.013
	.97	—	0.030	0.006	—	0.017	—	—	—	—	0.017	
	1.00	0.970	0.910	0.959	0.958	0.047	0.939	0.953	0.985	0.901	0.917	
	1.05	0.030	0.060	0.035	0.042	0.036	0.061	0.047	0.015	0.093	0.036	
	Obs. het.	0.061	0.157	0.080	0.050	0.070	0.088	0.092	0.030	0.171	0.106	
Exp. het.		0.058	0.166	0.079	0.080	0.101	0.112	0.080	0.029	0.169	0.102	
<i>Ala-2</i> Uii kn.	Sample	60	62	70	76	94	107	72	72	120	120	0.110 ± 0.019
	.96	0.050	0.016	0.043	0.039	0.030	0.020	0.027	0.028	0.041	0.042	
	1.00	0.950	0.984	0.886	0.883	0.961	0.970	0.950	0.972	0.933	0.914	
	1.10	—	—	0.071	0.078	0.009	0.010	0.014	—	0.026	0.044	
	Obs. het.	0.100	0.032	0.243	0.184	0.064	0.029	0.083	0.055	0.158	0.166	
Exp. het.		0.095	0.031	0.208	0.213	0.075	0.058	0.079	0.054	0.127	0.161	
<i>Adh</i> U	Sample	92	80	140	150	260	140	220	224	142	146	0.186 ± 0.000
	1.00	0.923	0.875	0.857	0.893	0.904	0.900	0.900	0.928	0.887	0.890	
	1.67	0.077	0.125	0.143	0.107	0.096	0.100	0.100	0.072	0.113	0.110	
	Obs. het.	0.152	0.200	0.200	0.133	0.177	0.371	0.182	0.125	0.141	0.192	
	Exp. het.	0.142	0.219	0.245	0.191	0.173	0.180	0.180	0.134	0.200	0.196	
<i>Ala-1</i> Unkn.	Sample	90	94	138	140	120	148	140	120	152	140	0.020 ± 0.010
	1.00	0.989	0.990	0.971	0.986	1.000	1.000	0.993	0.942	1.000	1.000	
	1.02	0.011	0.010	0.029	0.014	—	—	0.007	0.058	—	—	
	Obs. het.	0.022	0.021	0.058	0.028	—	—	0.007	0.053	—	—	
	Exp. het.	0.021	0.019	0.056	0.028	—	—	0.019	0.105	—	—	
<i>Ala-2</i> O	Sample	90	94	140	140	120	148	140	124	152	140	0.030 ± 0.011
	1.00	0.967	0.968	0.972	1.000	0.990	1.000	1.000	0.944	1.000	1.000	
	1.02	0.033	0.032	0.028	—	0.010	—	—	0.056	—	—	
	Obs. het.	0.067	0.064	0.057	—	0.017	—	—	0.050	—	—	
	Exp. het.	0.064	0.061	0.054	—	0.019	—	—	0.106	—	—	
<i>Aph-3</i> I	Sample	124	114	158	94	122	100	116	232	246	210	0.508 ± 0.007
	.73	0.548	0.570	0.481	0.585	0.434	0.570	0.439	0.517	0.467	0.438	
	1.00	0.452	0.430	0.436	0.404	0.557	0.410	0.551	0.470	0.533	0.547	
	1.27	—	—	0.083	0.011	0.009	0.020	0.010	0.013	—	0.015	
	Obs. het.	0.548	0.509	0.640	0.574	0.409	0.540	0.517	0.539	0.488	0.519	
Exp. het.		0.495	0.490	0.572	0.494	0.501	0.507	0.504	0.512	0.498	0.509	

TABLE 1. Continued.

Gene		Locality										Mean heterozygosity
Chrom.	Allele	1	2	3	4	5	6	7	8	9	10	
<i>Est-10</i>	Sample	58	64	64	64	32	42	100	94	76	70	0.061 ± 0.020
Unkn.	.95	—	—	0.109	0.062	—	—	0.030	0.021	0.060	0.020	
	1.00	1.000	1.000	0.891	0.938	1.000	1.000	0.970	0.979	0.934	0.971	
Obs. het.	—	—	—	0.156	0.125	—	—	0.060	0.064	0.132	0.057	
Exp. het.	—	—	—	0.194	0.116	—	—	0.058	0.061	0.123	0.056	
<i>α-Gpdh</i>	Sample	84	80	142	148	260	140	148	132	148	140	0.023 ± 0.012
U	.98	—	—	0.021	0.020	0.007	—	—	0.007	0.006	—	
	1.00	1.000	1.000	0.930	0.974	0.993	1.000	0.904	0.993	0.494	1.000	
	1.02	—	—	0.049	0.006	—	—	0.006	—	—	—	
Obs. het.	—	—	—	0.112	0.054	0.008	—	0.010	0.010	0.010	—	
Exp. het.	—	—	—	0.132	0.051	0.014	—	0.012	0.014	0.012	—	
<i>Gol-1</i>	Sample	—	40	70	60	—	60	54	00	60	—	0.117 ± 0.027
Unkn.	.95	—	0.025	0.057	0.033	—	—	0.055	0.060	0.050	—	
	1.00	—	0.975	0.872	0.901	—	1.000	0.927	0.934	0.950	—	
	1.05	—	—	0.071	0.066	—	—	0.018	—	—	—	
Obs. het.	—	—	0.050	0.200	0.133	—	—	0.148	0.133	0.100	—	
Exp. het.	—	—	0.049	0.231	0.183	—	—	0.137	0.123	0.095	—	
<i>Got-2</i>	Sample	90	78	106	86	146	94	80	128	138	144	0.509 ± 0.005
U	.80	0.555	0.615	0.537	0.593	0.541	0.563	0.412	0.476	0.543	0.541	
	1.00	0.411	0.385	0.424	0.384	0.445	0.437	0.551	0.507	0.435	0.444	
	1.10	0.034	—	0.039	0.023	0.014	—	0.037	0.017	0.022	0.015	
Obs. het.	—	0.488	0.461	0.500	0.560	0.465	0.447	0.475	0.546	0.492	0.403	
Exp. het.	—	0.524	0.474	0.530	0.500	0.509	0.492	0.525	0.516	0.513	0.510	
<i>Hk-1</i>	Sample	94	82	266	120	128	136	134	202	136	200	0.505 ± 0.036
E	.73	0.383	0.232	0.229	0.216	0.070	0.286	0.261	0.143	0.051	0.100	
	1.00	0.585	0.695	0.614	0.625	0.812	0.529	0.440	0.629	0.838	0.575	
	1.27	0.032	0.061	0.120	0.159	0.101	0.139	0.261	0.218	0.066	0.315	
	1.43	—	0.012	0.037	—	0.017	0.046	0.038	0.010	0.045	0.010	
Obs. het.	—	0.489	0.463	0.534	0.566	0.453	0.544	0.612	0.564	0.250	0.420	
Exp. het.	—	0.510	0.459	0.554	0.537	0.325	0.616	0.668	0.537	0.289	0.560	
<i>Hk-2</i>	Sample	80	84	146	120	128	124	126	48	124	206	
Unkn.	.96	0.062	0.024	0.096	0.083	0.039	0.089	0.015	0.061	0.008	0.092	
	1.00	0.687	0.797	0.828	0.833	0.883	0.790	0.897	0.837	0.943	0.757	
	1.05	0.251	0.179	0.076	0.084	0.078	0.121	0.088	0.102	0.049	0.151	

TABLE 1. *Continued.*

Gene		Locality										Mean heterozygosity
Chrom.	Allele	1	2	3	4	5	6	7	8	9	10	
<i>Aph-4</i>	Sample	—	—	108	56	118	—	90	96	100	134	0.054 ± 0.020
Unkn.	.5	—	—	0.222	0.142	0.186	—	0.155	0.187	0.120	0.097	
	1.00	—	—	0.778	0.858	0.814	—	0.845	0.813	0.880	0.903	
Obs. het.		—	—	0.417	0.250	0.229	—	0.222	0.292	0.160	0.157	
Exp. het.		—	—	0.345	0.250	0.303	—	0.262	0.304	0.211	0.175	
<i>Aph-5</i>	Sample	60	46	46	120	—	102	116	100	120	74	0.072 ± 0.015
Unkn.	.97	—	0.020	0.043	0.042	—	0.010	0.015	0.020	0.016	0.007	
	1.00	0.990	0.980	0.914	0.948	—	0.990	0.951	0.070	0.984	0.933	
	1.03	0.010	—	0.043	0.010	—	—	0.034	0.010	—	—	
Obs. het.		0.033	0.040	0.174	0.100	—	0.020	0.086	0.060	0.033	0.135	
Exp. het.		0.020	0.039	0.161	0.099	—	0.020	0.094	0.050	0.031	0.125	
<i>Aph-6</i>	Sample	66	50	46	90	—	96	116	100	120	74	0.056 ± 0.030
Unkn.	.98	—	—	0.174	—	—	—	0.077	0.010	0.016	0.013	
	1.00	1.000	1.000	0.826	1.000	—	1.000	0.923	0.990	0.984	0.987	
Obs. het.		—	—	0.343	—	—	—	0.121	0.020	0.033	0.027	
Exp. het.		—	—	0.287	—	—	—	0.142	0.020	0.031	0.026	
<i>Aph-7</i>	Sample	60	46	46	90	—	100	120	88	120	74	0.022 ± 0.008
Unkn.	1.00	1.000	0.979	1.000	1.000	—	-0.990	0.992	1.000	0.975	0.960	
	1.02	—	0.021	—	—	—	0.010	0.008	—	0.025	0.040	
Obs. het.		—	0.043	—	—	—	0.020	0.008	—	0.050	0.081	
Exp. het.		—	0.041	—	—	—	0.021	0.010	—	0.048	0.076	
<i>Est-5</i>	Sample	58	64	64	64	32	42	116	96	76	70	0.065 ± 0.012
O	.98	—	0.021	0.030	0.046	0.032	—	0.060	0.010	0.011	0.030	
	1.00	0.980	0.979	0.970	0.921	0.968	1.000	0.920	0.960	0.978	0.960	
	1.02	0.020	—	—	0.033	—	—	0.020	0.030	0.011	0.010	
Obs. het.		0.034	0.062	0.093	0.125	0.062	—	0.060	0.083	0.052	0.086	
Exp. het.		0.039	0.041	0.058	0.142	0.061	—	0.118	0.976	0.042	0.076	
<i>Est-7</i>	Sample	58	64	124	124	32	42	116	94	80	70	0.320 ± 0.019
1	.95	0.224	0.078	0.130	0.210	0.157	0.249	0.215	0.180	0.212	0.157	
	1.00	0.776	0.891	0.860	0.774	0.812	0.720	0.785	0.820	0.776	0.812	
	1.06	—	0.031	0.010	0.016	0.031	0.040	—	—	0.012	0.031	
Obs. het.		0.310	0.219	0.258	0.354	0.317	0.380	0.293	0.276	0.375	0.286	
Exp. het.		0.347	0.197	0.242	0.383	0.315	0.421	0.333	0.295	0.352	0.315	

TABLE 1. *C* continued.

Gene		Locality										Mean heterozygosity
Chrom.	Allele	1	2	3	4	5	6	7	8	9	10	
Obs. het.		0.425	0.333	0.205	0.166	0.234	0.306	0.174	0.292	0.113	0.388	0.286 ± 0.031
Exp. het.		0.461	0.332	0.229	0.292	0.213	0.353	0.187	0.285	0.108	0.396	
<i>Idh</i>	Sample	76	80	124	100	120	148	136	126	140	118	
1	.97	—	—	—	—	—	0.007	0.015	0.005	—	—	
	1.00	0.974	0.988	0.976	1.000	1.000	0.980	0.970	0.984	1.000	1.000	
	1.04	0.026	0.012	0.024	—	—	0.013	0.015	0.008	—	—	
Obs. het.		0.052	0.025	0.048	—	—	0.014	0.059	0.032	—	—	0.025 ± 0.007
Exp. het.		0.051	0.024	0.047	—	—	0.039	0.059	0.032	—	—	
<i>Lap-4</i>	Sample	90	102	110	140	126	148	140	120	180	148	
O	.86	0.044	0.068	0.037	0.057	0.054	0.027	0.036	0.025	0.013	0.020	
	1.00	0.844	0.843	0.854	0.830	0.866	0.932	0.907	0.925	0.940	0.912	
	1.11	0.112	0.089	0.109	0.113	0.080	0.041	0.057	0.050	0.047	0.068	
Obs. het.		0.222	0.274	0.214	0.271	0.253	0.095	0.128	0.133	0.120	0.175	0.208 ± 0.020
Exp. het.		0.277	0.280	0.257	0.294	0.239	0.130	0.173	0.142	0.125	0.165	
<i>Mdh-2</i>	Sample	90	94	108	140	120	140	140	124	152	148	
U	1.00	0.956	0.914	0.920	0.910	0.960	0.930	0.970	0.930	0.960	0.950	
	1.08	0.044	0.086	0.080	0.090	0.040	0.070	0.030	0.070	0.040	0.050	
Obs. het.		0.088	0.191	0.137	0.128	0.083	0.144	0.057	0.129	0.079	0.108	
Exp. het.		0.084	0.157	0.147	0.163	0.076	0.130	0.058	0.130	0.076	0.095	0.112 ± 0.011
<i>Lie-2</i>	Sample	—	—	116	120	120	—	—	120	140	132	
Unkn.	1.00	—	—	0.966	0.967	0.983	—	—	0.983	0.903	0.970	
	1.04	—	—	0.034	0.033	0.017	—	—	0.017	0.007	0.030	
Obs. het.		—	—	0.086	0.066	0.033	—	—	0.033	0.914	0.076	
Exp. het.		—	—	0.065	0.063	0.033	—	—	0.033	0.013	0.058	0.044 ± 0.00;
<i>Odh</i>	Sample	90	94	140	150	140	140	148	80	126	148	
O	.86	—	—	0.014	0.013	—	0.007	0.007	0.012	0.008	0.020	
	1.00	1.000	1.000	0.958	0.974	1.000	0.986	0.993	0.988	0.992	0.980	
	1.14	—	—	0.028	0.013	—	0.007	—	—	—	—	
Obs. het.		—	—	0.086	0.013	—	0.028	0.013	0.025	0.016	0.040	0.025 ± 0.007
Exp. het.		—	—	0.081	0.038	—	0.038	0.013	0.023	0.015	0.039	
<i>Pgm-1</i>	Sample	76	80	122	100	120	100	150	132	120	126	
1	.94	0.065	0.012	0.032	0.030	0.008	0.010	0.066	0.059	0.066	0.047	
	1.00	0.909	0.963	0.895	0.920	0.976	0.990	0.894	0.926	0.918	0.945	
	1.06	0.026	0.025	0.073	0.050	0.016	—	0.040	0.015	0.016	0.008	

TABLE 1. Continued.

Gene	Allele	Locality										Mean heterozygosity
		1	2	3	4	5	6	7	8	9	10	
Chrom.												
Obs. het.		0.157	0.075	0.213	0.140	0.050	0.020	0.173	0.151	0.166	0.111	
Exp. het.		0.168	0.071	0.193	0.150	0.047	0.020	0.195	0.139	0.153	0.105	0.124 ± 0.018
<i>Pgm-2</i>	Sample	76	80	—	—	—	100	150	74	116	114	
U	1.00	0.950	0.998	—	—	—	0.950	0.967	0.973	0.957	0.939	
	1.04	0.050	0.002	—	—	—	0.050	0.033	0.027	0.043	0.061	
Obs. het.		0.105	0.025	—	—	—	0.100	0.067	0.054	0.086	0.088	
Exp. het.		0.095	0.024	—	—	—	0.095	0.063	0.052	0.082	0.114	0.071 ± 0.013
Total loci:		44	45	46	46	42	45	46	47	47	46	47
Mean:		0.078	0.071	0.108	0.093	0.072	0.072	0.087	0.081	0.070	0.085	0.082 ± 0.004
Standard error:		0.023	0.019	0.023	0.021	0.020	0.023	0.022	0.020	0.018	0.021	0.019

Germany have analyzed the enzymatic polymorphism in populations of *D. subobscura* from different regions of Europe. In this paper comparisons are made between their enzymatic loci and ours. Such comparisons should be considered as merely tentative. The techniques and nomenclature used by the groups from Finland, Yugoslavia and Germany are practically the same with the exception of the denomination of the esterases (Marinković et al., 1978). We have also tried to adhere to this nomenclature with the only exceptions being that our *Est-7* seems to be the *Est-8* of Lakovaara and Saura (*Est-5* for Marinković et al.) and our *Hk-3* is the *Hk-3* of these authors. This correspondence has been based on the similarity of the majority of techniques used and on Dr. Ayala's comments on our data and photographic material. The comparisons with data from the Greek group and the others has been based on their own work (Zouros et al., 1974) and on the chromosomal localization of their loci and ours. For this reason we believe that their *Est-5* and 7 are precisely our *Est-5* and 7.

When the levels of enzymatic polymorphism permitted our doing so, statistical comparisons were made between populations within and between islands using a standard row \times column χ^2 test (Snedecor and Cochran, 1969) to determine if significant differences existed between allelic frequencies per locus.

RESULTS

The enzyme loci *Adh*, *Got-2* and *Hbdh* exhibit a cathodal migration; the remaining loci are anodal.

Aph-5 and *Aph-6* show individuals with low or no activity. Although this substantial variation may be clue to null alleles as described in *Aph-6* of *Drosophila pseudoobscura* (Hubby and Lewontin, 1966) we have not taken it into account and in individuals with no activity have not been included in this paper.

The *Got-2* locus, monomorphic or moderately polymorphic in most *Drosophila* species previously studied, are very poly

TABLE 2. χ^2 contingency tests for differences of allelic frequencies.

Loci	Within islands				Between islands
	Hierro	Palma	Tenerife	G. Canaria	
<i>Acph</i>	1.02 n.s. <i>d.f.</i> = 2	0.11 n.s. <i>d.f.</i> = 2	5.37 n.s. <i>d.f.</i> = 2	5.34 n.s. <i>d.f.</i> = 2	4.24 n.s. <i>d.f.</i> = 4
<i>Adh</i>	1.15 n.s. <i>d.f.</i> = 1	0.87 n.s. <i>d.f.</i> = 1	1.39 n.s. <i>d.f.</i> = 2	0.01 n.s. <i>d.f.</i> = 1	3.01 n.s. <i>d.f.</i> = 4
<i>Aph-3</i>	0.11 n.s. <i>d.f.</i> = 1	6.82 <i>P</i> < 0.05 <i>d.f.</i> = 2	4.71 n.s. <i>d.f.</i> = 4	3.78 n.s. <i>d.f.</i> = 2	15.78 <i>P</i> < 0.05 <i>d.f.</i> = 8
<i>Aph-4</i>	—	1.48 n.s. <i>d.f.</i> = 1	0.33 n.s. <i>d.f.</i> = 1	0.32 n.s. <i>d.f.</i> = 1	7.29 n.s. <i>d.f.</i> = 3
<i>Est-7</i>	6.69 <i>P</i> < 0.05 <i>d.f.</i> = 2	3.31 n.s. <i>d.f.</i> = 2	0.89 n.s. <i>d.f.</i> = 2	1.17 n.s. <i>d.f.</i> = 2	10.47 n.s. <i>d.f.</i> = 10
<i>Got-1</i>	—	0.44 n.s. <i>d.f.</i> = 2	3.95 n.s. <i>d.f.</i> = 4	—	10.34 n.s. <i>d.f.</i> = 6
<i>Got-2</i>	2.33 n.s. <i>d.f.</i> = 2	0.77 n.s. <i>d.f.</i> = 2	3.00 n.s. <i>d.f.</i> = 4	0.26 n.s. <i>d.f.</i> = 2	7.47 n.s. <i>d.f.</i> = 8
<i>Hk-1</i>	5.50 n.s. <i>d.f.</i> = 2	0.85 n.s. <i>d.f.</i> = 2	18.41 <i>P</i> < 0.005 <i>d.f.</i> = 4	25.16 <i>P</i> < 0.001 <i>d.f.</i> = 2	132.63 <i>P</i> < 0.001 <i>d.f.</i> = 14
<i>Hk-2</i>	3.08 n.s. <i>d.f.</i> = 2	0.17 n.s. <i>d.f.</i> = 2	7.79 n.s. <i>d.f.</i> = 4	19.48 <i>P</i> < 0.001 <i>d.f.</i> = 2	46.15 <i>P</i> < 0.001 <i>d.f.</i> = 10
<i>Lap-4</i>	0.74 n.s. <i>d.f.</i> = 2	0.62 n.s. <i>d.f.</i> = 2	0.76 n.s. <i>d.f.</i> = 4	0.85 n.s. <i>d.f.</i> = 2	21.33 <i>P</i> < 0.01 <i>d.f.</i> = 8
<i>Mdh-2</i>	1.25 n.s. <i>d.f.</i> = 1	0.70 n.s. <i>d.f.</i> = 1	3.21 n.s. <i>d.f.</i> = 2	0.11 n.s. <i>d.f.</i> = 1	6.00 n.s. <i>d.f.</i> = 4
<i>Pgm-1</i>	3.00 n.s. <i>d.f.</i> = 2	0.55 n.s. <i>d.f.</i> = 2	9.87 <i>P</i> < 0.05 <i>d.f.</i> = 4	0.83 n.s. <i>d.f.</i> = 2	29.10 <i>P</i> < 0.005 <i>d.f.</i> = 12
<i>Pgm-2</i>	2.02 n.s. <i>d.f.</i> = 1	—	0.74 n.s. <i>d.f.</i> = 2	0.39 n.s. <i>d.f.</i> = 1	1.18 n.s. <i>d.f.</i> = 2

n.s. = not significant at the 5% level.

morphic in all of the *D. subobscura* presented herein.

No variation, or only an occasional polymorphism, has been detected in the following 22 loci: *Ak-1*, *Ald*, *Aph-1*, *Aph-2*, *Cat*, *Est-1*, *Est-4*, *Fum-1*, *Funt-2*, *G3pdh*, *G6pdh*, *Hk-4*, *Hbdh*, *Lap-5*, *Lap-6*, *Lap-7*, *Me-1*, *6Pgdh*, *Phi*, *To*, *Tpi-2* and *Xdh*.

The remaining 25 loci were shown to be polymorphic in almost all populations. For each locus Table 1 shows the frequencies of the alleles detected in each population, the average heterozygosity observed, and the average heterozygosity expected in the Hardy-Weinberg equilibrium hypothesis. In no case did the χ^2 test reveal significant differences between

these. The average of the heterozygosities expected is 0.082 ± 0.019 , somewhat higher than, although not significantly different from the observed averages: 0.80 ± 0.020 . Table 2 groups those loci which because of their level of polymorphism permit the realization of a χ^2 test for contingency in order to evaluate the differences of allelic frequency between populations. Significant differences were found on or between islands in the following 6: *Aph-3*, *Est-7*, *Hk-1*, *Hk-2*, *Lap-4* and *Pgm-1*.

Aph-3 has the allele 1.27 with a frequency of 8% in population 3 whereas in population 4 the frequency is 1%, the most significant difference within the island of La Palma; in addition the most common

allele is not the same in all of the islands. The allelic frequencies of *Hk-1*, *Hk-2*, *Lap-4* and *Pgm-1* vary appreciably among populations even though the most common allele is the same for all of them. The remaining loci are less polymorphic; the most frequent allele is the same for all populations, and in some of these it is fixed. Although the small frequencies of certain alleles did not allow the evaluation of their differences by contingency χ^2 tests, direct examination of the data reveals clear differences between different populations. For example, *Ao-1* and *Ao-2* appear to be fixed for the populations on the island of Gran Canaria (9 and 10) and for one population on the island of Tenerife (6). The allele 1.10 of *Ak-2* does not appear in populations 1 and 2 (Hierro), whereas in 3 and 4 (La Palma) it is found with an average frequency of 7%. The allele 0.98 of *Aph-6* is absent in populations 1 and 2 (Hierro), in 4 (La Palma) and 6 (Tenerife), but has a frequency of 17% in population 3 (La Palma). The *Est-10* appears to be fixed in populations 1 and 2 (Hierro), in 5 (Gomera) and in 6 (Tenerife), being moderately polymorphic in the remaining populations.

The significant differences found within the islands for allelic frequencies of some loci do not seem to be correlated with differences in vegetation.

DISCUSSION

The enzyme polymorphism in natural populations of *D. subobscura* from the European continent has been investigated at some length: Lakovaara and Saura (1971) in marginal populations from Finland; Saura et al. (1973) in marginal populations from Finland, submarginal from Sweden and Denmark, and central from France and the Alps; Zouros et al. (1974), in meridional populations from Greece; Marinković et al. (1978) in a central population from Yugoslavia and Pinsker et al. (1978) in a central population of Germany.

In spite of the differences found between regions, Lakovaara and Saura (1971) and Saura et al. (1973) concluded that the enzyme polymorphism in *D. sub-*

obscura is rather uniform throughout the distributional range of the species, in contrast to what occurs in chromosomal polymorphism, where differences between marginal and central regions may be observed (Kunze-Mühl et al., 1958; Sperlich and Kunze-Mühl, 1963; Sperlich, 1964; Prevosti, 1966; Götz, 1967; Krimbas, 1967; Jungen, 1968; Sperlich and Feuchbach, 1969; Prevosti, 1971, 1972, 1974).

Pinsker et al. (1978) find differences in successive periods of time in the Gerinan population studied by them for the loci *Aph-3* and *Lap-4*.

Mean individual heterozygosity for continental regions (calculated from the enzymatic polymorphism given by the authors cited above) is higher than that measured for the populations of the Canary Archipelago (0.162 vs 0.082). However, notable differences exist in the number and set of loci analyzed by different authors. In order to reduce this source of variation we have based our comparison only on loci studied in the Canaries as well as in some other area (Table 3). This table shows 7 loci (*Adh*, α -*Gpdh*, *Hk-1*, *Lap-4*, *Mdh-2*, *Me-1* and *Odh*) that have been analyzed in all regions. *Ak-1* and *Ak-2* have been studied only in Yugoslavia and the Canaries and *Aph-7* only in Finland and the Canaries. Of the 33 loci that appear in this table for the Canary populations, 24 have also been studied by Marinković et al. (1978) in Yugoslavia, but only 15 by Pinsker et al. (1978) in Germany; the number of loci studied for the remaining regions varies between these two limits. In the row entitled "Canaries" the average heterozygosities of the seven groups of loci are given which in the Canary populations correspond to those of each of the seven regions compared. The average heterozygosity of the Canary populations continues to be lower in all cases than that of other regions. In the row entitled "Common loci" the average heterozygosities by region for the seven loci common to all of them are given. Here the heterozygosity of the Canary populations is more similar to that of the other regions.

When a locus by locus comparison is

TABLE 3. Mean heterozygosity per locus in *D. subobscura* populations from different geographic areas.

Loci	Canaries	Yugoslavia ¹	Greece ²	France ³	Alps ⁴	Germany ⁴	S. Scandinavia ⁵	Finland ⁵
<i>Adh</i>	0.185	0.035	0.012	0.040	0.000	0.060	0.035	0.046
<i>Ak-1</i>	0.000	0.043						
<i>Ak-2</i>	0.117	0.163						
<i>Ald</i>	0.003	0.000	0.000					
<i>Ao-1</i>	0.026	0.111						
<i>Ao-2</i>	0.030	0.607	0.411	0.538		0.157		0.202
<i>Aph-3</i>	0.508		0.494	0.510	0.462	0.513	0.183	0.507
<i>Aph-5</i>	0.072			0.131	0.033		0.046	0.128
<i>Aph-7</i>	0.021							0.096
<i>Est-1</i>	0.000		0.000	0.000	0.000		0.000	0.000
<i>Est-4</i>	0.000	0.000	0.000	0.000	0.000		0.000	0.000
<i>Est-5</i>	0.065		0.531					
<i>Est-7</i>	0.320	0.499	0.168	0.598	0.350		0.577	0.667
<i>Est-10</i>	0.066	0.049						0.234
<i>Fum</i>	0.005		0.000					
α - <i>Gpdh</i>	0.023	0.024	0.000	0.023	0.000	0.064	0.005	0.011
<i>G3pdh</i>	0.003	0.164						
<i>G6pdh</i>	0.007							0.000
<i>Hk-1</i>	0.514	0.412	0.603	0.316	0.460	0.518	0.585	0.526
<i>Hk-2</i>	0.285	0.417						
<i>Hk-3</i>	0.002	0.032		0.068	0.061	0.000	0.000	0.006
<i>Idh</i>	0.025	0.000		0.007	0.012	0.036	0.010	0.015
<i>Lap-4</i>	0.208	0.271	0.436	0.455	0.463	0.576	0.595	0.433
<i>Mdh-2</i>	0.111	0.016	0.020	0.056	0.040	0.053	0.061	0.068
<i>Me-1</i>	0.000	0.075	0.000	0.485	0.063	0.123	0.135	0.206
<i>Me-2</i>	0.044	0.045						
<i>Odh</i>	0.024	0.012	0.135	0.072	0.129	0.081	0.083	0.064
<i>Pgm-1</i>	0.143	0.149		0.105	0.128	0.134	0.111	0.121
δ <i>Pgdh</i>	0.000					0.076		
<i>Phi</i>	0.000					0.049		
<i>To</i>	0.000	0.018	0.000					0.042
<i>Tpi-2</i>	0.000	0.024		0.000	0.000	0.020	0.000	0.000
<i>Xdh</i>	0.003	0.000	0.457	0.470	0.460			0.470
Total loci:	33	24	17	18	17	15	16	22
hlean:	0.085	0.132	0.192	0.215	0.156	0.164	0.170	0.174
Canaries		0.088	0.117	0.119	0.124	0.118	0.132	0.102
Common loci:	0.152	0.121	0.172	0.207	0.165	0.211	0.214	0.193

¹ By Marinković et al. (1978).² By Zouros et al. (1974).³ By Saura et al. (1973).⁴ By Pinsker et al. (1978).⁵ By Lakovaara and Saura (1971).

made, certain continental as well as Canary Island loci are very to moderately polymorphic (*Aph-3*, *Est-7*, *Hk-1*, *Pgm-1*) or monomorphic or slightly polymorphic (*Ald*, *Est-1*, *Est-4*, *Fum*, α -*Gpdh*, *G6pdh*, *Hk-4*, *Idh*, δ *Pgdh*, *Phi*, *To*, *Tpi-2*). Nevertheless, certain differences do exist. *Adh* and *Mdh-2* are more polymorphic in the Canaries than in any of the continental populations, while *Ao-2*, *Est-5*, *Lap-4*, *Me-1*, *Odh* and *Xdh* are less variable in

the islands than is the mean on the continent. However in some continental regions some of these loci are as monomorphic as in the Canaries. Such is the case of *Me-1* in the Greek populations and *Xdh* in Yugoslavia.

Of the six loci for which we find significant differences in the allelic frequencies among some of our populations (Table 2) there are four for which differences are also found by Lakovaara and Saura (1971)

anri Saura et al. (1973), and in addition, for two of these same loci (*Aph-3* and *Lap-4*) Pinsker et al. (1978) find significant differences in successive periods of time. Of the two remaining loci (*Hk-2* and *Pgm-1*), *Hk-2* has different average heterozygosities in the Canaries and Yugoslavia (0.285 and 0.417), these being the only regions where they have been studied; on the contrary, *Pgm-1* shows similar average heterozygosities for all the regions studied.

Population 6 (Tenerife) is located 2,200 m above sea level, where the winters are relatively harsh, and during this season *D. subobscura* are not captured. All these facts lead us to hypothesize that the populations at this cite must be formed yearly by migration from the lower slopes, resulting in a possible founder effect. This supposition agrees with the fact that the samples from this population were found to be monomorphic for *Ao-1*, *Ao-2*, *Aph-6*, *Est-5*, *Est-10*, α -*Gpdh* and *Got-1*, some of which were moderately polymorphic in other populations of the same island.

The average heterozygosities of the ten Canary populations are very similar (Table 1) with an average 0.082 ± 0.019 and a range of variation of 0.070–0.111. This pattern accords with observations of chromosomal variation made by Prevosti (1971) in eight Canary populations, the majority captured in the same places as ours.

The least polymorphic loci in the Canaries (compared to the continental populations) are found in the O chromosome. This chromosome is practically monomorphic in all of the islands for the O_{3+4} arrangement, but is very polymorphic in the continental populations. Precise associations between alleles and certain inversions have been found in some of these loci: *Ao* and *Xdh* (Zouros et al., 1974), *Est-5* and *Lap-4* (García et al., 1977), *Lap-4* (Charlesworth et al., 1977). Nevertheless, this linkage does not seem to be constant in all populations, since for *Lap-3* Zouros et al. (1974) and Pinsker et al. (1978) do not find significant differences for the associations between alleles and arrangements in their respective studies. In spite of all this, it seems that for chro-

mosome O a global tendency relating reduced heterokaryosis with reduced heterozygosity is consistent.

On the other hand, the *Adh* and *Mdh-2* loci, more polymorphic in the Canaries than on the Continent, are localized on the U chromosome which is less heterokaryotic in the Canaries. In spite of this, Pinsker et al. (1978) found a complete association between the *Mdh-2* alleles and two different chromosomal sequences (U_{+2} and U_{1+2+8}) in such manner that the 1.00 allele always appears with the U_{1+2} arrangement, and 1.08 with U_{+2+8} . These gene and arrangement sequences are observed in the Canaries with frequencies of 0.94 and 0.06 for 1.00 and 1.08 alleles and 0.804 and 0.196 for the $1+2$ and $1+2+8$ arrangement. Although either of these inversions is very frequently encountered in the continental populations studied, neither of them is noted in proportions similar to that of the Canaries (Krimbas and Alevizos, 1973; Zouros et al., 1974; Pinsker et al., 1978). The greater frequency of Canary *Mdh-2* polymorphism may be associated with a determined set of inversions for the chromosome where this locus is situated. We do not know if a similar type of association exists in the Canary populations for the locus *Adh*. Pinsker et al. (1978) did not find one in their German population, but we have observed that in population cages the frequency of the allele 1.08 of *Mdh-2* has a tendency to grow significantly, and this increase is accompanied by a similar increase for the allele 1.67 of *Adh*.

The loci for which we and other authors find differences between regions are very polymorphic in the whole range of distribution of the species and that with the exception of *Lap-4*, no association with inversions is found.

Although we have made no detailed analysis of linkage disequilibrium between alleles and inversions, the differences in enzyme allele frequencies found in the Canary and continental populations may be due, at least partially, to differences in chromosomal polymorphism.

Differences in the techniques employed

also may have contributed to increase or decrease population allele frequencies. For example, the electrophoretic procedures followed in this study for *Xdh* locus are different from those of other studies. (cf. Singh et al., 1976).

SUMMARY

This study presents the genetic variation of 47 enzyme loci in ten natural populations of *D. subobscura* from the Canary Archipelago obtained by starch gel electrophoresis. The mean individual heterozygosity of 0.082 is lower than the average of 0.162 found by other authors in populations from the European continent. All of the loci contributing to this lower polymorphism in the Canaries are located on the O chromosome, being practically monomorphic within the Archipelago. The *Mdh-2* and *Adh* loci are more polymorphic in the Canaries than on the Continent.

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