Electrophoretic variability in natural popuiations of *Drosophila melanogaster* and *Drosophila simulans*

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Abstract

Genetic variation at 59 gene loci coding for enzymes (50) and larval proteins (9) has been studied in sympatric populations of *Drosophila melanogaster* and *D. simulans* from insular and continental origin. The average nurnber of alleles per locus, the rnean proportion o i polymorphic loci and the mean heterozygosity are similar both within and between species. There are however sorne significant differences betueen *D. simulans* populations in the genotypic frequencies for four polymorphic loci.

Introduction

The sibling species *Drosophila melanogaster* and *Drosophila simulans* are extended worldwide. The former is more common in colder regions while the latter is more abundant in warmer areas (Bock & Wheeier. 1972). but it seerns that both are of tropical African origin (Tsacas & Lachaise. 1974). However it is not known if both species utilized equivalent adaptive mechanisrns ior their worldwide expansion. On one hand. similar rnorphological clines have been found for some of their characters (David & Bocquet. 1975a. b: Tantauy er *al.*. 1964); on the other. while *D. melanogaster* shows a rich chromosomal polymorphism with different cosmopolitan inversions. some of them endemic (Stalker. 1976: Langley *et al.*. 1974: Ashburner & Lemeunier. 1976). *D. simulans* seems to be nearly rnonomorphic (Carson. 1965: Ashburner & Lemeunier. 1976).

There are observations that point to a positive correlation between chromosomal and enzymatic polymorphisms. the *D. melanogaster* populations being according to this view also molecularly more polymorphic than those ot *U. simulans* (O'Brien & Macintyre. 1969; Berger, 1970; Triantaphyllidis er *al.* 1980). but Kojima *et al.* (1970) find that molecularly *D. simulans* is at least as polymorphic as *D. melanogaster*.

Lewontin (1974) suggests that this apparent disagreement may be due to the small size of the sample of loci utilized to compare the enzymatic polymorphism of both species.

It would be interesting to clarify uhether or not these species have comparable enzymatic polyrnorphisms when a greater number of loci are studied than have been up to now. With this aim in mind we haie analyzed the molecular polymorphism at 59 gene loci (50 enzyrne and Ÿ larval protein loci) in sympatric populations of *D. melanogaster* and *D. simulans* from the isiand of Tenerife (Canary Islands) and irom the Province of Cordoba (South of the Iberian Peninsula).

Material and methods

All the samples were gathered throughout 1978. Those from Tenerife were collected at a large fruit yarden neara wine press in Güimar. a locality in the south oíthe isiand. *D. simulans* (sG) being captured in March and *D. melanogaster* (mG in Table 1) in

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Scptcrnber. The sarnples of *D. melanogaster* frorn the Province of Córdoba were collected in October at a wine press in the locality of Fernán-Nuñez (mFN) and those of *D. siniulons* in Septernber in a ncighbouring pinc and oak forest at Villares (sV). Every sarnple was cornposed of at least 80 wild fernales. Each fernale was placed in an individual 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 frorneach fernale was latcrassaycd. A sarnple of the 'Oregon' strain of *D. melanogaster.* hornozygous for the standard polytene chrornosorne band arrangernent of this species. obtained frorn the Departrnent of Genetics of Bowling (Ohio). and maintained at our laboratory since 1973. was also analyzed.

For each locus an average number of IZ0 genomes (with a minimum of 601 for natural populations and of 20 genomes for the 'Oregon' strain were tested.

The nomenclature for the gene loci coding for enzyrnes and their alleles are those used by González *et al.* (1982).

Preparation of sarnples. clectrophoretic techniques and the preparation of the starch gel followed Ayala *et al.* (1972). The preparation of the polyacrylamide gel is as indicated in González *et al.* (1982). The following loci have been analyzed: Acid phosphatase *(Acph)*; Alcohol dehydrogenase *(Adh)*; Adenylate kinase *(AA-2* and -3): Aldolase *(Ald)*; Arnylase *(Amy)*: Aldehyde oxidase *(Ao)*; Alkaline phosphatase. *(Aph.3, 4,* 7. and 8): Catalase *(Coi):* Diaphorase. four loci *(Dio-l* 2.3 and *4):* Esrerases. nine loci *(Est-1, 4. 6, 8, 10. 11. 12. 13* and *15)*; Furnarase *(Fum)*; Glutamate osaloaceiate transaminase. two loci *(Got-1* and *2)*; α-Glyerophosphate dehydrogenase *(α-Gpdh)*. Glucose-6-phosphate dehydrogenase *(G6pdh)*; Hydroxybutyrate dehydrogenase *(Hbdh)*; Hesokinase. three loci *(Hk-1, 3* and *5)*: Isocitrate dehydrogenase *(Idh)*; Lrucine aminopeptidase. two loci *(Lap-2* and *3)*; Malate dehydrogenase *(Mdh)*; Malic enzyme *(Me)*; Octanol dehydrogenase *(Odh)*: Peptidase. rhree loci *(Pept-1, 2* and *3)*; 6-Phosphogluconate dehydrogenase *(6Pgdh)*; Phosphoglucomutase *(Pgm)*; Phosphohexose isornerase *(Phi)*; Pyranosidase *(Pyr)*; Tetrazoliurn oxidase *(To)*: Tyrosinase *(Tyr)*; Xanthine dehydrogenase *(Xdh)*; Larval proteins. ninc loci *(Pt-1, 2, 4, 6, 7, 8, 10, 12* and *15)*. The stage in which these enzymes have been studied, together with the buffers. gels and dyes used have becn cited in González ei *al.* (1982) cxcept for *Aph-8* and *Est-1* which were analyzed in the adult stape and *Lap-3* in the pupa stage.

Statistical cornparisons between populations uithin species to determine if significant differences of phenotype frequencies existed between sarnples were rnade using a standard row X colurnn χ^2 contingency test (Snedecor & Cochran. 1969).

Results

Table 1 gives allelic frequencies. observed proportion of heterozygous individuals and that expected in the Hardy-Weinberg equilibrium hypothesis. for each gene locus found to be polyrnorphic in at least one of the sarnpled populations of *D. melanogaster* and *D. simulans*. At the end of the same table the nurnber of gene loci analyzed, the average expected heterozygosity, the proportion of polymorphic loci at the 95 and 99 per cent levels, and the average nurnber of alleles per locus are given for each population.

Neither of these two species were found to be polyrnorphic for any of the following 23 gene loci at the 99 per cent level: Ak-3. *Cat. Dio-l* and *2: Est-1, 4.* 13and *15; Got-1.* Hbdh. Hk-3 and *5; Idh. Me. Pept-1* and *2: Phi. Pyr. Xdh.* and *Pt-1, 4.6* and *12.*

Variation in D. melanogaster

Besides rhe above rnentioned 23 gene loci. variation uas also lacking for the following four loci in this species: *Acph, Ak-2, Ald* and *Ao*, two of which *(Acph* and *Ao)* are very polyrnorphic in *D. simulans.*

Table 2 lists the χ^2 values for these gene loci which showed significant differences between o b served genotypic frequencies and those expected ir, HH' equilibriurn. In the population frorn Güirnar the loci u ith significant differences were *Adh, Est-6* and *Pt-8*. and in the one frorn Fernan Nuñez they were *Aph-7* and *Tyr.* In all cases the significance is due to an excess oí homozygotes save *Pt-8* which shows excess of heterozygotes.

The observed phenotypic frequencies do not show significant differences betueen the two popu-

Table 1. Allelic frequencies and observed and expected proportions of heterozygotes for polymorphic gene loci. For each population number of loci analyzed, mean expected heterozygosity with standard error, percentage of polymorphic loci at the 99 and 95 per cent level, and average number of alleles per locus with standard error are given. Abbreviations cf text p 192.

Locus	Allele	mG	mEN	0080	0266	Mean heterozygosity *melanogaster*	Mean heterozygosity *simulans*
	95			0.810	0 661		
	1.00	1.000	1.000	u 010	0 073		
Obs. het		0000	0.000	**0.300**	0 468		
Exp. hci.		0.000	0.000	0.311	0 487	0.000 ± 0.000	0.399 ± 0.062
Adh	.79				0 C09		
	.87			1.000	0.991		
	.9<	0.119	0 067				
	1.00	0.881	0 933				
Obs. hci		0.153	0.105	0.000	0.019		
Exp net.		0 210	0.125	0.000	0.018	0 168 ± 0.030	0 009 ± 0.006
Ak-2	.97				0.038		
	.98			1.000	0 9h2		
	1.00	1.000					
Obs het.		0.000		0.000	0 045		
Exp. hcr.		0000		0.000	0 073	0 000	0.019 ± 0.013
Ald	.96			0 037	0 027		
	1 00	1.000	1.000	0.963	0.973		
Obs. her		0.000	0 C00	0 037	0 055		
Exp het		0 C00	0.000	0 071	0 053	0 000 ± 0.000	0 062 ± 0.006
Amy	.Y?			0.195	0 092		
	.94			0.–so	0 875		
	.96			0.052	0.033		
	.98	0.021					
	1 00	0 99	1.000				
Obs. hei		0.043	0000	0 15	0 183		
Exp. hct		0.041	0.000	0.395	0 225	0 021 ± 0.014	0.310 ± 0.060
Ao	1.00	0.991	1 000				
	1.03	0 009		0 780	0 754		
	1 05			0 220	0 246		
Obr hci		0 018	0 000	0 280	0 261		
Exp het.		0 018	*0 000*	0 343	0 371	0 009 ± 0 006	0 357 ± 0.010
Aph-3	96	0.206	0 143	1 000	0 9h7		
	1.00	0 794	0 857		0 ni:		
Obs. het		u 294	0 163	0 000	0 025		
Exp. he;.		u 327	0 245	0 000	0 026	0 286 ± 0.029	0 013 ± 0.009
Aph-4	90		0 042	0 059	0 053		
	9'		u 010				
	1 00	0908	0.854	0 941	0 917		
	1 04	0 092	0 091				
Obs. hei		0 102	0 *16*	0 061	0 100		
Exp he:		0 167	0 260	0 111	0 152	0 214 ± 0 033	0 132 ± 0.014
Aph-5	98			0 094	0 071		
	1 00	0 674	0.579	0 859	0 929		
	1 02	0 314	0 421	0 047			
	1 04	0 012					
Obs. het.		0 302	0.246	0 156	0 047		
Exp. het.		0 447	0 487	0 251	0 132	0 467 ± 0.014	0 192 ± 0.042

Table 1 (Continued)

Locus	Allele	mG	mEN	sG	sN	Mean heterozygosity *melanogaster*	Mean heterozygosity *simulans*
Aph-8	98			0.023	0.014		
	1.00			0.773	0.868		
	1.02		-	0.204	0.118		
Obs. het.				0.182	0.088		
Exp. het.				0.360	0.232		0.296 ± 0.045
Dia-3	Y3		0.083				
	1.00		0.917	1.000	1.000		
Obs. het.			0.011	0.000	0.000		
Exp. het.			0.152	0.000	0.000	0.152	0.000 ± 0.000
Dia-4	94	0.019	0.010	-			
	1.00	0.942	0.990	1.000	0.992		
	1.01	0.039	-		0.008		
Obs. het.		0.115	0.019	0.000	0.016		
Exp. het.		0.111	0.020	0.000	0.016	0.066 ± 0.032	0.008 ± 0.006
Est-6	1.00	0.793	0.753	0.098	0.281		
	1.02		0.006	0.010			
	1.05	0.185	0.235	0.892	0.708		
	1.07	0.022	0.006	-	0.011		
Obs. het.		0.19h	0.272	0.137	0.333		
Exp. het.		0.336	0.378	0.195	0.420	0.357 ± 0.015	0.308 ± 0.080
Est-8	99		0.021				
	1.00	1.000	0.958				
	1.01		0.021				
	1.04		-	1.000			
Obs. het.		0.000	0.083	0.000			
Exp. het.		0.000	0.081	0.000		0.041 ± 0.029	0.000
Est-10	96			0.074	0.280		
	1.00		-	0.926	0.720		
Obs. het.				0.147	0.240		
Exp. het.			-	0.137	0.403		0.270 ± 0.094
Est-11	1.00	0.854	1.000	0.957	1.000		
	1.03	0.146		0.043			
Obs. het.		0.125	0.000	0.087	0.000		
Exp. het.		0.249	0.000	0.082	0.000	0.061 ± 0.044	0.041 ± 0.029
Est-12	98		0.040				
	1.00		0.960	0.957	1.000		
	1.01			0.043			
Obs. het.			0.077	0.008	0.000		
Exp. het.			0.038	0.082	0.000	0.038	0.041 ± 0.029
Fum	98	0.020					
	1.00	0.980	1.000	1.000	1.000		
Obs. het.		0.041	0.000	0.000	0.000		
Exp. het.		0.039	0.000	0.000	0.000	0.020 ± 0.014	0.000 ± 0.000
Got-2	98	0.010		1.000	1.000		
	1.00	0.990	1.000				
Obs. het.		0.020	0.000	0.000	0.000		
Exp. het.		0.020	0.000	0.000	0.000	0.010 ± 0.007	0.000 ± 0.000
6Gpdh	1.00	0.581	0.582				
	1.02	0.419	0.418	1.000	1.000		
Obs. het.		0.581	0.418	0.000	0.000		
Exp. het.		0.487	0.487	0.000	0.000	0.487 ± 0.000	0.000 ± 0.000

Table 1. (Continued)

Locus	Allele	mG	mEN	sG	sV	Mean heterozygosity *melanogaster*	Mean heterozygosity *simulans*
G6pdh	.96	0.012	0.038	1.000	1.000		
	1 00	0 972	0 962				
	1.0?	0012	-				
	1.04	0 004	-				
Obr. hei		0 057	0 070	0.000	0 000		
Exp. het		0 055	0 073	0 000	0.000	0.064 ± 0.006	0.000 ± 0 000
Hk-1	1.00	0 750	0.620	1.000	0 991		
	1 06				0.009		
	1.10	0 250	0.380				
Obs. het		0 346	0.457	0 000	0.019		
Exp. het.		0 175	0 471	0.000	'3019	3423 ± 0.034	0 009 ± 0.006
Lap-2	96			0.019	0 176		
	.98	0 115	0.058	0.981	0 h4h		
	.Y9	0 090	0 081	-			
	1.00	0 7b2	0.861		0 028		
	1.04	0 013	-				
Oba. het.		0 231	0.209	0.039	0 187		
Exp. hei.		0 367	0.249	0.037	0.268	0.308 ± 0.012	0.153 ± 0.082
Mdh	93			-	0 009		
	1.00	u 985	1.000	1.000	0.991		
	1.09	0.015	-				
Obr. het.		0 030	0 000	0.000	0 018		
Exp. het		0 030	0.000	0 000	0.018	0 015 ± 0.011	0.009 ± 0.006
Odh	.94	0 026	0.018	-			
	1 00	0 974	0.982	1.000	1 000		
Obr het.		0 053	0.035	0.000	0.000		
Exp. hei		0.051	0.035	0 000	0 000	0 043 ± 0 006	0 000 ± 0.000
Pep-3	9?		0.006				
	1.00	0 991	0.974	0.216	0 144		
	1.0?	u 009	0.020	0.784	0.856		
Obr hei.		0 018	0.052	0.314	0 156		
Exp hsi.		0 018	0.051	0 339	0 247	0.035 ± 0.012	0.293 ± 0.033
6Pgdh	96		0.012	0.988	0 988		
	98		0 012				
	1.00	0 949	0 976	0 012	0 012		
	1 02	0 038					
	1 04	0 011					
Obs. het		0 107	0 067	0 032	0 000		
Exp. hei		0 098	0.047	0 024	0 024	0 073 ± 0 018	0.024 ± 0.000
Pgm	90	0 019	0.038				
	Y4	u 095	0 075	0 148	0 082		
	96		-	0 037	0 062		
	1.00	0 880	0 887	0 815	0 856		
	1.05	0 006					
Obs. het		0 241	0 226	0 259	0 250		
Exp. hei		0 216	0 206	0 313	0 257	0 211 ± 0.004	0.285 ± 0 020
To	85	0 023					
	94	0 091	0 082				
	1 00	0 886	0 918	1 000	1 000		
Obs. het.		0 136	0 139	0 000	0 000		
Exp. hei.		0 206	0 151	0 000	0 000	u 179 ± 0.019	0.000 ± 0.000

populations of Drosophila

has been studied in ... heterozygosity between D.

Table 1 (Continued)

Locus	Allele	mG	mEN	sG	sV	Mean heterozygosity *melanogaster*	Mean heterozygosity *simulans*
Tir	1.00	0 986	0 X6X	0.907	0 846		
	1 03	0014	0 132	0 093	0 ira		
Obs het.		0 028	0 053	0 111	0 154		
Exp hei.		0 028	0 229	0.169	0 261	0 129 ± 0.071	0.215 ± 0.033
Pt-2	1 00	0 990	0 967	0 914	u 942		
	1 02	0 010	0 033	0 086	0 058		
Obs hci		0019	0 067	0 103	0 115		
Exp. hei		U 020	0 064	0 157	0 109	0 042 ± 0.016	0 133 ± 0.017
Pt--	1 00	1.000	0 9.12	0 971	1 000		
	1 01		0 018	0 029			
Obs hci		0 000	0 037	0 029	U 000		
Exp hei		0.000	0 035	0 056	0 000	0 018 ± 0.012	0.028 ± 0.020
Pt-8	1.00	0 *606*	0 614		0 048		
	1.01			0.825	0 865		
	1.03	0.383	0.372				
	1 04	0.011	0.014	0 175	0 087		
Obs het		0 745	0 65*i*	0 211	0 096		
Exp het		0 486	0 4b1	0.289	0 242	0.485 ± 0.001	0 *266* ± 0.017
Pt-10	"9	0 019	0.027				
	1.00	0.981	0.973	0.961	1.000		
	1 02			0 039			
Obs het		0 039	0.055	0 026	0 000		
Exp het.		0 037	0 053	0 075	0 000	0.045 ± 0.006	0.038 ± 0.026
Pt-15	1.00	1 000	0 979	1 000	1 C00		
	1.04		0.021				
Obs. het.		0 000	0.043	0.000	0 000		
Exp het		0.000	0.041	0.000	0 000	0 021 ± 0014	0.000 ± 0.000
Toia! Loci		52	*52*	5"	55	56	*59*
Mean heterozygosity		0.085	0 087	0 067	0 074	0 081	0 067
Standard error:		0.020	0.020	0.015	0.017	0.018	0 015
% Polymorphic loci (99)		0.442	U 462	0.351	0 327	0 500	U 408
% Polymorphic loci (95)		0.269	0 250	0.228	0 255	0 250	0 203
No Alleles locus		1.63	1 60	1.46	45	1 63	1.46
Standard error		0 12	0 10	0 10	0 10	0 10	0 09

Table 2. χ^2 values for differences between observed phenotypic frequencies and those expected in Hardy-Weinberg equilibrium (only loci with significant differences, in parentheses DF) Abbreviations cf. text p. 192

Locus	mG	mEN	SG	SV
Adh	6.46 (1)*			
Amy			51.19 (3)***	
Aph-7		14.04 (1)***		
Aph-8				8.68 (1)**
Est-6	11.43 (3)**			
Lap-2				4.56 (1)*
Tir		6.11 (1)*		
Pt-8	14.72 (3)**			12.15 (1)***
Pt-10			3.87 (1)*	

* significant at the 5% level
** significant at the 1% level
*** significant at the 0.1% level

lations ar any iocus.

The same allele is found in boih populaiions a: every rnonornorphic gene locus. In the polymorphic loci. the most frequenr allele is always ihesarne. ana has u similar frequency in the two populaiions. although in many of them (Table 1) ihere are some rare alleles exclusive to only one ofihe popuiations.

The 'Oregon' strain is rnonornorphic for nearly all the loci with the escepiions of *Aph-7*, *To* ana *Pt-8*, bur nor always is the fixed aliele of 'Oregon' :he most common in ihe natural popuiarions studied. as is the case for *Aph-3* (0.96).*6 Pgdh* ***(0.96)*** and *Idh* (1.13).

Variation in D.simulans

In addition to the 23 monomorphic gene loci common to both species. the following shoued no variation in *D. simulans: Adh. Dia-3* and *4; Est-8. Fum. Got-2, o-Gpdh. G6pdh. Hk-1. Lap-3. Mdh. Odh, To,* and *Pt-15,* from which four *(Adh. α-Gpdh, Hk-1* and *Tu)* are very polymorphic in *D. melanogaster.*

When observed and expected genotypic frequencies in Hardy-Weinberg equilibrium were compared. significant differences were found for the loci *Amy* and *Pt-10* in the popuiation from Güirnar and for the loci *Aph-8. Lap-2* and *Pt-8* in the population from Villares. Table 2 shous the respective levels of significanee. in all cases the significant differenees may be attributed to a smaller frequency of heterozygotes.

Only four gene loci showed significant differences between populations uith respect to phenotypic freauences: *€si-6* ($\chi^2 = 11.54$. $P < 0.05$). *Est-10* ($\chi^2 = 7.31$. $P < 0.05$). *Lap-2* ($\chi^2 = 18.69$. $P < 0.001$) and *Pt-8* ($\chi^2 = 11.13$. $P < 0.05$).

The rnostcornmonallelcis thesarne ineachofthe two populations studied. although. just as in *D. melanogaster,* alleles with frequencies srnaller than 0.05 can be found in only one of them. It is uorth mentioning that the rare alleles for *Adh* and *Mdh* in Villares uere only found in the offspring of a single female.

Genetic distances resulting frorn our data between sibling species *D. melanogaster* and *D. simulans* as well as betueen each ofthern and *D. mauritiana* have becn treated in another paper (Gonzalez *et al.*, 1982.

Diseussion

We find similar levels of genetic variation in both species. *D. melanogaster* and *D. simulans.* when cornparing the populations irorn Tenerife and those frorn the Iberian Peninsula. uhether measured by mean heterozygosities. by the percent of polymorphic loci. or by average number ot alleles per locus (Table1). Steiner *et al.* (1976). however. finds that the mean heterozygosity in *D. simulans* frorn Hawaii (0.077) is only about half that found in continental populations frorn Texas (0.162). A possible explanation could be that the Canary Archipelago is much older and nearer to the Continent ihan the Hawaiian Archipelago. and repeated migration of new founder individuals. with subsequent recolonizations. could therefore have been more frequent in the former. which would have maintained or produced levels of variation similar lo those of the continental populations.

Our values of mean heterozygosities. pereentages of polymorphic gene loci. and average number of alleles per locus are among the smailest found by different authors in both species (Tables 3 and 5). This is largely because we have analyzed a higher number of gene loci: our values become intermediate when only the gene loci in common with other studies are taken into account (Tables 4 and 6). In spite of this. when we center our attention on those gene loci common to all studies. clear differences of the mean heterozygosities per locus among populations can be observed. Thus u e find that our populations. rogether with the one from Corfu (Triantaphyllidis *et al.*. 1980). are less polymorphic for *Adh* and more for *α-Gdph* than the others compared (Table 4). w hile for the *Pgm* locus of *D. melanogaster.* u hich display a striking variation between populations. our vaiues are intermediate (Table 4). In addition. our populations are less polymorphic at the *Ao* and *Odh* loci than are those from other geographical regions (Band. 1975; Kojima *et al.*. 1970: Langley *et al.*. 1974). Nevertheless. the differences at the *.40* locus may be imputed to differences in enzyrne assay techniques (Langley *et al.*, 1974).

Also in *D. simulans* polymorphic gene loci shou variation arnong regions. Thus. if the gene loci common to all studies are reconsidered. the heterozygosity at the *Pgm* locus is higher in our populations than in the rernainder. u hile the *Adh* locus is more polymorphic in the samples frorn Texas; likewise in our insular population the *Est-6* locus is less polymorphic than in the continental one from Villares. w hich for this enzyme shows a heterozygosity similar to the other regions (Table 6).

Some gene loci appear to deviate from Hardy-Weinberg equilibrium in our insular as well as our continental populations frorn *D. simulans* and *D. melanogaster* (Table 2). However. these gene loci do not coincide in both geographical areas. nor with those found out of equilibrium by other authors. Moreover. uhen they do coincide. the surplus phenotype may be different. as is the case for *Est-6* in *D. melanogaster* uhere we find excess of

Table 5 Number of gene loci analyzed, mean expected heterozygosity with standard error, percentage of polymorphic loci at the 99 and 95 per cent levels and average number of alleles per locus for *D. simulans* from different regions

	Texas[1]	Greece[2]	Hawaii[3]	Oahu[1]	Canaries	Iberian Peninsula
Total loci.	18	11	15	15	57	55
Mean heterozygosity:	0.156	0.174	0.095	0.096	0.067	0.074
Standard error.	0.051	0.007	0.043	0.046	0.015	0.017
% Polymorphic loci (99):	0.611	0.455	0.400	0.333	0.351	0.327
% Polymorphic loci (95):	0.389	0.455	0.267	0.333	0.228	0.255
No. Alleles locus	2.06	2.18	1.53	1.40	1.46	1.45

[1] Kojima *et al.* (1970)
[2] Triantaphyllidis *et al.* (1980)
[3] Steiner *et al.* (1976)

Table 6 Mean expected heterozygosities at gene loci of *D. simulans* analyzed in all the regions considered. Cf. Table 5

Loci	Texas[1]	Greece[2]	Hawaii[3]	Canaries	Iberian Peninsula	Mean heterozygosity
Acph	0.415	0.396	0.422	0.311	0.467	0.40h ± 0.025
Adh	0.240	0.000	0.036	0.000	0.018	0.059 ± 0.041
Est-6	0.548	0.177	0.340	0.195	0.420	0.396 ± 0.054
α-Gpdh	0.027	0.012	0.066	0.000	0.000	0.021 20011
Mdh	0.006	0.000	0.000	0.000	0.018	0.005 ± 0.003
Me	0.000	0.000	0.019	0.000	0.000	0.004 ± 0.003
Pgm	0.064	0.119	0.152	0.313	0.257	0.181 ± 0.041
Xdh	0.053	0.000	0.000	0.000	0.000	0011 ± 0.009
Total loci:	8	8	8	8	8	8
Mean heterozygosity:	0.169	0.126	0.129	0.102	0.150	0.15 ± 0.010
Standard error	0.069	0.065	0.054	0.048	0.068	
% Polymorphic loci (99):	0.750	0.375	0.750	0.375	0.375	
% Polymorphic loci (95):	0.375	0.375	0.500	0.375	0.375	
No. Alleles locus:	2.25	1.63	1.88	1.75	1.75	

[1] hoiirna *et al.* (1970)
[2] Triantaphyllidis *et al.* (1980)
[3] Steiner *et al.* (1976)

homozygotes, as do Johnson and Shaffer (1974), but Hand (1975) finds a surplus of heterozygotes. Generally, however, significant differences are due to escess of the former, perhaps imputable to lack of representativeness of some samples, because of their small size, or to the genetic structure of the populations (Wahlund, 1928).

When we compare genotypic frequencies per locus between insular and continental populations from each species, both *D. melanogaster* samples appear to be very similar (none of 48 gene loci show significant differences), while those from *D. simulans* show greater variation (4 gene loci among 53 show significant differences). A possible explanation would be that local populations within *D. simulans* are more isolated from each other than are those from *D. melanogaster*. Perhaps this is so because at temperate latitudes it is easier to find *D. melanogaster* indoors than in fields and forests, which might favour their migration by man, while the contrary is the case for *D. simulans*.

It would be interesting to know if the rare alleles fixed in the 'Oregon' strain are frequent or not in the natural American populations from which this strain proceeds.

Some authors have found lower molecular variation in *D. simulans* than in *D. melanogaster* revealed as mean heterozygosity, or as percentage of

polymorphic gene loci at the 99 per cent level (O'Brien & MacIntyre, 1969; Berger, 1970). Triantaphyllidis *et al.* (1980) in turn find similar values of heterozygosity and oinurnber of alleles per locus in both species. but confirrn a smaller number of polymorphic gene loci at the 99 per cent level in *D. simulans.* While these differences between species could be attributed to a smaller size of the *D. simulans* samples analyzed (Berger, 1970). they may also be correlated with the known differences in chromosomal and biometrical polymorphism between both species (Triantaphyllidis *et* al.. 1980). On the contrary. Kojirna *et al.* (1970) observe similar levels of polymorphisrn in populations of both species. And we find that mean heterozygosity. as well as percentage of polymorphic loci. and average number of alleles per locus in the pooled populations of *D. melanogaster* (0.081, 0.464 (99%), 0.264 (95%), 1.61) are slightly higher. but not significantly different from the values found in *D. simulans* (0.060. 0.359 (99%). 0.226 (95%), 1.41). It could be that these slight differences were due, as has been mentioned above, to a larger migration flow among local populations of *D. melanogaster,* which in turn would give this species a larger population size. In any case. differences in rnolecular polymorphism between both species do not seem to be nearly as great as their differences in chromosomal polymorphism. since u hile *D. melanogaster* is chromosomally highly polyrnorphic all over the World. *D. simulans* is nearly rnonornorphic (Ashburner & Lemeunier, 1976).

In spite of this resemblance in rnean molecular polymorphisrn. it should be pointed out that *D. melanogaster* is more successful in cold regions and in highly alcoholic substrates (David & Bocquet. 1975). as well as in laboratory conditions (Moore. 1952). On the other hand. *D. simulans* is superior in nature to *D. melanogaster* in ternperare regions (Wallace. 1968). Perhaps the slight differences in polymorphisrn in certain gene loci are sometimes enough to give one or another species a higher adaptive value.

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