Genetic polymorphism and high detrimental load in natural populations of *Drosophila melanogaster* from cellar and vineyard

A. González and
J. L. Ménsua

Departamento de Genética, Facultad de C.
Biológicas, Universidad de Valencia, Dr. Moliner, 50.
Burjassot, Valencia, Spain.

Two Spanish natural populations of *Drosophila melanogaster* have been analysed with respect to genetic variability in third chromosome viability. The two populations, although from the same locality, belong to relatively different habitats: the inside of a cellar and a vineyard. The patterns of homozygote and heterozygote viability are similar in both populations. The homozygous detrimental loads estimated are very high and the values for the D:L (detrimental/lethal) ratio close to 2.5, which is higher than any previously found. The environmental variance of viability, average degrees of dominance of lethal genes and of viability polygenes and effective population sizes were estimated in each population. The mechanisms which could maintain variability in these populations, the causes of the high detrimental loads and the possibility that cellar and vineyard could be two subpopulations of a larger population are discussed.

INTRODUCTION

Genetic variability with respect to viability in natural populations of *Drosophila melanogaster* has been studied by many investigators (e.g., Greenberg and Crow, 1960; Mukai and Yamaguchi, 1974; Choi, 1978).

Recent work on natural populations of *Drosophila melanogaster* indicate that there is a north to south cline in the extent of genetic variability in viability (Mukai and Nagano, 1983; Tachida *et al.*, 1983; Kusakabe and Mukai, 1984b) and that some kind of balancing selection may be operating in southern populations, (although a mutation-selection balance operates at the majority of loci).

The adaptedness of a population is a measure of its ability to survive and reproduce in its own environment, which may encompass many niches. Although theoretical aspects of the relation between genetic variability and ecological niche has been extensively analysed (Levene, 1953; Levins and MacArthur, 1966; Levins, 1968), the variability of genes affecting viability in relation to niche parameters has not been studied.

This paper presents the patterns of viability, genetic load and population size of a population of *Drosophila melanogaster* from a cellar and a nearby exterior population in a vineyard in an attempt to measure the extent of genetic variability of viability in Spanish populations and the mechanisms which may operate in its maintenance.

MATERIAL AND METHODS

Late in October of 1979 a capture of *D. melanogaster* was sampled in two sites with different environmental conditions, a cellar and a vineyard separated by 4 km in Requena (Valencia) in the east of Spain.

Extraction and maintenance of third chromosomes

Male flies were individually mated to 3 virgin females of the *Ubx/Sb* [*TM2 h ca/CSb*] strain (the X, II, IV chromosomes and the cytoplasm of *Ubx/Sb* flies had been substituted with chromosomes and cytoplasm from the cellar population). A single *Ubx* male fly from each cross was mated again with 3 *Ubx/Sb* virgin females in the next generation. Thus, 300 third chromosomes were

extracted and were maintained, as lines, at 19°C in the following way: 15 *Ubx* males were mated to 15 *Ubx/Sb* females. The 15 pairs of flies were distributed at random in 3 vials, establishing 3 replicates. In the next and following generations, 15 males of *Ubx* phenotype were selected at random from the offspring of the 3 replicates, and were mated again with 15 *Ubx/Sb* females establishing 3 new replicates.

Estimation of relative viabilities

Homozygote and heterozygote relative viabilities were estimated as follows: Crosses were made between 4 *Ubx*/+ *i* females and 4 *Ubx*/+ *i* males with two simultaneous replications in each chromosome line, where “*i*” indicates line number. In the offspring, *Ubx*/+ *i* and + *i*/+ *i* flies segregate at an expected ratio of 2:1. The relative viabilities of random heterozygotes were estimated in a way similar to the above, combining two successively numbered lines, i.e., *Ubx*/+ *i* × *Ubx*/+ *i*₊₁ in order to secure random combination of different chromosome lines. As in the case of homozygotes, four-pair matings were conducted with two simultaneous replications. In both cases, four days after the crosses were made, all 8 flies in a vial were transferred to a second vial. Four days after the transfer, all flies were discarded. In both vials, the original and the transferred, all flies were counted 3 times until the 18th day after the cross or transfer was made. *Ubx* flies and wild type flies from the pair of vials were respectively pooled and considered as a single observation. Relative viability was expressed as the ratio of the number of wild type flies to the number of *Ubx* flies + 1 (cf. Haldane, 1956).

The crosses were made at different times (cycles). Fifteen lines were employed per cycle (except two cycles in which 20 were used). The chromosomes for each cycle were chosen at random from chromosomes that originated from males since line numbers were assigned randomly. Homozygote and heterozygote relative viabilities were estimated at the same time within each cycle. Before the analyses were made, all relative viabilities were standardised by cycle to the average heterozygote relative viability of that cycle.

Estimation of environmental variance

The expected sampling variance (V_s) of viability indices is given by the following formula (Mukai *et al.*, 1982a; Mukai and Nagano, 1983):

$$V_s = 4n(m+n)/m^3 \quad (1)$$

where “*m*” and “*n*” indicate the number of *Ubx*/+ and +/+ flies emerged, respectively. Thus, environmental variance (V_{EN}) is given by subtracting the average of the expected sampling variances ($\bar{V}_s$) of viability indices for respective replicates from the observed error variance ($\hat{\sigma}_E^2$) of viability indices among replicates within lines of crosses.

$$\hat{V}_{EN} = \hat{\sigma}_E^2 - \hat{\bar{V}}_s \quad (2)$$

Formula (1) was appropriately modified to take into account the standardization of viability indices.

Estimation of the average degree of dominance for viability polygenes

The average degree of dominance for viability polygenes was estimated by the regression method of Mukai and Yamaguchi (1974). The expected regression of heterozygote viability (Y) on the sum of the two constituent homozygote viabilities (X) on chromosome basis ($\beta_{X,Y}$) estimates the harmonic mean of degree of dominance of newly arisen mutant viability polygenes ($\bar{h}_N$); at the same time, this gives the average degree of dominance of viability polygenes in an equilibrium population ($\bar{h}_E$). The regression coefficient of X on Y ($\beta_{X,Y}$) gives approximately the inverse of arithmetic mean of the average degree of dominance of newly arisen mutant viability polygenes.

RESULTS

155 third chromosomes were extracted from the cellar and 145 from the vineyard, and all homozygote and heterozygote relative viabilities were examined.

The cosmopolitan inversions, *In*(3*R*)*P* and *In*(3*L*)*P* are present in both populations. Their frequencies are: 5.81 per cent for *In*(3*L*)*P* and 8.14 per cent for *In*(3*R*)*P* in the cellar and 8.00 per cent and 17.33 per cent for *In*(3*L*)*P* and *In*(3*R*)*P* respectively in the vineyard (González, 1985).

Viability data were collected so as to permit partition of the total variance into (a) the variance between groups of chromosomes tested at the same time (cycles); (b) that between chromosomes within these groups or cycles; (c) that between replicates within the chromosomes.

Table 1 shows a summary of two-level nested anovas which were carried out for homozygote (excluding lethals) and heterozygote viabilities in

Table 1 Summary of the two-level nested anovas of homozygote and heterozygote viabilities of third chromosomes in cellar and vineyard populations

		$F_{C,CR}$	df	$F_{CR,E}$	df
CELLAR	Homozygotes	2.2650*	9, 107	28.5899**	107, 117
	Heterozygotes	0.0001 ns	9, 145	1.7276*	145, 155
VINEYARD	Homozygotes	1.4890 ns	8, 96	16.9500**	96, 105
	Heterozygotes	0.0001 ns	8, 136	1.3338*	136, 145

F: Value of F test

C: Cycles (groups of chromosomes analysed simultaneously)

CR: Chromosomes (within cycles)

E: Error (replicates within cycles)

*: $p < 0.05$; **: $p < 0.01$

both populations. Significantly greater mean squares are due to chromosomes rather than to replicates in the four groups of analysed data, indicating there is a real genotypic difference between the chromosomes within populations. The differentiation depending on cycles is not significant for homozygotes or heterozygotes in the vineyard nor for the heterozygotes in the cellar. For homozygote viabilities from the cellar, the added variance component among cycles is significant ($F_{C,CR}$) at the 5 per cent level: we think that the detected differences, bearing in mind that the number of chromosomes analysed by cycle is not excessively large, are because in given cycles, simply by chance, chromosomes of which the homozygous viabilities are more related to each other than they are to the viabilities of the other cycles may coincide. An additional Student-Newman-Keuls test, comparing the average homozygote viabilities of all cycles from cellar confirmed that all averages could be grouped into the same range.

Linear regression analyses complementing

those of variance were carried out to test the relationship between the viabilities of different cycles (groups of chromosomes the viability of which is simultaneously tested) and the time at which the viabilities of these chromosomes are estimated (represented by the generations of maintenance of the chromosomes since these were isolated from nature). There were no significant linear regressions of the homozygote viabilities on the time of maintenance of the chromosomes in either of the populations (table 2). The variance analyses carried out previously showed the unlikelihood of significant lineal regression of heterozygous viabilities on time in either population since:

$$SS \text{ cycles} < MS \text{ (chromosomes)}$$

$$\times F(1, \sum_{i=1}^a n_i - a)$$

$$\text{Cellar } 1.43 \times 10^{-5} < 0.0183 \times F(1, 145)$$

$$\text{Vineyard } 7.80 \times 10^{-6} < 0.0151 \times F(1, 136)$$

Where:

n_i = number of chromosomes per cycle and

a = number of cycles

Table 2 Completed anova table with regression of the homozygote viability of third chromosomes on the time passed until their analysis. Cellar and vineyard populations

	Source of variation	df	SS	MS	F
CELLAR	Among cycle	9	1.4103	0.1567	2.2650*
	Lineal regression	1	0.2951	0.2951	2.1167 ns
	Deviations from regression	8	1.1152	0.1394	2.0153 ns
	Within cycles	107	7.4016	0.0691	
	Total	116	8.8119		
VINEYARD	Among cycle	8	0.8558	0.1070	1.4890 ns
	Lineal regression	1	0.1669	0.1669	1.6960 ns
	Deviations from regression	7	0.6889	0.0984	1.3699 ns
	Within cycles	96	6.8961	0.0718	
	Total	104	7.7519		

df: Degrees of freedom

SS: Sums of squares

MS: Mean squares

F: Values of F test

*: $p < 0.05$

Distributions of relative viabilities

Relative homozygote and heterozygote viabilities for the third chromosomes are shown in fig. 1. Using the Kolmogorov-Smirnov non parametric test, there were no significant differences between the distribution patterns of the two populations for homozygote or for heterozygote viabilities. The frequency of lethal-carrying chromosomes (Q) was $0.2452 (=38/155)$ in the cellar and $0.2759 (=40/145)$ in the vineyard.

In the cellar, the average relative viability of homozygotes was 0.2452 ± 0.0256 , of homozygotes excluding lethal-carrying chromosomes lines, 0.5194 ± 0.0255 , and of heterozygotes, 1.0001 ± 0.0075 . In the vineyard the average relative viability obtained for homozygotes was 0.3678 ± 0.0257 , for homozygotes excluding lethal-carrying chromosome line, 0.4928 ± 0.0266 , and for heterozygotes, 1.0001 ± 0.0070 .

Homozygous load

The genetic loads caused for genes of viability were calculated using the methods of Greenberg and Crow (1960) (table 3).

Relative viabilities in lethal and semilethal heterozygotes

(Table 4). The estimate of the average degree of dominance of lethal genes ($\bar{H}_E$) was made assuming that the lethal genes are distributed on the chromosomes according to a Poisson distribution and that the heterozygous effects of non-homologous lethal genes are additive. The value estimated for the cellar population was ($\bar{H}_E = 0.0076$).

Average degree of dominance of viability polygenes

All heterozygotes of which the constituent chromosomes have both viability indices larger than 0.6 ($n = 20$ in the cellar; $n = 12$ in the vineyard) were used in these estimations which were performed as indicated in materials and methods. For the calculation of the regression coefficients, analyses of variance and covariance were performed to estimate the genetic variances and genetic covariances between the viabilities of random heterozygotes and the sums of the viabilities of the respective

Figure 1 Frequency distributions of homozygote and heterozygote viabilities of the third chromosomes from the cellar and vineyard populations. The average viability of heterozygotes is assumed to be 1.0000.

Table 3 Homozygous genetic loads, expressed in lethal equivalents, for the third chromosome in cellar and vineyard populations

Population	Total load	D	Dm	Ds	L	Dm/L	D/L
Cellar	0.9146	0.6544	0.3025	0.3526	0.2602	1.1661	2.515
Vineyard	1.0003	0.7077	0.2963	0.4114	0.2926	1.0129	2.420

Load components are: Dm for mild detrimental (viability > 0.5); Ds for severe detrimental (0.1 < v < 0.5) and L for lethals (v < 0.1). D = Dm + Ds.

homozygotes for their constituent chromosomes. On the basis of these estimated parameters, the average degrees of dominance of mildly deleterious genes in the approximately equilibrium population ($\bar{h}_E$) and for newly arisen mutations ($\bar{h}_N$) were estimated. The results for the vineyard population do not give any significant information due to the small sample ($n = 12$), so they were disregarded. The values estimated for the cellar population were $\bar{h}_E = 0.152 \pm 0.084$ and $\bar{h}_N = 0.992 \pm 0.320$.

Environmental variance

Environmental variances of viabilities of all homozygotes and heterozygotes with viability indices larger than 0.6 were estimated, using formulas (1) and (2). The environmental variance was used as an index of developmental homeostasis.

Tables 5(a, b) and 6(a, b) show the environmental variances for the cellar and vineyard populations and the correlation coefficient between viabilities and environmental variances. In the vineyard population, the values of the average environmental variance for homozygotes and heterozygotes are similar ($F = 1.0461$; $df = 50, 144$; $0.75 > p > 0.5$). In the cellar population, the average environmental variance of homozygotes is

significantly smaller than those of heterozygotes ($F = 1.9313$; $df = 154, 64$; $p < 0.01$).

Allelism rates of lethal chromosomes and effective population size

All lethal chromosomes from both populations were used for calculating the inter and intrapopulation allelism rates. The number of crosses made within cellar, within vineyard and between cellar and vineyard were 703, 780, and 1520 respectively; the respective rates of allelism were 0.00853 ± 0.00347 ; 0.00641 ± 0.00286 and 0.00329 ± 0.00147 . There are no significant differences among them.

The fraction of the lethal allelism due to mutations (I_N) and the fraction due to consanguinity (I_F) were estimated using the method of Wallace (1966) (table 7).

I_N is of the same order in both populations. Therefore, the greater frequency of lethal allelism observed (I_C) in the cellar with respect to that in the vineyard, although the difference is not significant, is principally due to the greater consanguinity of that population.

The effective sizes of both populations (N_e) was estimated by the following formula (Nei, 1968) where the degrees of dominance of lethal genes and the mutation rates to lethals (u) per locus are

Table 4 Average viabilities of: (a) lethal-free, single-lethal and double-lethal heterozygotes. (b) semilethal-free, single-semilethal and double-semilethal heterozygotes

Genotype	Cellar		Vineyard	
	N	Average viability	N	Average viability
(a) Non-lethal/Non-lethal'	89	1.0039 ± 0.0091	79	1.0016 ± 0.0093
Non-lethal/Lethal	56	0.9970 ± 0.0145	51	1.0099 ± 0.0122
Lethal/Lethal'	10	0.9829 ± 0.0161	15	0.9648 ± 0.0210
Total	155		145	
(b) Non-semilethal/Non-semilethal'	29	1.0316 ± 0.0170	21	0.9924 ± 0.0217
Non-semilethal/Semilethal	39	0.9847 ± 0.0129 *	33	1.0059 ± 0.0115
Semilethal/Semilethal'	21	1.0014 ± 0.0178	26	1.0046 ± 0.0171
Total	89		80	

N stands for the number of crosses

*: $p < 0.05$

Table 5 Environmental variance for third chromosomes extracted from the cellar population

Viability range	No. of lines	Mean viability	Environmental variance	Average no. of flies per observation
A. Homozygotes (no. of lines tested = 65)				
0.5-0.6	13	0.5441693 ± 0.0091309	0.0041751 ± 0.0001092	480.96
0.6-0.7	12	0.6402916 ± 0.0089540	0.0054703 ± 0.0000953	491.17
0.7-0.8	17	0.7518941 ± 0.0064223	0.0031527 ± 0.0002681	477.03
0.8-0.9	15	0.8472334 ± 0.00603069	0.0044375 ± 0.0002040	511.17
0.9-1.0	7	0.9370714 ± 0.0125306	0.0030010 ± 0.0003250	477.29
1.0-1.1	0	—	—	—
1.1-1.2	0	—	—	—
1.2-1.3	1	1.2258000	-0.0050570	364.00
Mean	65	0.7201215 ± 0.0180210	0.0039389 ± 0.0001997	486.59
Correlation between environmental variance and viability = -0.5071600 ± 0.1085831; $t = -4.671^{***}$				
B. Heterozygotes (no. of crosses = 155)				
0.6-0.7	1	0.6141000	-0.0025699	279.50
0.7-0.8	2	0.7809500 ± 0.0012449	0.0276729 ± 0.0024332	336.25
0.8-0.9	16	0.8733626 ± 0.0054588	0.0095235 ± 0.0001592	546.59
0.9	57	0.9556824 ± 0.0037312	0.0061584 ± 0.0001630	522.54
1.0-1.1	61	1.0405430 ± 0.0039816	0.0074841 ± 0.0001536	546.93
1.1-1.2	15	1.1467600 ± 0.0063123	0.0107661 ± 0.0002421	545.63
1.2-1.3	3	1.2377670 ± 0.0160812	0.0016525 ± 0.0004358	490.50
Mean	155	1.0001000 ± 0.0075142	0.0076075 ± 0.0002546	532.26
Correlation between environmental variance and viability = -0.0723247 ± 0.0806335; $t = -0.897$				

***: $p < 0.001$

the same for all loci:

$$\hat{N}e = (1 - \hat{I}g) / 4(\hat{I}gU - u)$$

(Ig) stands for the allelism rate of lethal genes and can be estimated by $-\ln(1 - I_C Q^2) / \ln(1 - Q)^2$, where (I_C) stands for the allelism rate of lethal

chromosomes); (U) is the total lethal mutation rate, using Wallace's (1968) estimate for the third chromosome of 0.005.

Values of 10^{-5} and 0.20×10^{-5} were used in our calculations for (u); the former estimate comes from assuming that the number of lethal-producing

Table 6 Environmental variance for third chromosomes extracted from the vineyard population

Viability range	No. of lines	Mean viability	Environmental variance	Average no. of flies per observation
A. Homozygotes (no. of lines tested = 51)				
0.5-0.6	8	0.5523000 ± 0.0070915	0.0063380 ± 0.0002010	402.56
0.6-0.7	13	0.6594693 ± 0.0069038	0.0059753 ± 0.0002404	425.88
0.7-0.8	15	0.7636667 ± 0.0063457	0.0066335 ± 0.0003222	463.13
0.8-0.9	8	0.8483876 ± 0.0114843	0.0223759 ± 0.0003783	482.94
0.9-1.0	5	0.9244200 ± 0.0085386	0.0081152 ± 0.0006848	410.90
1.0-1.1	2	1.0327000 ± 0.0089902	0.0012345 ± 0.0001093	593.00
Mean	51	0.7435510 ± 0.0181040	0.0088223 ± 0.0008551	477.20
Correlation between environmental variance and viability = 0.3113898 ± 0.1357546; $t = 2.294^*$				
B. Heterozygotes (no. of crosses = 145)				
0.7-0.8	3	0.7882667 ± 0.0029263	0.0152445 ± 0.0000308	544.17
0.8-0.9	14	0.8641358 ± 0.0069368	0.0071703 ± 0.0004218	457.07
0.9-1.0	51	0.9598254 ± 0.0041240	0.0080180 ± 0.0001152	526.36
1.0-1.1	61	1.0371180 ± 0.0036184	0.0076714 ± 0.0001144	532.88
1.1-1.2	14	1.1336500 ± 0.0083707	0.0146242 ± 0.0003869	471.39
1.2-1.3	2	1.2289500 ± 0.0180433	-0.0024201 ± 0.0009576	431.50
Mean	145	1.0001011 ± 0.0070112	0.0084337 ± 0.0002317	516.16
Correlation between environmental variance and viability = 0.1079144 ± 0.0831359; $t = 1.298$				

*: $p < 0.05$

Table 7 Estimate of the lethal allelism due to chance mutations and consanguinity

	I_C	I_N	I_F
VINEYARD			
n = 500		0.0027	0.0037
n = 2400	0.0064	0.00057	0.0058
CELLAR			
n = 500		0.0026	0.0059
n = 2400	0.0085	0.00055	0.0079

I_C = Rate of lethal allelism observed; I_N = lethal allelism due to mutation; I_F = allelism due to consanguinity; n = no. of lethal producing loci per third chromosome.

loci per third chromosome is similar to the second chromosome, $n = 500$ (cf. Wallace, 1950) and the latter is based on $n = 2,400$ (Judd *et al.*, 1972).

The estimate of effective sizes for both populations are presented in table 8.

Table 8 Estimates of the effective population sizes for cellar and vineyard populations

Population	Cellar	Vineyard
Q	0.2452	0.2759
I_C	0.0085	0.0064
I_g	0.0065	0.0047
N_e : $u = 10^{-5}$	11000	18500
$u = 0.2 \times 10^{-5}$	8000	11500

Q = Frequency of lethal chromosomes; I_C = allelism rate of lethal chromosomes; I_g = allelism rate of lethal genes; N_e = effective population size; and u = lethal mutation rate per locus

DISCUSSION

The frequency distributions of homozygote and heterozygote viabilities of *Drosophila melanogaster* from cellar and vineyard populations are similar, in spite of their habitats being relatively different. Factors peculiar to the cellar do not influence the fitness of its population of *D. melanogaster* (at least, as regards viability, which is the component of fitness studied here).

In the cellar population, the lethals in the heterozygous condition show a very slight deleterious effect while in the vineyard population neither dominance nor overdominance is manifest. Detrimental heterozygous effect of lethal genes in our populations can also indirectly be estimated from the frequency of lethal-carrying chromosomes. Under the null hypothesis (that heterozygous lethal genes are not deleterious to their carriers), the equilibrium frequency of lethal-carrying chromosomes can be predicted. The equilibrium frequency of a lethal is (u) (where (u)

is the lethal mutation rate). If the number of lethal-producing loci is (n), then the average number of lethal genes per chromosome is $n\sqrt{u}$. Assuming that the lethal genes are distributed on the chromosome according to a Poisson distribution, the expected frequency of lethal-carrying chromosomes (Q) can be estimated to be: $E(Q) = 1 - e^{-n\sqrt{u}}$.

Values of (u) and (n) have been reported as 10^{-5} /locus/generation and 500 respectively (as a minimum estimate); thus, $E(Q) = 0.79$. If $n = 2,400$; then $E(Q) = 0.97$. The estimates of (Q) in our populations are 0.24 and 0.27, which are significantly smaller than the minimum estimate of 0.79. This finding rejects the null hypothesis and clearly supports the alternative hypothesis that the fitness of lethal heterozygotes is significantly smaller than of lethal-free individuals in both habitats (cf. Mukai and Yamaguchi, 1974; Mukai and Nagano, 1983).

The semilethals in heterozygous condition in both populations show the same behaviour as the lethals and the average degree of dominance of viability polygenes estimated in the cellar population, though it is low ($\bar{h}_E = 0.152$) is within the estimated values for natural populations of *Drosophila melanogaster* made by Mukai (1969) and close to the estimates of Mukai (1964) and Mukai and Yamaguchi (1974).

Which mechanisms may act to maintain genetic load and genetic variability of viability in these populations?

Such a high detrimental load might indicate that some type of balancing selection is acting at some loci. However, heterosis was not observed for lethals nor for detrimentals, which together with the slight rate of lethal allelism, does not support associative overdominance as a mechanism operating to maintain these deleterious genes.

Simmons *et al.*, (1980) observed pleiotropic effects on fitness of mutations affecting viability in *D. melanogaster*. However, our experimental results do not support the action of associative overdominance on other components of fitness. Also, the experiments of Marinkovic (1967) and Rose and Charlesworth (1981) indicate no overdominance with respect to fertility.

The estimated values for elimination rates of lethals due to homozygosity depend on $IQ^2[(Q)$ being the frequency of lethal-carrying chromosomes and (I) their allelism rate] and were 0.00050 and 0.00048 for the cellar and vineyard respectively. These values are much smaller than the average chromosome mutation rate (0.0005) established by Wallace (1968) for the third chromosome.

Therefore, in our populations, bearing in mind that selective neutrality of the lethal heterozygotes has been rejected, purifying selection must be acting, (although at a low intensity) to eliminate lethals through heterozygotes.

Dominance (Mukai *et al.*, 1974; Mukai *et al.*, 1980; Mukai *et al.*, 1982b; Tachida and Mukai, 1985) or frequency dependent selection (Dolan and Robertson, 1975; Yoshimaru and Mukai, 1979) are not satisfactory mechanisms for maintaining genetic variability in random mating populations: diversifying selection may be a better candidate. However, the present study does not support the view that this type of selection is acting in our populations. In the vineyard, differences in the environmental variances of viability between homozygotes and heterozygotes is not observed; while in the cellar, developmental homeostasis for viability is manifested in heterozygotes even more than in homozygotes. This is different from the results in the Raleigh (Mukai *et al.*, 1982), the Florida (Mukai and Nagano, 1983) and the Ishigakijima (Tachida and Mukai, 1985) populations. The vineyard population is more similar, as regards the environmental variances of viability, to the Japanese population of Aomari studied by Kusakabe and Mukai (1984a) showing a rather similar situation to that of the newly arisen mildly deleterious mutations, which have not experienced natural selection at all (Mukai *et al.*, 1982a).

The smaller environmental variance of homozygotes compared to that of heterozygotes in the cellar population might simply result from the higher endogamy of this population, a circumstance which induce homozygosis. As the stability in viability of heterozygotes over homozygotes is a product of natural selection (Mukai *et al.*, 1982a), if this selection is not positively acting on the newly arisen mildly detrimental mutations which are homeostatic in heterozygotes, or if the selection pressure is very low, the gene frequency of homozygously homeostatic genes increases in this population. Thus, we find homozygote stability in respect of the viability in the chromosomes from the cellar population.

Recent work (Mukai *et al.*, 1974; Mukai and Nagano, 1983; Tachida *et al.*, 1983; Kusakabe and Mukai, 1984b) suggest that the principal mechanism responsible for the maintenance of genetic variability of viability on the majority of loci in the natural populations is likely to be a mutation-selection balance. These works also indicate that is a north-to-south cline in the magnitude of genetic variability of viability, which suggests that selection operates more actively in southern popu-

lations. If the high detrimental load in the populations from cellar and vineyard indicates the existence of a great number of concealed deleterious genes in our populations, then their behaviour would be more similar to that of more southern Japanese and American populations, where selection is more active. However, in our populations, the detrimental load is even higher than that of such southern populations.

The lethal load from the cellar and vineyard populations is within the range of that of natural populations (e.g., Greenberg and Crow, 1960; Kusakabe and Mukai, 1984a). The frequencies of lethal-carrying chromosomes in our populations are similar to the frequencies observed for this species in Mediterranean populations (Golschmidt *et al.*, 1955; Dawood, 1961; Sperlich and Karlik, 1963; Mourad *et al.*, 1976). Nevertheless, our values for the D:L relation are higher than those estimated in natural populations for *D. melanogaster*, and are closer to the values of other *Drosophila* species (Dobzhansky and Spassky, 1953; Malogolowkin-Cohen *et al.*, 1964).

What could be the cause of the high detrimental load in the populations studied? We think that two circumstances may be present: a higher mutation rate of mildly deleterious genes compared to that of lethals, the normal ratio of 20:1 (Mukai, 1964), and very low average degrees of dominance of lethals and detrimental compared to that of the majority of natural populations, the difference being relatively more accentuated for the average degree of dominance of detrimental genes. Therefore, the elimination by selection through heterozygotes will be low, more particularly for detrimental; the accumulation of these will hence be higher.

The possible effect of newly induced mutations in the processes of establishment and maintenance of chromosome lines might also be considered (Mukai, 1964; Mukai *et al.*, 1972). However the means and the variances in the different sets of experiments (cycles) did not decrease or increase as the experiments advanced, suggesting that this effect is not important.

It is unlikely that estimated viabilities are disturbed as a consequence of recombinational phenomena in *Ubx/+* heterozygous females due to the TM2 balancer (McIntyre and Wright, 1966). This chromosome also carry out the *h* and *ca* mutations which were used as a control, and no recombination was observed in any of the lines analysed. Moreover, this phenomenon occurred when the *Ubx/+* females also were heterozygous for an X-chromosome inversion. In natural popu-

lations of *D. melanogaster*, inversions on the X chromosome are rare (Inoue *et al.*, 1984) and, in our populations no inversion has been detected on this chromosome (González, 1985).

We cannot reject out the chromosome cytoplasmic interaction known as hybrid dysgenesis (Kidwell *et al.*, 1977). If this were present during the extraction of the third chromosome, the homozygous load due to detrimental genes estimated in the present study might be overestimated. However in a study on male recombination in the same populations (Botella and Ménsua, 1983), male recombination frequencies typical of hybrid dysgenesis were not detected.

The high detrimental load may exist in our populations throughout the year, but can also be a circumstance due to the moment of capture, which was at the end of the seasonal cycle of *D. melanogaster*. There might be a higher frequency of detrimental genes then due to their accumulation over the seasonal, as a consequence of the low pressure against detrimental heterozygotes.

The fact that cellar and vineyard populations behave in the same way suggest that they are subpopulations of a large population.

Lethal allelism and the population size estimate support this supposition. Wallace (1966) and Paik (1968) observed that the frequency of allelism of lethals declines linearly with the square root of distance. However, for the relatively great distance that separates the cellar and the vineyard (4 Km) the rate of allelism although it decreases does not do so markedly. The effective population sizes estimated in the cellar and vineyard populations are not very different; both populations are relatively large and the smaller size estimated in the cellar is essentially due to the consanguinity of this population (as suggested by the lethal allelism due to endogamy).

However, the level of interpopulational allelism of lethals is very low suggesting that each population has a characteristic complement of lethals. This indicates a certain independence of the two populations, which calls into question whether they really are two subpopulations of a larger population.

We think, in spite of the low frequency of interpopulation allelism, that there is gene flow between cellar and vineyard. McKenzie and Parsons (1974) observed in the *D. melanogaster* population from "Chateau Tahbilk", the existence of genic flow between the cellar and the exterior. Jones *et al.* (1981) estimated migration for *D. pseudoobscura* and their work suggests that migration between populations to the extent inferred

here is quite feasible. Moreover, it must be taken into account that the frequencies of interpopulation allelism are not excessively low if they are compared with the frequency of intrapopulation allelism and as Spieth (1974) said, "in terms of gene flow, the distinction between absolutely none and almost none is enormous".

REFERENCES

- BOTELLA, L. M. AND MENSUA, J. L. 1983. Analysis of male recombination in third chromosomes of *Drosophila melanogaster*. *Genetica*, 61, 197-203.
- CHOI, Y. 1978. Genetic load and viability variation in Korean natural populations of *Drosophila melanogaster*. *Theor. Appl. Genet.*, 53, 65-70.
- DAWOOD, M. M. 1961. The genetic load in the second chromosomes of some populations of *Drosophila melanogaster*. *Egypt Genetics*, 46, 239-246.
- DOBZHANSKY, T. AND SPASSKY, B. 1953. Genetics of natural populations. XXI. Variability in two sympatric species of *Drosophila*. *Genetics*, 38, 471-484.
- DOLAN, R. AND ROBERTSON, A. 1975. The effect of conditioning the medium in *Drosophila*, in relation to frequency-dependent selection. *Heredity*, 35, 311-316.
- GOLDSCHMIDT, E., WAHRMAN, J., LEDERMANN-KLEIN, A. AND WEISS, R. 1955. A two years survey of population dynamics of *Drosophila melanogaster*. *Evolution*, 9, 353-366.
- GONZALEZ, A. 1985. Ph.D. Thesis, University of Valencia.
- GREENBERG, R. AND CROW, C. F. 1960. A comparison of the effect of lethal and detrimental chromosomes from *Drosophila* populations. *Genetics*, 45, 1154-1168.
- HALDANE, J. B. J. 1956. Estimation of viabilities. *J. Genetics*, 54, 294-296.
- INOUE, Y., WATANABE, T. AND WATANABE, T. K. 1984. Evolutionary change of the chromosomal polymorphism in *Drosophila melanogaster* populations. *Evolution*, 38, 753-765.
- JONES, J. S., BRYANT, S. H., LEWONTIN, R. C., MOORE, J. A. AND PROUT, T. 1981. Gene flow and the geographical distribution of a molecular polymorphism in *Drosophila pseudoobscura*. *Genetics*, 98, 157-178.
- JUDD, B. H., SHEN, M. W. AND KAUFMAN, T. C. 1972. The anatomy and function of a segment of the Y chromosome of *Drosophila melanogaster*. *Genetics*, 71, 139-156.
- KIDWELL, M. G., KIDWELL, J. F. AND SVED, J. A. 1977. Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility and male recombination. *Genetics*, 86, 813-833.
- KUSAKABE, S. AND MUKAI, T. 1984a. The genetic structure of natural populations of *Drosophila melanogaster*. XVII. A population carrying genetic variability explicable by the classical hypothesis. *Genetics*, 108, 393-408.
- KUSAKABE, S. AND MUKAI, T. 1984b. The genetic structure of natural populations of *Drosophila melanogaster*. XVIII. Clinal and uniform genetic variation over populations. *Genetics*, 108, 617-632.
- LEVENE, H. 1953. Genetic equilibrium when more than one ecological niche is available. *Amer. Nat.*, 87, 331-333.
- LEVINS, R. 1968. *Evolution in changing environments*. Princeton Univ. Press. Princeton.

- LEVINS, R. AND McARTHUR, R. 1966. Maintenance of genetic polymorphism in a heterogeneous environment: variations on a theme by Howard Levene, *Amer. Nat.*, 100, 580-590.
- MALOGOLOWKIN-COHEN, C., LEVENE, H., DOBZHANSKY, N. P. AND SIMMONS, A. S. 1964. Inbreeding and the mutational and balanced loads in natural populations of a *Drosophila willistoni*. *Genetics*, 50, 1299-1311.
- MARINKOVIC, D. 1967. Genetic loads affecting fecundity in natural populations of *Drosophila pseudoobscura*. *Genetics*, 56, 61-71.
- McINTYRE, R. J. AND WRIGHT, T. R. F. 1966. Recombination in FM4/+; SM1/+; *Ubx*¹³⁰/+ heterozygotes. *Dros. Inf. Serv.*, 41, 141-142.
- McKENZIE, J. A. AND PARSONS, P. A. 1974. Microdifferentiation in a natural population of *Drosophila melanogaster* to alcohol in the environment. *Genetics*, 77, 385-394.
- MOURAD, A. H., TANTAWY, A. D. AND MOSTAGA, Y. A. 1976. Studies on natural populations of *Drosophila*. XIX. Concealed genetic variability in a marginal population of *Drosophila melanogaster* and its fate under laboratory conditions. *Egypt. J. Genet. Cytol.*, 5, 346-358.
- MUKAI, T. 1964. The structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. *Genetics*, 50, 1-19.
- MUKAI, T. 1969. The genetic structure of natural populations of *Drosophila melanogaster*. VIII. Natural selection on the degree of dominance of viability polygenes. *Genetics*, 63, 467-478.
- MUKAI, T. AND NAGANO, S. 1983. The genetic structure of natural populations of *Drosophila melanogaster*. XVI. Excess of additive genetic variance of viability. *Genetics*, 105, 115-134.
- MUKAI, T. AND YAMAGUCHI, D. 1974. The genetic structure of natural populations of *Drosophila melanogaster*. XI. Genetic variability in a local population. *Genetics*, 76, 339-366.
- MUKAI, T., CHIGUSA, S. I., METTLER, L. E. AND CROW, J. F. 1972. Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. *Genetics*, 72, 335-355.
- MUKAI, T., CARDELLINO, R. A., WATANABE, T. K. AND CROW, J. F. 1974. The genetic variance for viability and its components in a local population of *Drosophila melanogaster*. *Genetics*, 78, 1195-1208.
- MUKAI, T., TACHIDA, H. AND ICHINOSE, M. 1980. Selection for viability at loci controlling protein polymorphisms in *Drosophila melanogaster* is very weak at most. *Proc. Nat. Acad. Sci. (USA)*, 77, 4857-4860.
- MUKAI, T., CHIGUSA, S. I. AND KUSAKABE, S. 1982a. The genetic structure of natural populations of *Drosophila melanogaster*. XV. Nature of developmental homeostasis for viability. *Genetics*, 101, 279-300.
- MUKAI, T., YAMAGUCHI, O., KUSAKABE, S., TACHIDA, H., MATSUDA, M., ICHINOSE, M. AND YOSHIMARU, H. 1982b. Lack of balancing selection for protein polymorphisms. Kimura, M. (ed.), in *Molecular evolution, protein polymorphisms and the neutral theory*, Japan Scientific Societies Press, Tokyo/Springer-Verlag, Berlin.
- NEI, M. 1968. The frequency distribution of lethal chromosomes in finite populations. *Proc. Nat. Acad. Sci. (USA)*, 60, 517-524.
- PAIK, Y. K. 1968. Behaviour of lethals in *Drosophila melanogaster*. *Proc. XII Intern. Congr. Genet.*, 2, 164-165.
- ROSE, M. R. AND CHARLESWORTH, B. 1981. Genetics of life history in *Drosophila melanogaster*. I. Sib analysis of adult females. *Genetics*, 97, 173-186.
- SIMMONS, M. J., PRESON, C. R. AND ENGELS, W. R. 1980. Pleiotropic effects on fitness of mutations affecting viability in *Drosophila melanogaster*. *Genetics*, 94, 467-475.
- SPERLICH, D. AND KARLIK, A. 1963. Frequenz und Alleliengrad autosomaler Letalfaktoren in Insel- und Festlandpopulationen von *Drosophila melanogaster*. *Verh. Deutsch. Zool. Ges. München*, 73-78.
- SPIETH, P. 1974. Gene flow and differentiation. *Genetics*, 78, 961-965.
- TACHIDA, H. AND MUKAI, T. 1985. The genetic structure of natural populations of *Drosophila melanogaster*. XIX. Genotype-environment interaction in viability. *Genetics*, 111, 43-55.
- TACHIDA, H., MATSUDA, M., KUSAKABE, S. AND MUKAI, T. 1983. Variance component analysis for viability in an isolated population of *Drosophila melanogaster*. *Genet. Res. Camb.*, 42, 207-217.
- WALLACE, B. 1950. Allelism of second chromosome lethals in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. (USA)*, 36, 654-657.
- WALLACE, B. 1966. Distance and the allelism of lethals in a tropical population of *Drosophila melanogaster*. *Amer. Nat.*, 100, 565-578.
- WALLACE, B. 1968. Mutation rates for autosomal lethals in *Drosophila melanogaster*. *Genetics*, 60, 389-393.
- YOSHIMARU, H. AND MUKAI, T. 1979. Lack of experimental evidence for frequency-dependent selection at the alcohol dehydrogenase locus in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. (USA)*, 76, 876-878.