LETHAL FREQUENCIES ON SECOND AND THIRD CHROMOSOMES IN POPULATIONS OF *DROSOPHILA MELANOGASTER*¹

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THERE is no doubt that natural populations of *Drosophila* carry hidden genetic variation. Few naturally-occurring flies are free of genes, or gene complexes, that lower fitness when brought together in homozygous condition. The number of drastic (lethal and semilethal) chromosomes carried by a population in heterozygotes varies considerably. IVES (1954) observed an average of 36.2% second chromosome lethals in *D. melanogaster* with a range from 25.6% to 65.3% for flies tested from nine different areas of the United States. The lethal frequency for second chromosomes in several Japanese populations of *D. melanogaster* (OSHIMA and KITAGAWA 1961) was about 12.9% with little variation between populations. *D. melanogaster* from the American tropics showed an average frequency of 18.2% second chromosome lethals with a range of 9.2% to 25.3% for five different Colombian populations (HOENIGSBERG and deNAVAS 1965). When semilethals are included for these populations, the frequency of drastics averaged 37.8% with a range of 25.3% from Bogota to 48.1% from one site at Caracolisito. WALLACE, ZOUROS and KRIMBAS (1966) reported 44.7% drastics for chromosome 2 and 55.3% for chromosome 3 from Bogota, Colombia, using a translocation (2;3) marker stock. PAIK (1966) examined three Korean samples and reported an average of 10.1% and 14.5% lethals for chromosomes 2 and 3, respectively, when tested simultaneously; while if semilethals were included the total frequency of drastics was 15.2% and 18.4% for 2 and 3, respectively. Simultaneous tests on chromosomes 2 and 3 of *D. pseudoobscura* from several localities revealed 25.3% and 16.4% drastics, respectively (SPASSKY, DOBZHANSKY and ANDERSON 1965). This difference is expected if based on relative lengths for the two chromosomes.

When various populations are compared, flies do not carry the same number of drastics on any particular chromosome. DOBZHANSKY (1933) observed a significant difference between percentages of drastic third chromosomes of *D. pseudoobscura* from Mexico and Guatemala (30.0 ± 4.2) and California (13.9 ± 1.0) populations. However, flies from the same location should carry similar genetic viability factors on chromosomes of about equal length (BAND and IVES 1963; WALLACE *et al.* 1966; PAIK 1966) or proportional to their lengths (SPASSKY *et al.* 1965) if the presence of a drastic is a chance event.

Viability tests of *D. melanogaster* for chromosomes 2 and 3 have shown a rather

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consistently higher number of drastics on chromosome 3 than on 2. This difference has not been significant for results of particular tests. In the following report, lethal frequencies for chromosomes 2 and 3 from several populations were not consistent from population to population. However, when results from all populations were combined, chromosome 3 had a higher number of lethals than did chromosome 2. These results are not expected for chromosomes of about equal length. (BRIDGE's maps show 108 and 110 map units for chromosomes 2 and 3, respectively.)

MATERIALS AND METHODS

Samples of flies were taken from one laboratory population and six natural populations. Populations and samples were as follows: *Cage*—A population cage (lucite $6 \times 6 \times 18$ in) was started with flies from twenty egg samples from WALLACE's (1951) control population #3, originally free of lethals on chromosome 2. After an estimated 160 generations of this reestablished population, tests were made on flies from 4 different samples by SPIESS and ALLEN (1961). *Austin*—Several samples of flies were collected with fermenting banana traps during October, 1961, from three sites within the city limits of Austin, Texas, from about $\frac{1}{2}$ to $2\frac{1}{2}$ miles apart. Results of tests on the first 100 chromosomes were reported by ALLEN (1966). *Samoa*—Two samples were collected by PROFESSOR WHEELER, one in the spring and one in the summer of 1962 in or near the city of Pago Pago, Tutuila Island, American Samoa. Tests were made on males or single sons of females from those collections. *Tenn.*—A total of 72 males was successfully tested from a collection made in mid-November, 1962, by PROFESSOR STEVENSON near Johnson City in east Tennessee. Since several heavy frosts had occurred prior to collection, these flies probably represented the population during the last of the season. *Lubbock*—The 177 successful tests from this locality represent small collections taken within the city limits of Lubbock, Texas, during 1964, 1965, and 1966 from watermelon or banana traps, or from fallen fruits. Males or a single son each from females were tested. Large populations of flies are rare in this area, perhaps due to the semi-arid conditions, reaching a peak in numbers in late summer or early fall. Most of the collections were made in that season.

When several samples are taken over a period of years from single localities the data are combined. A homogeneity chi-square test shows no significant difference between the occurrence of drastics from year to year and from sample to sample (Lubbock, $\chi^2 = 3.8$ with 4 df, $P > 0.30$; Hondo, $\chi^2 = 12.7$ with 6 df, $P \approx 0.05$; N.C., $\chi^2 = 8.2$ with 4 df, $P > 0.05$). *Hondo*—This population was from an area of irrigated commercial fruit farms in Bonita Valley, Hondo, New Mexico. The trees are sprayed with a variety of insecticides (among them DDT, dieldrin, and arsenic) throughout the season. Samples were collected in late August or early September during 1964, 1965, and 1966 from several sites. This area is also semi-arid, and large populations are rare. Samples were taken by sweeping over fallen or discarded fruit. The combined samples represent about half of the total original sample. Sterility or too few generation 3 flies were the major reasons for failures. *N.C.*—Samples from this area came from large populations on a farm 6 miles from Marion, N.C., in the foothills of the Blue Ridge Mountains. Collections were made in the summer or early fall of 1966 and 1967 by sweeping over discarded fruit or with yeasted traps.

A total of 960 flies were successfully tested from all localities. The tests extended over a period of years from 1958 to 1967. Experiments were conducted and flies maintained in a controlled temperature at about 25°C . SPASSKY's medium was used for the 1st, 5th, 6th and 7th populations and a standard cornmeal medium of the Genetics Foundation, Austin, Texas, for the 2nd 3rd and 4th populations described above.

Figure 1 shows the general mating scheme, the genotypes of tester stocks, and the expected proportions in the third generation. The balancer chromosomes were *In(2LR)SM¹,al²Cy cn²sp²* for chromosome 2 and *In(3LR)Ubx¹³⁰²⁸* for chromosome 3. Only one of the second and third chromosomes from wild type males, 2?/2?, 3?/3?, in generation 0 was balanced in generation 1 males by choosing a single male, *Cy*/2?, *Ubx*/3?. In order to obtain males and females with the same balanced 2? and 3? this single male was crossed with three virgin tester stock females. The

GEN.

$$0 \quad \frac{Cy}{Pm} \quad \frac{Ubx}{Sb} \quad \text{♀♀} \times \frac{2?}{2?} \quad \frac{3?}{3?} \quad \text{♂♂}$$

$$1 \quad \frac{2?}{Cy} \quad \frac{3?}{Ubx} \quad \text{♂♂} \times \frac{Cy}{Pm} \quad \frac{Ubx}{Sb} \quad \text{♀♀}$$

$$2 \quad \frac{2?}{Cy} \quad \frac{3?}{Ubx} \quad \text{♀♀} \times \frac{2?}{Cy} \quad \frac{3?}{Ubx} \quad \text{♂♂}$$

3 OFFSPRING EXPECTED

$$\frac{4}{9} \frac{2?}{Cy} \quad \frac{3?}{Ubx} : \frac{2}{9} \frac{2?}{Cy} \quad \frac{3?}{3?} : \frac{2}{9} \frac{2?}{2?} \quad \frac{3?}{Ubx} : \frac{1}{9} \frac{2?}{2?} \quad \frac{3?}{3?}$$

FIGURE 1.—Mating scheme to obtain homozygotes for chromosomes 2 and/or 3, and the expected proportions in generation 3.

mating was expected to produce 4/9 *Cy,Ubx* : 2/9 *Cy*,+ : 2/9 +,*Ubx* : 1/9 +,+ individuals. Routinely, a random 100 or more generation 3 flies were classified. Care was exercised in all experiments not to include flies from a 4th generation and not to classify only the first-emerging flies. If less than 90 flies were produced in generation 3, either the cross was repeated (starting with a balanced *Cy*/2?, *Ubx*/3? male at generation 1, or the data were omitted.

RESULTS

The relative viability of chromosomes 2 and 3 homozygotes was estimated from data collected in generation 3 (Figure 1) for seven populations (Table 1). Actual numbers of chromosomes tested and F_3 counted are given in Table 2. These relative values are based on an expected value of 33.3% wild type because twice as many *marker*/+ flies are expected as homozygous +/+ flies. The total values indicate a difference between the two heterologous autosomes, both including and excluding lethals (0% wild type in generation 3). This difference is not obvious when populations are taken separately. A homogeneity chi-square test was highly significant ($\chi^2 = 72.3$ with 18 df, $P < .001$), which indicates a difference between populations. All populations except the two Texan populations had lower values for chromosome 3 than for 2. However, since this may be a differential marker effect (*Cy*/+ vs. *Ubx*/+) on viability, no conclusions can be drawn from a comparison between nonlethal chromosomes 2 and 3. The lethal class probably best represents natural conditions. It is likely that this class would be lethal in homozygous condition independent of the technique, whether point mutation or gross aberration is involved.

The total number of lethals was greater for chromosome 3 than for 2, 21.1% vs. 16.5%, respectively (Table 3). This difference is not obvious for each population taken separately. Samples from the two Texan populations displayed more lethals on chromosome 2 than on 3 with equal numbers on each for Tennessee. However, a test for heterogeneity indicated no significant difference between populations

TABLE 1
*Relative viabilities of homozygous second and third chromosomes from
different populations of D. melanogaster*

Population sampled	Number of chromosomes tested	Percentage* including lethals		Number of chromosomes 2	Percentage* excluding lethals		Number of chromosomes 3
		Chromosome 2	Chromosome 3		Chromosome 2	Chromosome 3	
Cage	104	25.5 ± 1.4	18.3 ± 1.4	88	30.1 ± 1.1	26.0 ± 1.0	73
Austin, Texas	93	20.6 ± 1.4	20.7 ± 1.3	73	26.2 ± 1.1	25.4 ± 1.0	76
Samoa	109	22.3 ± 1.3	17.4 ± 1.3	93	26.1 ± 1.0	24.6 ± 1.0	77
Tennessee	72	22.4 ± 1.6	16.9 ± 1.3	59	27.3 ± 1.2	20.6 ± 1.0	59
Lubbock, Texas	177	18.5 ± 0.9	21.5 ± 1.7	156	21.0 ± 0.9	23.7 ± 1.8	161
Hondo, New Mexico	147	22.6 ± 1.1	19.1 ± 1.0	133	25.0 ± 1.1	22.8 ± 0.8	123
N. Carolina	258	19.9 ± 0.9	14.6 ± 0.8	200	25.8 ± 0.7	20.1 ± 0.7	188
Total	960	21.4 ± 0.4	18.2 ± 0.5	802	25.4 ± 0.4	22.9 ± 0.5	757

* ± Standard error.

TABLE 2

Numbers of flies in Generation 3, including and excluding (italics) counts with drastics on either or both chromosomes

Population sampled	Number of chromosomes tested	<i>Cy Ubx</i>	<i>Cy</i>	<i>Ubx</i>	+	Total
Cage	104	6,297	1,404	2,141	503	10,345
	50	2,411	959	1,145	485	5,000
Austin	93	5,831	1,556	1,541	372	9,300
	42	2,024	777	866	333	4,000
Samoa	109	6,972	1,464	2,000	428	10,864
	50	2,574	965	1,064	387	4,990
Tenn.	72	4,627	968	1,363	242	7,200
	31	1,585	568	741	205	3,099
Lubbock	177	11,127	3,156	2,541	644	17,468
	70	3,470	1,468	1,400	429	6,767
Hondo	147	8,600	2,330	2,795	623	14,348
	83	3,903	1,527	1,756	545	7,731
N.C.	258	18,971	3,384	4,750	779	27,884
	77	4,467	1,548	1,847	600	8,462
Total	960	62,425	14,262	17,131	3,591	97,409
	403	20,434	7,812	8,819	2,984	40,049
Percent		64.1	14.6	17.6	3.7	
		51.0	19.5	22.0	7.5	

for number of lethals ($\chi^2 = 9.2$ with 6 df, $P > .10$) whether or not the cage population was included in the calculations (excluding the cage $\chi^2 = 7.1$ with 5 df, $P > .20$). Similar results were observed when all drastics (lethals + semilethals) were included in the calculations, which seemed to justify pooling the data.

TABLE 3

Viability classes for chromosomes 2 and 3 in different populations

Population sampled	Number of quasinormal chromosomes		Number of semilethal chromosomes		Number of lethal chromosomes		Percentage of lethal chromosomes	
	2	3	2	3	2	3	2	3
Cage	79	63	9	10	16	31	15.4	29.8
Austin	62	66	11	10	20	17	21.5	18.3
Samoa	78	67	15	10	16	32	14.7	29.4
Tenn.	50	41	9	18	13	13	18.1	18.1
Lubbock	106	120	50	41	21	16	11.9	9.1
Hondo	112	95	21	28	14	24	9.5	16.3
N.C.	164	127	36	61	58	70	22.5	27.1
Total	651	579	151	178	158	203	16.5	21.1

Since the total data indicated a difference in number of lethals on chromosomes 2 and 3, a consideration of joint distributions of lethals was made for the chromosomes from different populations under two hypotheses; H_1 , that number of lethals on chromosome 3 equals the number of lethals on 2, and H_2 , that lethals on 3 exceed those on 2 at the 5% significance level. Viability classes for the seven populations are divided into four classes:

$$l_2l_3, l_2n_3, n_2l_3, n_2n_3$$

where l = number of lethals, and n = number of nonlethals.

The frequency of lethals on chromosome 2 in the i^{th} population, $p_i = (l_2l_3 + l_2n_3)/N_i$ (where N_i = the number of males tested in the i^{th} population) and frequency of nonlethals, $q_i = 1 - p_i$, or $(n_2l_3 + n_2n_3)/N_i$. Likewise, for chromosome 3, the frequency of drastics, $r_i = (l_2l_3 + n_2l_3)/N_i$, and nonlethals, $s_i = 1 - r_i$. Then the expected number in each class can be estimated by:

Class	Expected
l_2l_3	$= N_i p_i r_i$
l_2n_3	$= N_i p_i s_i$
n_2l_3	$= N_i q_i r_i$
n_2n_3	$= N_i q_i s_i$

Table 4 gives the distribution of classes.

Based on chance, we expect the frequencies of drastics to be the same for chromosomes 2 and 3; r_i takes the value of p_i and s_i takes the value of q_i . The pooled data indicated a significant departure from H_1 . While four populations show this departure, three do not, and, since the cage population was initially free of lethals on chromosome 2, but not 3, a difference would be expected.

By substituting $p_i + 1.96 \sigma_{p_i}$ for r_i , the difference between the frequency of lethals on chromosomes 2 and 3 is eliminated (in italics, Table 5), except for the

TABLE 4

Distribution of lethals and nonlethals* occurring together and separately on chromosomes 2 and 3 in D. melanogaster

Population sampled	l_2l_3	l_2n_3	n_2l_3	n_2n_3
Cage	6	10	25	63
Austin	2	18	15	58
Samoa	8	8	24	69
Tenn.	4	9	9	50
Lubbock	3	18	13	143
Hondo	3	11	21	112
N.C.	20	38	50	150
Total	46	112	157	645

* l_2 or l_3 = lethal-bearing second or third chromosomes, respectively, n_2 or n_3 = nonlethal second or third chromosomes, respectively.

TABLE 5

Contributions to chi-square under two hypotheses, H_1 ($r_i = p_i$) and H_2 ($r_i = p_i + 1.96\sigma_{p_i}$)
 H_2 is in italics

Population sampled	l_1l_2	l_1n_2	n_1l_2	n_1n_2	Total	df	P
Cage	4.9	0.9	9.6	1.8	17.2	2	<.001
	<i>1.6</i>	<i>0.5</i>	<i>1.5</i>	<i>0.4</i>	<i>4.0</i>	<i>2</i>	<i>>.10</i>
Austin	1.2	0.3	0	0	1.5	2	>.30
Samoa	13.1	2.4	7.7	1.3	24.5	2	<.001
	<i>6.2</i>	<i>1.7</i>	<i>0.9</i>	<i>0.2</i>	<i>9.0</i>	<i>2</i>	<i><.05</i>
Lubbock	0.1	0	1.7	0.2	2.0	2	>.30
Hondo	2.2	0.2	5.6	0.6	8.6	2	<.02
	<i>0.5</i>	<i>0</i>	<i>0.2</i>	<i>0</i>	<i>0.7</i>	<i>2</i>	<i>>.70</i>
N.C.	3.8	1.1	0.6	0.2	5.7	2	$\approx .05$
Pooled	15.1	3.1	4.6	0.9	23.7	2	<.001
	<i>8.7</i>	<i>2.1</i>	<i>0.2</i>	<i>0.1</i>	<i>11.1</i>	<i>2</i>	<i><.01</i>

Samoa population and for pooled data. Therefore, the pooled data indicate a difference in number of lethals between these autosomes in a number of dispersed samples that is not obvious in samples from one location.

DISCUSSION

Experiments with *D. melanogaster* have indicated a greater number of drastics on chromosome 3 when both second and third chromosomes were tested from single populations (BAND and IVES 1963; WALLACE *et al.* 1966; PAIK 1966; ALLEN 1966). However, when confidence limits were determined, the lower limit for drastics on chromosome 3 overlapped the upper limit for chromosome 2. This overlap rules out a real difference between these autosomes, or indicates that the samples are too small to demonstrate a difference if one exists. Most cases indicated that a very large sample would be required. The present report of samples of a reasonable size shows a difference for lethals and semilethals between the chromosomes when all populations were considered together. Taken separately, some of the populations obviously do not show a difference. The cage population was expected to have a higher number of lethals on chromosome 3 because chromosome 2 lethals were screened out of the Oregon-R flies used to start the population. That population is probably not representative of what might be seen in nature. The natural populations, on the other hand, display a high degree of heterogeneity, both between and within, for relative viability values. Only the within-population variation for proportion of drastic chromosomes does not vary from collection to collection or from year to year. This did not apply to lethals or drastics in general from different populations. The total picture of viability certainly indicates a difference from one population to the next (SPASSKY *et al.*

1965). This gives identity to local populations that is hidden when viewed together. The question remains then, would a sufficiently large sample of the local populations show a consistent difference between the number of lethals on heterologous chromosomes?

An explanation for a difference, if one actually exists, between the two major autosomes of *D. melanogaster* is not clear. Different mutation rates can be ruled out (WALLACE 1968). Two possibilities may be: (1) Linkage complexes differ for the two chromosomes. That is, lethals or semilethals may have been more closely linked to selectively superior genes on chromosome 3 to give a heterotic effect. Or, drastics alone may have been more heterotic in their own right on chromosome 3; (2) small inversions were in higher frequency on chromosome 3 and may have included lethals along with selectively superior genes. The latter explanation is less likely than the first, considering the paucity of reported inversions in this species. The first explanation seems more feasible in the light of the findings of SPASSKY *et al.* (1965). They reported positive epistatic interaction in diverse populations of *D. pseudoobscura* after making a comparison between homozygotes and heterozygotes of chromosomes 2 and 3 that had been tested simultaneously. Perhaps interaction was greater on chromosome 3 for the present populations of *D. melanogaster*.

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SUMMARY

Collections of flies from one laboratory and six natural populations were tested for homozygote viability of chromosomes 2 and 3 simultaneously. Balanced markers *Cy/Pm* and *Ubx/Sb* were used for chromosomes 2 and 3, respectively, to obtain homozygotes. The totals for all populations demonstrated that chromosome 3 had a higher frequency of lethals and semilethals than chromosome 2. The upper 95% confidence limit for the total frequency of lethals on chromosome 2 did not overlap the lower limit for chromosome 3 when samples were pooled. This difference held true for only one population when samples were examined individually. It cannot be assumed that populations of *D. melanogaster* carry lethals on particular chromosomes in proportion to the length of the chromosome, as might be expected. However, many, if not most, natural populations probably would not show this difference between chromosomes. The reason it occurs in some is not clear. Some mechanisms of natural selection that control the frequencies of lethals under given local conditions may be different for these two heterologous autosomes of approximately equal size.

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