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Genetic Load and Viability Variation in Korean Natural Populations of *Drosophila melanogaster*

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Summary. Natural populations of *Drosophila melanogaster* from Anyang and Susac (suburbs of Seoul) have been analyzed with respect to viability variation on the second chromosome. Homozygotes as well as random heterozygotes for wild chromosomes were studied. The frequency of lethal factors was about 16 per cent, that of drastics 26 per cent. The average viability of homozygotes was 0.650 including lethal lines and 0.858 for quasinormals; that for random heterozygotes was 1.125. Allelism tests have been performed for the lethals. The allelism rate turned out to be as high as 0.036 and 0.0214, respectively. Using a formula by Nei, the effective population size can be estimated from these data. Korean *D. melanogaster* populations proved as small as 2000 to 3000 individuals. No correlation between homozygous and heterozygous viabilities could be found. According to these observations, along with the fact that partly big clusters of identic lethals could be found in the allelism tests, it is concluded that in Korean populations quite a large part of the hard genetic load is balanced. The connection between population size, population structure and associative or genuine overdominance is discussed.

Key words: *D. melanogaster* – Natural populations – Variability variations – Genetic load

Introduction

Studies on the genetic load concealed in natural populations of *Drosophila* have been conducted by a great number of different investigators. The first data on wild populations of *D. melanogaster* was reported by Ives as early as 1945 for America and by Dubinin in 1946 for Russia. Later, quite a big number of publications dealing with this *Drosophila* species followed. In addition, other species were used for studies (e.g. *D. pseudoobscura* by Dobzhansky and Spassky 1963, *D. willistoni* by Krimbas 1959, *D. subobscura* by Sperlich et. al. 1977).

For *D. melanogaster* it is now clear that all populations so far studied contain lethal factors in all chromosomes but differencies exist with respect to their relative frequencies. Whereas American populations (Band and Ives 1968, Greenberg and Crow 1960) and Mediterranean populations (Goldschmidt et. al. 1955, Dawood 1961, Sperlich and Karlik 1963) show similar lethal frequencies, those for populations from Eastern Asia (Minamori et. al. 1973, Paik 1960, 1966, Watanabe 1969) are generally much lower. On the other hand, Band (1972a) found that the frequencies of recessive drastics have changed in populations of U.S.A., and Minamori et. al. (1973) and Watanabe et. al. (1976) reported upon a sudden increase of lethal genes in Japanese natural populations of *D. melanogaster*.

Two main questions seem to be of outstanding importance in these connections. One is the general problem whether the genetic load is mainly due to mutation alone or whether it is balanced to a certain degree. The other question is how the differencies in lethal frequencies between the Asian and the American-European populations of *D. melanogaster* can be explained. This paper will present data which may contribute to the clarification of these problems.

Materials and Methods

Wild male flies of *Drosophila melanogaster* were collected in August 1974 at two localities (Anyang and Susac) near Seoul. Both trapping sites are about ten kilometers apart and ecologically very similar. They are situated in a large vine-growing region of Korea with many vine-yards around.

Each wild male sampled from the natural populations was individually crossed to three virginal *Cy/Pm* females, the genetic background of which had been already previously substituted by chromosomes from the same natural population. From each

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F_1-progeny a single *Cy/+* male was back-crossed to *Cy/Pm* females. Finally, the test crosses *Cy/+* × *Cy/+* were established from the various F_2-progenies and the offspring of these cultures counted. Because of the lethality of the *Cy/Cy* genotypes, *Cy/+* and *+/+* were expected to appear in the offspring in a 2:1 ratio. Any deviation from this expected proportion was considered to be due to viability genes on the wild second chromosomes tested. Hence, these chromosomes could then be classified according to the relative frequency of non-*Cy* flies in the test offspring using the following classification:

lethal (le): less than 1 per cent +/+ flies
semilethals (sle): less than 16.6 per cent +/+ flies
subvitals (sv): 16.7 to 26.7 per cent +/+ flies
quasinormals (nor): 26.8 to 39.9 per cent of +/+ flies
supervitals (spv): more than 39.9 per cent.

In addition to homozygous viabilities, random heterozygous viabilities were derived by applying almost the same crossing procedure with the exception of the last test crosses $Cy/+^n \times Cy/+^{n+1}$ (in the above mentioned crosses all *Cy/+* -flies of each test cross came from the same single male culture (*Cy/Pm* × single $Cy/+^n$ ♂) and hence carried the same wild chromosome). For estimating random heterozygous viabilities the test crosses were performed with *Cy/+* -flies from different single male cultures ($Cy/+^n \times Cy/+^{n+1}$). Again from these pairings *Cy/+* and *+/+* genotypes arose. The *+/+* genotypes, however, represented now random heterozygous combinations of wild chromosomes ($+^n/+^{n+1}$). The proportions of these wild genotypes were then used as a measure of viability and subjected to the same mode of classification as above.

Finally, allelism tests were performed for all lethals from the two samples from Anyang and Susac. The lethals were kept in balance over *Cy* and all possible interstrain crosses ($Cy/l_i \times Cy/l_j$) made. Those crosses which yielded no normal flies l_i/l_j were considered to carry allelic lethal factors.

Experimental Results

From the local populations of Anyang 223 and Susac 214 wild second chromosomes of *D. melanogaster* were tested with respect to their effect on viability in the homozygous condition. The results are shown in Table 1. Since a homogenity test did not reveal significant differences between the two populations, the data were pooled. The frequency of lethal-carrying chromosomes proved to be about 16 per cent. As can be seen from Table 2, however, quite a great number of chromosomes proved semilethal, among them a considerable group with extremely low viabilities.

In a second experiment the viabilities of genotypes were tested which carried two different quasinormal wild second chromosomes. Among these random heterozygotes no lethal and no semilethal combinations were found at all and the total distribution of viabilities is shown to shift in the direction of higher viability classes (Table 2).

In accordance with the usual results of many other investigations on viability determination in *Drosophila*, the distribution curve of homozygotes proved bimodal with one peak at the lethal and another peak at the normal viability class. The viability distribution of random heterozygotes, however, is a normal distribution with only one peak. If one wants to compare homozygote to heterozygote viabilities, two ways are possible. Either means and variances of all the variabilities or only those of quasinormal homozygotes can be used. The latter include the subvital, normal and supervital classes but omit the semilethal and lethal classes.

Table 1. Viabilities of *D. melanogaster* flies homozygous for second wild chromosomes extracted from two local populations in Korea (for classification criterions see 'Materials and Methods')

Population		Viability classes					Total number of chromosomes tested
		le	sle	sv	nor	spv	
Anyang	No observed	41	27	43	109	3	223
	per cent	18.39	12.11	19.28	48.88	1.34	–
Susac	No observed	30	17	35	128	4	214
	per cent	14.02	7.94	16.36	59.81	1.87	–
Total	No observed	71	44	78	237	7	437
	per cent	16.25	10.07	17.85	54.23	1.60	–
Homogenity test (χ^2)		1.282	1.880	0.524	2.407	0.187	
	$\Sigma \chi^2 = 6.280$ d.f. = 4 P = 0.2 – 0.1						

Table 2. Distribution of relative viabilities of homozygotes and heterozygotes for second chromosomes, expressed in viability ratios (wild type observed: wild type expected)

Viability ratio	Homozygotes	Heterozygotes
0.00-0.05	80	0
0.05-0.15	14	0
0.15-0.25	9	0
0.25-0.35	11	0
0.35-0.45	13	0
0.45-0.55	9	0
0.55-0.65	13	17
0.65-0.75	51	27
0.75-0.85	65	48
0.85-0.95	68	91
0.95-1.05	58	101
1.05-1.15	25	120
1.15-1.25	9	105
1.25-1.35	3	86
1.35-1.45	9	59
1.45-1.55	0	33
1.55-1.65	0	17
1.65-1.75	0	7
1.75-1.85	0	14
Total	437	725

In Table 3 the means and variances of quasinormal heterozygotes, of all homozygotes and of quasinormal homozygotes are shown. As expected, the random heterozygotes are considerably more viable than the homozygotes. With respect to variances, however, the quasinormals are less variable than the random heterozygotes. The F-value of 1.434 corresponds to a probability of less than 5 per cent. Thus, the difference in variances seems to be a reality. The high variance for the group of all homozygotes is, of course, due to the many included lethal chromosomes.

The question whether all observed lethal chromosomes carry different lethal factors or are allelic to each other was investigated by allelism tests among all lethal chromosomes available. In total, 1633 intercrossses between balanced lethal strains were performed. The allelism rates for lethals of the two populations do not differ very much from each other (Table 4). The common average intrapopulation allelism rate is 3.14, a value which can be considered as rather high and significantly different to the 0.4 interpopulation allelism rate. A more detailed analysis, however, reveals additional information. As can be seen from Figure 1, there exists a cluster of 8 allelic lethals in the Anyang population. A further cluster of 4 lethals is present in Anyang and a cluster of the same size and one for three lethals is present in Susac. Also, there exsists allelism between this cluster of three lethals from Susac and a single lethal from Anyang.

Finally, a direct comparison was made in order to find out whether a correlation exists between the viabilities of the wild second chromosomes in the homozygous and heterozygous condition. This can be done by calculating the observed viability of a given heterozygous combination ($+^i/+^i$) with the sum of the observed viabilities of the two corresponding homozygotes ($+^i/+^i$ and $+^j/+^j$). After having listed all i/j: $(i/i + j/j)$-pairs, a correlation table was drawn which is shown in Figure 2. There is no obvious positive

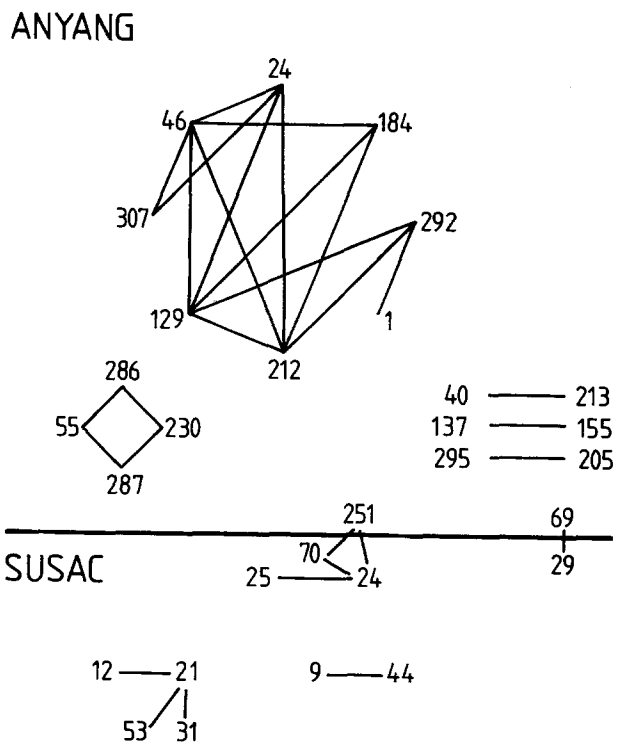

Fig. 1. Graphic demonstration of clusters of allelic lethals in two Korean wild populations of *D. melanogaster*

Table 3. Mean viabilities and variances for quasinormal heterozygotes and homozygotes for second wild chromosomes of *D. melanogaster*

Genotype	Number of crosses	Mean viabilities	Variances
Quasinormal heterozygotes	725	1.125 ± 0.010	0.0793
All homozygotes	437	0.650 ± 0.020	0.1674
Quasinormal homozygotes	322	0.858 ± 0.013	0.0553

Table 4. Allelic rates between second chromosome lethals isolated from natural populations of *D. melanogaster* from Anyang and Susac

Locality	Number of lethals	Number of crosses	Number of allelic lethals	% allelism
Anyang	41	611	22	3.60 ± 0.75
Susac	30	281	6	2.14 ± 0.86
Intrapopulation	71	892	28	3.14 ± 0.58
Interpopulation	71	741	3	0.40 ± 0.23

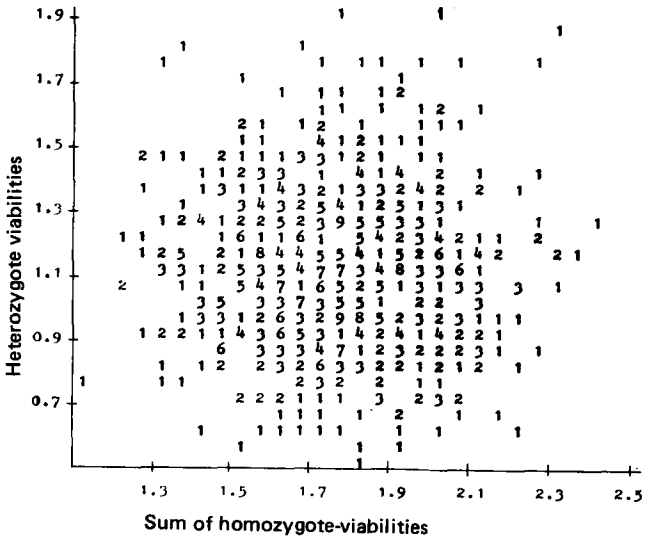

Fig. 2. The relationship between heterozygote viability (viab. $+^i/+^j$) and the sum of the viabilities (viab. $+^i/+^i$ and viab. $+^j/+^j$) of the corresponding homozygotes (lethals excluded)

Table 5. Frequencies of detrimentals (lethals + semilethals) and their allelism rates in Korean natural populations of *D. melanogaster* from 1957 to 1976

Year		Chromosomes tested	% le	% sle	% detrimentals	Number of allelic crosses	% allelism
1957	1)	611	8.7	2.5	11.2	1,002	4.49
1962	2)	474	10.1	5.1	15.2	865	1.04
1967	3)	1430	17.3	8.1	25.4	10,076	1.36
1971	4)	195	22.1	5.6	27.7	903	0.55
1972	4)	90	17.8	8.9	26.7	120	0.83
1973	4)	200	23.0	6.5	29.5	276	1.45
1976	5)	437	16.3	10.1	26.4	892	3.14

1) Paik (1960)
2) Paik (1966)
3) Paik and Sung (1969)
4) Choo and Lee (1976)
5) Present experimental results

or negative correlation. The coefficient of correlation was estimated to be 0.0712. It is not very much different from zero and its deviation from zero is far from being significant ($p > 0.5$). Hence, viability genes seem on the average to be recessive.

Discussion

Lethal frequencies and their allelism rates are not constant in natural populations of *Drosophila melanogaster*. A number of studies are available in which clear frequency changes in the second chromosome of this species are described. Ives (1954) found a reduction in the frequency of drastics after a period of hot and dry summers. Later, Band (1972b) presented a review of genetical changes from 1960 to 1970 in the South Amherst, Massachusetts (U.S.A.) natural population of *D. melanogaster*. In this period the frequencies of drastics fluctuated between 16 and 43 per cent. In Japan similar changes were observed from 1961 to 1971 by Minamori et. al. (1973) for a population from Hiroshima. In this case a general trend for increased frequencies of drastics from 13 per cent up to more than 30 per cent could be registered. More recently, Watanabe et. al. (1976) found for a Japanese population a doubling of the lethal frequency even in a period of only two years. A comparison of lethal and semilethal frequencies for Korean populations of *D. melanogaster* from 1957 to 1976 is given in Table 5. In accordance with other long term investigations, frequency changes are apparent. Whereas extremly low frequencies are noticeable for 1957 and 1962, an increase occurs between 1962 and 1967. From then on, only slight fluctuations are observable. A linear regression analysis shows a significant deviation from zero ($Y = 23.14 \pm 0.961\ (x - \bar{x})$, $t_{(5)} = 3.38$, $p = 0.02 - 0.01$). After omitting the data from 1957, however, the regression is no longer significantly different from zero. Together with the frequency increase, a decrease of allelism rates can be found. The only exception is the allelism rate of 0.0314 from the present investigation. Since only full lethals are usually used in calculating allelism rates, it would be better to compare the rates with lethal and not with drastic frequencies. As can be seen from Table 5, however, the lethal frequency of 1976 was only 16.3 per cent, a very low value.

Another point which should be made in this connection is related to the geographic differencies. It has been pointed out by several authors (e.g. Minamori and Azuma 1962) that American *Drosophila* populations have higher lethal frequencies at the average than Korean and Japanese populations. Allelism rates behave in the reverse way. Finally, the mean viabilities of quasinormals relative to random heterozygotes of the populations studied are negatively correlated to the frequency of drastics (Watanabe 1969). Our present data from Table 2 and 3 fit well in this line (frequency of detrimentals $\approx$ 26 per cent, viab. quasinormals/viab. het. ≈ 0.76).

Taking all these observations into consideration, the question arises as to what factors of population dynamics are those differencies due. Mukai and Yamaguchi (1974) in their excellent investigation on lethals in the second chromosome of *D. melanogaster* from a local population from North Carolina/U.S.A. deduced quite different population characteristics than we found in our investigation. Their lethal frequencies approach almost 40 per cent whereas our figures are less than 20 per cent. They find allelism rates at a magnitude of 0.005 whereas our figures are about 0.03. Finally they find a positive correlation between homozygous and heterozygous viabilities whereas in our investigation no correlation could be found (see Figure 2). With respect to mean quasinormal viabilities,

Table 6. Estimates of the effective population size for Korean populations of *D. melanogaster*

	Q	I_c	I_g	N_e ($u = 10^{-5}$)
Anyang	0.1839	0.0360	0.0295	1,800
Susac	0.1402	0.0214	0.0184	3,000
Average	0.1625	0.0314	0.0264	2,000

however, our data agree quite well (0.72 in Mukai's and 0.76 in our data).

According to Nei (1968) the effective population size (N_e) can be estimated by the following formula under the assumption that the degrees of dominance of the lethal genes (H) and the mutation rates to lethals (u) per locus are the same for all loci:

$$N_e = (1 - I_g) / 4(I_g U - u)$$

where I_g stands for the allelism rate of any individual gene locus; U is the total lethal mutation rate per chromosome and had been estimated by Mukai and Yamaguchi (1974) as 0.005; u is the lethal mutation rate per locus and was taken to be 10^{-5} in our calculations. I_g can be estimated from the experimental data by:

$$I_g = \frac{-\ln(1 - I_c q^2)}{[\ln(1 - q)]^2}$$

In this formula I_c stands for the allelism rate of lethal chromosomes and q for their frequency. By use of this formula, population size estimates were deduced from our data (Table 6). Comparing our findings to Mukai and Yamaguchi's data another unexpected difference becomes apparent. Whereas their N_e estimates for American populations are as large as 20,000 to more than 100,000, our values are between 2000 and 3000 only.

According to the differencies in the observation data our interpretations and explanations also have to be different. It is quite clear that Mukai and Yamaguchi (1974) explain the mechanism of maintenance of genetic load and variability in populations to be basically due to a balance between mutation and selection pressures allowing only a little effect from associative overdominance. Our data, on the other hand, are quite clearly much more in favor to an explanation which puts forward the importance of balancing selection. Not only do we find high allelism rates and no correlation between homozygous and heterozygous viabilities, but there is also the high amount of identical lethals (see Figure 1). Hence our impression is that the lethal load in the Korean populations studied by us is mainly due to a small number of individual lethal factors which are heterotic. Sperlich and Karlik (1970, 1972 and unpl.) and Karlik and Feuerbach-Mravlag (1977) could show that the incorporation of lethals into experimental populations of *D. melanogaster* is different in homozygous and heterozygous populations. In homozygous lethal free populations, the probability for a spontaneous or newly induced lethal to become heterotic is much higher than in a population containing a high genetic variability. Whether the heterotic effect is due to associative overdominance has been investigated by Pfriem (1978). There are most probably hitch-hiking effects involved in the phenomenon. Mukai and Yamazaki (1968) found also overdominance for viability mutations when they are on the same chromosome and in heterozygous combination with a 'normal' chromosome. This overdominant effect increases with the accumulation of linked mutants until a maximum of about 11 mutants is reached; then it decreases.

Whatever the case may be, if heterotic lethals (due to associative overdominance or not) can appear in a population which is large enough to prevent strong random effects then these heterotic lethals will persist in the population with a higher probability than others.

It is clear that the differencies between our present findings and those published by Mukai and Yamaguchi are mainly produced by the effect that they investigated large populations with strong migration effects whereas our data stem from relatively small populations. Whether this is only due to ecogeographic differencies between America and Eastern Asian populations or also partly to the sampling methods remains questionable. In any case, the largest problem appears to be the difficulty in collecting *Drosophila* samples. If local populations of *Drosophila* are small and migration reduced, the collections over a broad area will not allow the detection of all dynamic processes inside of the panmictic unities. On the other hand, samples from a single place in an area of continous distribution will increase the chance for collecting relatives.

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