EFFECTS OF TEMPERATURE ON THE VIABILITIES OF HOMOZYGOTES AND HETEROZYGOTES FOR SECOND CHROMOSOMES OF *DROSOPHILA MELANOGASTER*

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BAND (1963) has reported that in *Drosophila melanogaster* significant viability changes among random heterozygotes for lethal, semilethal and normal chromosomes could be induced when the same chromosome combinations were tested in wide and narrow temperature range conditions having a mean temperature of 25°C. In a study utilizing only lethal and normal chromosomes, TOBARI (1966) found that viabilities of lethal heterozygotes were higher than those of normal heterozygotes at an above standard temperature, analogous to the results of BAND obtained in the narrow range conditions; however, viabilities of the lethal heterozygotes were lower than those of normal heterozygotes both in the standard laboratory temperature conditions and at the lower temperature of 17°C. In neither study by BAND (1963) nor TOBARI (1966) were the viability effects of the homozygous chromosomes scored at temperatures other than the standard laboratory temperature.

On the other hand, BAND and IVES (1963) studied the correlation between viabilities of homozygotes and heterozygotes at the standard laboratory temperature of 25°C and at a lower temperature of 17°C, using the second chromosomes isolated from a natural population of *D. melanogaster*. They obtained negative but nonsignificant values at both temperatures. This and other evidence led them to conclude that the heterozygous effects of second chromosomes on viability are largely independent of the homozygous effects of these same chromosomes when experiments are carried out at the standard laboratory temperature and at a lower temperature, and that the genetic load is primarily of balanced type.

The present investigation was undertaken to study the homozygous and heterozygous effects of second chromosomes isolated from a laboratory population of *D. melanogaster* at the standard laboratory temperature and the higher temperature of 29°C.

MATERIALS AND METHODS

The base population employed in this study was the same as that used in the previous experiment (TOBARI 1966). This base population was set up by intermixing 20 males and 20 females from each of 24 different laboratory stocks of *D. melanogaster*. Before starting the experiment, this base population had been kept for about two years at constant temperature (25°C) in a plastic population cage (30 × 40 × 13 cm) provided with 15 cups containing medium.

In the test of homozygous effect of second chromosomes, 159 males taken from the base popu-

lation were individually crossed to *Cy/Pm* females having the X and third chromosomes originating from the base population. In the F_1 generation, a single *Cy/+* male fly taken from each cross was backcrossed to *Cy/Pm* females. In the F_2 generation, ten pairs of *Cy/+* flies taken from each backcross were introduced into a vial (3×10 cm) having agar, sugar and yeast medium. They were allowed to lay eggs for a period of a day and transferred to a next vial. Such procedures were repeated six times in a constant temperature (25°C) room. The first, third and fifth vials thus obtained were placed at 25° , and the remaining vials in an incubator at 29° . In the next generation, two types of flies, non-Curly and Curly, were counted separately before the offspring emerged. If the chromosome tested is normal, the frequency of wild-type flies is expected to be 33.3%. In practice, this frequency varies from 0 to more than 33.3% according to the viability of the wild-type homozygotes. It should be noted here that 25°C is the optimum growth temperature for *D. melanogaster* and 29°C creates an adverse environment.

In the test of heterozygous effect, 10 *Cy/+* F_2 females taken from the backcross progeny from an F_1 male fly were crossed to 10 *Cy/+* F_2 males derived from another F_1 male fly. As in the test of homozygous effect, six vials with eggs deposited by the same parents were obtained. Three vials (1st, 3rd and 5th) were placed at 25° and the remaining three (2nd, 4th and 6th) in an incubator at 29° . The wild-type flies in the progeny thus carried two different second chromosomes in heterozygous condition. The total number of random heterozygous combinations produced was 158.

RESULTS

Viabilities of the random heterozygotes: The average viabilities of the random heterozygotes of second chromosomes at the optimum and above-optimum temperature, 25° and 29°C , are presented in Table 1. The average viability is higher at 29° than at 25° , though the difference is not statistically significant.

The frequency distribution of heterozygote viability is given in Table 2. It is seen that the distribution is of normal type at both temperatures. The correlation

TABLE 1

Viabilities of heterozygotes and homozygotes tested at 25° and 29°C

	Temperature	Viability (%)	No. of flies counted	<i>n</i>
All homozygotes	25°C	23.8 ± 1.06	70,296	159
	29°C	21.9 ± 0.89	55,146	159
Quasinormal homozygotes	25°C	30.9 ± 0.38	57,194	119
	29°C	29.9 ± 0.41	41,528	113
Heterozygotes	25°C	34.1 ± 0.21	76,308	158
	29°C	35.4 ± 0.28	66,669	158

n = Number of chromosomes or chromosome combinations tested.

TABLE 2

Frequency distribution of viability of random heterozygotes tested at 25° and 29°C

	Viability															Total
	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	
25°C				1	2	7	22	39	49	29	8	0	0	1		158
29°C		1	0	1	1	6	12	34	47	26	14	9	3	3	1	158

FIGURE 1.—Correlation between the viabilities of random heterozygotes at 25° and 29°C.

coefficient between the viabilities at the two temperatures is 0.2217, which is significant at the 1 % level (cf. Figure 1).

Viabilities of the homozygotes: The average viabilities of the homozygotes at 25° and 29° are given in Table 1. The average viability of all homozygotes is markedly lower than that of the heterozygotes at both the temperatures. The difference in viability between the homozygotes and heterozygotes is statistically significant at 29° but not at 25°. This result indicates that if the population studied suddenly became homozygous for the second chromosomes, the resulting loss of fitness (inbreeding load), expressed in percentage of the viability of the random heterozygotes, would be 30.2% and 38.1% at 25° and 29°, respectively. That is, the inbreeding load is larger in adverse environments than in favourable ones.

The frequency distribution of homozygote viability is given in Table 3. The distribution is bimodal, similar to that obtained in the previous study (TOBARI 1966) and also to those reported by other workers (DOBZHANSKY and SPASSKY 1953, 1954; KRIMBAS 1959; GREENBERG and CROW 1960; DAWOOD 1961; OSHIMA 1963). The relation between the viabilities of the homozygotes at the two tem-

TABLE 3

Frequency distribution of viability of homozygotes for second chromosomes tested at 25° and 29°C

	Viability																				Total	
	0	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36		38
25°C	27	6	0	1	2	1	0	0	2	2	1	3	5	5	12	16	21	28	21	4	2	159
29°C	30	4	2	2	2	3	1	1	1	4	2	4	6	7	11	18	18	18	12	10	3	159

FIGURE 2.—Correlation between the viabilities of homozygotes at 25° and 29°C.

peratures is presented graphically in Figure 2. The correlation coefficient is 0.9318 ($P < .001$).

Further, it may be noted that the difference in viability between the quasi-normal homozygotes and random heterozygotes is larger at 29° than at 25° (cf. Table 1).

Frequencies of lethal, semilethal and subvital chromosomes: In the present investigation, lethal chromosome was defined as a chromosome giving no wild-type flies in homozygous condition, and semilethal chromosome as a chromosome giving less than half the expected frequency of wild-type flies, that is, $0\% < +/+ < 16.7\%$. At 25°, the frequencies of lethal and semilethal chromosomes were 17.0% and 8.2%, respectively, while at 29° they were 18.9% and 10.1%. Thus, the frequency of drastic chromosomes, i. e., lethal and semilethal chromosomes, is larger by 4% at 29° than at 25°, though the difference is not statistically significant. As shown in Figure 2, four of the 30 chromosomes which are lethal at 29° have become semilethal and one of them even normal at 25°. On the other hand, only two of the 27 chromosomes which are lethal at 25° have become semilethal at 29°. Furthermore, seven of the 16 semilethal chromosomes at 29° have become subvital or normal at 25°, while only one of the 13 semilethal chromosomes at

25° has become subvital at 29°. Similar increases in the frequency of drastic chromosomes at higher temperatures have been observed by BAND and IVES (1963) and SPASSKY *et al.* (1960) for the chromosomes of *D. melanogaster* and *D. pseudoobscura*, respectively.

The quasinormal chromosomes are divided into three classes, namely subvital, normal and supervital. A statistical method for estimating the frequencies of these three classes has been developed by WALLACE and MADDEN (1953) (see DOBZHANSKY and SPASSKY 1953). In this method of estimation, subvital chromosomes are defined as those yielding homozygotes with viability below the average heterozygote viability by more than $2\sigma_{g,het}$, and supervital chromosomes as those yielding homozygotes with viability above the average viability of heterozygotes by more than $2\sigma_{g,het}$, where $\sigma_{g,het}$ denotes the genetic standard deviation of viability of the heterozygotes. The genetic variances for the heterozygotes and homozygotes are shown in Table 4, together with the environmental variances (including the sampling variance).

The proportions of the three classes of quasinormal homozygotes obtained are as follows:

	Subvital	Normal	Supervital
25°C:	44.5%	53.2%	2.3%
29°C:	57.1%	42.8%	0.1%

It is seen that the proportion of subvital chromosomes is higher at 29° than at 25°, the difference being significant at the 5% level. This means that some chromosomes which are normal at a favourable environment become subvital at an adverse environment. A similar behavior was found by SPASSKY *et al.* (1960) for the second and third chromosomes from a Texas population of *D. pseudoobscura*, but not for those from the California population. In the experiment of BAND and IVES (1963), the proportion of subvital chromosomes was almost the same for the both temperatures of 17° and 25°.

Correlation between the heterozygote viabilities and the mean viabilities of homozygotes: Comparison of the heterozygote viabilities with the mean viabilities of two corresponding homozygotes has been conducted at each temperature. The results obtained are presented in Figure 3. The correlation coefficients computed are: at 25°C, 0.0176 ± 0.0806 ; and at 29°, -0.2271 ± 0.0782 . The correlation at 25° is not significant. This is in agreement with the results obtained by DOBZHANSKY and SPASSKY (1963) and BAND and IVES (1963). On the other hand, the

TABLE 4

Observed variance (σ_{ges}^2), environmental-sampling variance (σ_{es}^2), and genetic variance (σ_g^2) among heterozygotes and among quasinormal homozygotes

Temperature	Heterozygotes			Homozygotes		
	σ_{ges}^2	σ_{es}^2	σ_g^2	σ_{ges}^2	σ_{es}^2	σ_g^2
25°C	7.18	3.79	3.39	17.13	4.92	12.21
29°C	11.78	6.24	5.54	27.08	7.99	19.09

FIGURE 3.—Correlation between the heterozygote viabilities and the mean viabilities of two corresponding homozygotes. Numbers on the left in the squares refer to viability at 29°C, those on the right to viability at 25°C.

correlation at 29° is negative and significantly different from 0. This indicates that the chromosomes, which are deleterious when homozygous, may produce superior heterozygotes, that is, overdominance is involved.

Viabilities of the three classes of random heterozygotes: The viabilities of the heterozygotes for drastic chromosomes (d/d; lethals + semilethals), drastic and nondrastic (d/nd) and nondrastic chromosomes (nd/nd), together with the variance components for viability are shown in Table 5. At 25°C the d/d heterozygotes are less viable than the d/nd and nd/nd heterozygotes, while at 29° the

TABLE 5

Mean and variances of the viability for three different classes of random heterozygotes

	25°C			29°C		
	nd/nd	nd/d	d/d	nd/nd	nd/d	d/d
Mean viability (%)	34.2	34.1	32.9	35.1	35.8	35.4
No. of chromosome combinations	88	61	9	79	67	12
No. of flies per chromosome combination	501.7	466.2	413.9	412.4	431.8	430.1
σ^2_{ges}	6.76	7.61	10.49	12.69	11.09	11.53
σ^2_{es}	3.50	3.93	6.97	5.43	7.48	4.72
σ^2_g	3.26	3.68	3.52	7.26	3.61	6.81

d = drastic; nd = non-drastic.

d/nd heterozygotes show the highest viability. The differences in viability among the three classes are not significant at either temperature. Nevertheless, the result suggests that the drastic chromosomes show overdominance at temperature higher than the optimum, agreeing with the previous results (TOBARI 1966). The selection coefficient for the heterozygotes for the drastic chromosomes may be measured by the reduction in viability of the drastic heterozygotes relative to the viability of the nondrastic heterozygote. The selection coefficient thus obtained is 0.0205 at 25°C and -0.0114 at 29°.

The genetic variances for the nd/nd and d/d classes at 29° are about two times larger than those at 25°. For the d/nd class, it is almost the same for the both temperatures of 25° and 29°. These results suggest that the developmental homeostasis of the heterozygotes may be realized in a slightly higher degree at 25° than 29°.

DISCUSSION

BAND and IVES (1963) reported that the average viability of the random heterozygotes is almost independent of the temperature at which the flies are grown. In the present case, however, the viability of the random heterozygotes was higher at above-optimum temperature than at the optimum temperature, though the difference was not statistically significant. This disagreement may be due to the fact that BAND and IVES performed the experiment at 17° and 25°C, whereas we did at 25° and 29°. TOBARI (1966) found that the viability of lethal heterozygotes is almost the same for the groups grown at 17° and 25°, while it shows overdominance at 29°. Another possible cause of the disagreement may be in the fact that the experimental material used is not the same. BAND and IVES used the chromosomes extracted from a natural population, while we used those taken from a laboratory population.

The overdominance observed at 29° is considered to have arisen primarily by a higher degree of mortality of the homozygotes than the heterozygotes. As seen from Table 1, the number of wild-type flies grown (counted) per culture at 29° is 78% of the number of those at 25° for the homozygotes, while it is 87% for heterozygotes. This higher mortality of the homozygotes than the heterozygotes at 29° has certainly contributed to the higher viability of the heterozygotes relative to that of the homozygotes at this temperature. A higher degree of developmental homeostasis of the heterozygotes than the homozygotes at 29° is also suggested by a smaller genetic variance of viability of the heterozygotes than the homozygotes (cf. Table 4).

KRIMBAS (1959) has utilized the correlation between the viabilities of the homozygotes and heterozygotes for discriminating between the mutational and balanced loads. DR. NEI (personal communication) has shown that the expectation of the correlation is given by

$$r = \frac{\Sigma sh + \Sigma qs(s - 2h)}{\sqrt{\frac{1}{2}\Sigma s^2\{2\Sigma(1 - 2pq)h^2 - 4\Sigma q^2hs + \Sigma q(1 + q)s^2\}}}$$

approximately, where s and h refer to the selection coefficients of the homozygotes and heterozygotes for a less fit allele at individual loci, respectively, and q is the

frequency of the allele. At equilibrium, this correlation is always positive when the genetic variation is maintained by mutation and selection, though it could be small if h is 0 or very small. On the other hand, when overdominance is involved, the correlation becomes 0.

In the present investigation, the correlation at 25°C was very small and not statistically significant. Thus, no discrimination can be made by this correlation between the mutational and balanced loads, though the former is advocated by the positive value of the estimate of h (0.0205) previously mentioned. The correlation at 29° was negative, which fits neither model of mutation-selection and overdominance at equilibrium. However, this negative correlation suggests that h becomes negative at 29°, while it is positive at 25°. Note that the value of r becomes negative when h is negative and q is smaller than the equilibrium frequency under overdominant selection, i.e. $-h/(s-2h)$ for most loci. Our basic population has been kept at 25° for a long time, so that the frequencies of deleterious genes are considered to have become much smaller than the value of $-h/(s-2h)$ at 29°.

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SUMMARY

One hundred fifty-nine second chromosomes were extracted from a laboratory population kept at 25°C and the preadult viabilities of the homozygotes and heterozygotes for these chromosomes were determined by *Cy*-technique at 25° and 29°. The average viability of the heterozygotes is higher at 29° than at 25°, while that of the homozygotes is lower at 29° than at 25°. This means that the inbreeding load is larger in adverse environments than in favourable ones. There is no correlation between the viabilities of the homozygotes and heterozygotes at 25°, while it is negative and significantly different from 0 at 29°, suggesting that overdominance is involved in the chromosomes tested at a temperature higher than the optimum. The selection coefficient estimated from the reduction in viability of the heterozygotes for drastic chromosomes relative to that for nondrastic chromosomes is .0205 at 25° and $-.0114$ at 29°.

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