A COMPARATIVE STUDY OF LETHAL-BEARING HETEROZYGOTES IN A LOCAL POPULATION OF *DROSOPHILA MELANOGASTER*

by

ROBERT VAUGHN ROBINSON, B.S.

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CHAPTER I

INTRODUCTION

Beginning with the pioneer work of Dobzhansky and Wright (1941), the extent of lethal frequency, degree of allelism, concealed genetic load, and viability of lethal-bearing chromosomes in Drosophila populations have been under investigation.

Wide variations have been observed by different workers. Some of the heterogeneity of results may be due to the various techniques which have been used. For instance, experiments involving x-rayed laboratory populations of Drosophila melanogaster (Dyer, 1969; Salceda, 1967) gave significantly different results compared with those involving natural populations (Epling, Tinderholt, and Mattoni, 1961; Wallace, 1966; Dobzhansky and Spassky, 1953). However, results obtained from work done on strictly natural populations have shown a wide range of often conflicting data. The major unsolved question remains: "What is the origin and mechanism operating to maintain the variability?" The ambiguous results from tests made on natural populations may be due to a number of reasons such as the size of the population, the length of time used in collecting flies, the environmental conditions under which the test was performed, and the type of natural environment the flies came from. Crossing

natural populations with laboratory marker stocks may not be a true representation of what is found in the natural habitat.

Evolutionary forces in local populations have been shown to affect the frequency of lethal-bearing chromosomes compared with the total population. In "small" populations the elimination or accumulation of lethals may be determined by genetic drift. This may cause a mutation, which has deleterious effects on viability, to reach a high frequency (Wright and Kerr, 1954). Such an effect would likely take place in locations such as Lubbock, due to decreased population size in winter, as well as the semi-arid conditions in this area. Due to these factors, a recessive lethal that is preserved during the winter is expected to become widely distributed during the summer months. A larger number of flies carrying lethals would mean more matings between individuals heterozygous for the same recessive lethal factor. The newly arising lethal zygotes will cause a reduction in the frequency of the mutated chromosome; thus the equilibrium is once more shifted towards the chromosomes which are free of lethal factors. Dubinin (1946) postulated such a seasonal cycle for the frequency of lethal factors for Russian populations of Drosophila melanogaster. To what extent the incidence of lethal factors in local populations is influenced by mutagenic climatic conditions,

differences in the spontaneous mutability, and fluctuations in population size has not been determined. These mechanisms probably act simultaneously with selection pressure to keep the frequencies of lethal mutations fluctuating around a certain equilibrium. This equilibrium is attained if in each generation the number of chromosomes carrying newly arised lethal factors is equal to the number of chromosomes with lethal effect which are eliminated from the population through the death of the zygotes containing them.

If it is certain that the frequencies of existing lethal factors in a population are constantly decreased by elimination, then how can newly arisen lethal factors gain a foothold in a population? Under conditions of random mating, the survival of an individual lethal factor through several generations has a low probability. Several Drosophila geneticists have estimated the relative proportions of identical or allelic lethal factors from all the possible combinations of chromosomes originating from a wild population. If two flies from a local population are allelic for the same lethal gene, it is highly possible that this is due to their having a common origin. It seems logical that lethals from the same breeding population would be allelic more often than those miles apart. This was first shown to be true for non-lethal genes by Wright, Dobzhansky, and Hovanitz (1942), and since then, confirmed by many. Wallace

(1966) demonstrated that flies disperse in a manner such that the numbers of them found at various distances decrease exponentially with the square root of distance. Therefore, lethal genes may be distributed in like manner.

From the point of view of population genetics, it is important to know whether those lethal factors which do not produce any demonstrable novel phenotype in single dose are recessive with regards to viability. The tendency for such factors to lower viability in the heterozygous condition has been noted by many workers examining large natural populations and lethals induced by irradiation. Some investigations have, however, shown that recessive lethal factors have favorable influence on the viability of heterozygotes. The gradual increase of viability of lethal heterozygotes in D. melanogaster was noted by Wallace (1962). The type of population being studied seems to be an important factor. This factor has been demonstrated in experiments with two non-allelic lethals. Conflicting reports have been introduced by Cordeiro (1952) and Oshima (1963), the latter having shown that double lethal heterozygotes were higher than either the single lethal heterozygote or lethal free heterozygotes.

For many of the reasons given, a systematic analysis similar to that of Dobzhansky and Spassky (1963) was performed. Only naturally occurring chromosomes were used,

therefore eliminating any introduction of laboratory chromosomes which would not give a good representation of the local population. Since the flies were collected within a one month period, they are assumed to be from the same breeding population. From those collected and demonstrating a balanced lethal system, a diallelic analysis of the second chromosome was performed. The main purpose in this experiment was to see if the viability of the double lethal heterozygotes (l^1/l^2) differed from that of the single lethal heterozygotes ($l^{1 \text{ or } 2}/+$). In order to support the findings, other data, such as the allelic frequency was obtained.

CHAPTER II

MATERIALS AND METHODS

Samples of Drosophila melanogaster from four different locations (A, B, C, and D--all near local residences) in Lubbock, Texas, were collected from one to two miles apart. These were transferred to the laboratory and stored in one-half pint milk bottles with a medium of molasses-wheat-yeast. The cultures were kept at 25° C ± 1° C.

Those cultured and producing only curly-winged offspring were assumed to have a balanced lethal system. This is because curly is always lethal in the homozygous condition (Cy/Cy dies). The absence of any straight-winged flies means that there is an allelic lethal condition on that pair of chromosomes also. From location A there were four balanced lethal systems (A9, A15, A8, and A18); location B had six (B15, B14, B23, B2, B19, and B4); location C only had two (C10 and C24); and location D had five (D15, D24, D27, D7, and D3). These balanced systems were chosen to be crossed with each other in all possible combinations giving k(k-1)/2 crosses. The crosses involved the following:

$$P_1 \quad Cy/l^{1 \text{ or } 2} \quad X \quad Cy/l^{1 \text{ or } 2}$$

$$F_1 \quad 1\ Cy/Cy\ (\text{dies}) \ : \ 2\ Cy/l^{1 \text{ or } 2} \ : \ 1\ l^{1 \text{ or } 2}/l^{1 \text{ or } 2}$$

The expected ratio is 2/3 Curly to 1/3 wild-type (straight) unless the lethals (1) are allelic in the homozygous state, thereby giving another balanced lethal system. The Curly chromosome contains inversions that suppress crossovers, and in the homozygous state the Cy chromosome is lethal. The presence of a sub-vital or semi-lethal factor in the test chromosome will result in a reduction of surviving wild-type flies.

The basic design of the experiment consisted of crosses between flies from inter- and intralocalities. This provided a method by which the degree of allelism as well as the viability of the F_1 generation could be obtained. Virgins were collected from the original balanced systems and stored for 5 days in 3.5 by 10 cm. vials. The crosses were made, using 3 females and 2 males, in one-half pint milk bottles. After 18-20 days flies were scored for each cross. The flies used in this study include both lethals and semilethals with the following standard: 0-2% surviving wild-type was classified as a lethal and from 2-16% surviving was semilethal. In calculations on viability, alleles were excluded from the calculations since the test of viability is restricted to the two types of surviving heterozygotes.

CHAPTER III

RESULTS

DEGREE OF ALLELISM FOR LETHALS: The results of the lethal tests for allelism are summarized in Table 1. It lists the number of tests and the appropriate combination of each cross. The number of lethal and semilethals are included and final percentages for both within and between localities are shown. The difference in the percentages is not statistically significant, but the tendency toward an increase in allelism between groups can be noted. The need for more data is very crucial at this point in order for a significant difference to be confirmed. Combining within and between crosses, seven were found allelic from 136 crosses, giving an allelic frequency of 5.1%. This percentage of allelism is very revealing because there seems to be little chance of finding this many allelic lethals in a natural population with number of crosses. The relationship of distance and allelism cannot be confirmed, but it appears that they are correlated.

VIABILITY OF HETEROZYGOTES: Since all surviving offspring are heterozygotes, either carrying one lethal with a marker chromosome or two non-allelic lethals, the distinction between them will be made by calling them single or double lethals. The ideal frequency of 66.7 to 33.3

TABLE 1

OBSERVED ALLELIC FREQUENCY FOR RECESSIVE LETHALS IN CROSSES WITHIN AND BETWEEN LOCATIONS

Interlocalities

Type of Cross	No. tests	No. allelic
within A	6	1
within B	15	1
within C	1	0
within D	10	0
	32	2

Intralocalities

Type of Cross	No. tests	No. allellic
A X B	24	2
A X C	8	2
A X D	20	0
B X C	12	1
B X D	30	0
C X D	10	0
	104	5

Allelic frequency within = 6.2%
Allelic frequency between = 4.8%

percent would be found if no effect on viability is found. In Table 2 the total results of viability counts are summarized for within and between groups. It should be noted that if the crosses demonstrated any degree of lethality, they were excluded from the calculations of viability. Using the G-test for goodness of fit (Sokal and Rohlf, 1969) revealed values that were not significant. The percentages of 65.8:34.2 for within and 65.5:34.5 for between, do show a tendency toward the increased viability of the double lethal heterozygote (l^1/l^2).

Table 3 shows the G-statistic values obtained from a G-test for accumulated data indicating the result of crossing each chromosome with all the other possible combinations both within and between. Two chromosomes gave a significant deviation from the expected 2 to 1 ratio. These were B19 and B4--both involved in the intralocality crosses. These showed an increased viability for the double-lethal heterozygote. Because the G-statistic values are completely additive when employing the G-test, a test of homogeneity was performed. The results of these tests are shown in Table 3. Although these tests did not reveal heterogeneity of the values at .05 probability, it was significant at the .10 level. Only 3 of the interlocality tests showed an increased viability in the single lethal heterozygote, as well as three in intralocality tests.

TABLE 2

TOTAL VIABILITY COUNTS FOR SINGLE AND DOUBLE LETHAL BEARING HETEROZYGOTES AND THEIR CALCULATED RATIOS

Interlocality crosses

Type of cross	total count
within A	364-177
within B	812-442
within C	60-31
within D	598-302
	1834-592

Intralocality crosses

Type of cross	total count
A X B	999-515
B X D	1622-891
B X C	490-270
A X D	1061-538
A X C	241-125
C X D	602-294
	5015-2633

Ratio for within-- 65.8%:34.2%
Ratio for between- 65.5%:34.5%

TABLE 3

G-STATISTIC VALUES OBTAINED FROM A G-TEST FOR GOODNESS OF FIT AND HETEROGENEITY FOR ACCUMULATED DATA INDICATING THE RESULT OF CROSSING EACH CHROMOSOME WITH ALL THE OTHER POSSIBLE COMBINATIONS BOTH WITHIN AND BETWEEN

Test chromosome	G-statistic(within)	G-statistic(between)
A9	.36	.06
A15	1.71	.01
A-8	.21	3.41
A18	.09	.09
B15	1.24	.12
B14	.09	.00
B23	.01	.01
B2	1.04	.00
B19	.93	8.75***
B4	1.93	6.15**
C10	.00	2.33
C24	.00	.78
D15	.01	1.54
D24	.01	.69
D27	.10	.57
D7	.36	.01
D3	.21	.63
	8.30	25.15
(Pooled)	- .84	- 4.21
	7.46 ns	20.94 ns

$X^2.05_{[1]} = 3.841$

$X^2.05_{[15]} = 24.996$

CHAPTER IV

DISCUSSION

Experiments with Drosophila have given a great degree of evidence that the viability of the heterozygote is reduced when a recessive lethal is carried on their autosomes (Sankaranarayanan, 1964; Salceda, 1967; Crow and Temin, 1964). Other experiments revealed that a x-ray radiation dose of 2000r caused no significant reduction in the viability of the chromosomes affected (Falk and Ben-Zeev, 1966). Still, deleterious genes that are lethal in homozygotes may even be favorable in the heterozygous condition in some populations (Dobzhansky and Spassky, 1968). Watanabe and Oshima (1970), working with persistent lethal genes on the second chromosome in Japanese natural populations of D. melanogaster, observed that when the genetic background consisted of unrelated chromosomes, the viability of heterozygotes for lethal chromosomes was less than that of heterozygotes for quasinormal chromosomes. On the other hand, the viability of lethal heterozygotes was equal to, or higher than, that of normal heterozygotes if their genetic backgrounds consisted of coadapted chromosomes from the same natural population. Their experiments also revealed that persistent lethal-bearing chromosomes showed no deleterious effects, but rather a heterotic effect on preadult viability

in heterozygous condition. Wallace (1962) observed that the average viability for lethal heterozygotes improved during the course of his experiment and rose above the average viability of normal heterozygotes after many generations. However, their results may be due to the local populations being studied, and no generalizations can be made.

How do other populations relate to the population studied from Lubbock? It appears from the data collected that the lethals present in the local population increase the viability of their carriers. However, a closer study of the Lubbock population is necessary in order to support the conclusion that lethal genes are giving a heterotic effect.

Lubbock populations are relatively small due to the semi-arid environment. Allen (1969) has found a percentage of 11.9 for second chromosome lethals for Lubbock. This was considerably lower than the percentages found in Austin (21.5) and North Carolina (22.5). The allelic frequency is expected to be high when the lethal frequency is low, as well as, the allelic frequency is expected to be low if the lethal frequency is high. This is due to the rate of elimination becoming too high if these failed to interact each other. Since Lubbock has a low frequency of lethal genes on the second chromosome, the values for the allelic rate are higher than those of most populations. A comparable

study by Dobzhansky, Hunter, Spassky, and Wallace (1963), in which they studied the genetics of a isolated population D. pseudoobscura near Bogata, Colombia, showed a frequency of allelism at 3.60%. The higher percentage in Lubbock is probably due to a smaller population.

How does this pertain to the suggested hypothesis that in the Lubbock population there is a increased viability for the heterozygote carrying lethals? It appears that due to the high number of alleles and low frequency of lethal genes in the population there is little probability that these lethals are new lethals. While most recessive mutations are deleterious in both homozygous and heterozygous states and are quickly eliminated from the population, it appears that some of the mutations may be helpful and are therefore retained in the heterozygote condition or due to "good" linked genes that carry them. In a finite population, a mutant gene is either fixed in the population or lost from it within a finite length of time. Kimura and Ohta (1969) have devised a theory by which the average number of generations until fixation can be calculated. For non-deleterious genes to be incorporated into a population in large numbers is quite feasible for a population such as that found in Lubbock. Due to the reduced population size in the winter, any lethal retained during the winter will become widely spread in the summer. In a large population,

the survival of a recessive lethal is very slim under conditions of random mating.

Lerner (1954) postulated that a lethal factor could produce a degree of heterosis and that there may be an optimum level of heterozygosity. Oshima (1963) was first to show evidence that double lethal heterozygotes were superior in viability to both the single lethal and non-lethal heterozygotes. It appears that both lethal genes are heterotic and the presence of them together gives higher viability than when either is present alone. Huang (1967) working with laboratory populations of D. melanogaster demonstrated that double non-allelic lethal heterozygotes yielded normal viability compared with that of single lethal heterozygotes. He further showed, however, that when compared to non-lethal bearing chromosomes, all heterozygotes (double and single lethal) showed increased viability. Although significant results were not obtained in the tests on the Lubbock population, it does seem that lethals do increase viability in this population. Homogeneity tests indicate that a possible optimum level of heterosis has been reached. It is evident that the viability of the heterozygote is dependent on its genetic background. It may be concluded, therefore, that the viability of newly-arisen lethal heterozygotes may be slightly less than those of normal heterozygotes, but those that have been incorporated into

the genetic load often are equal or greater in viability than those of quasinormal heterozygotes.

CHAPTER V

SUMMARY

Natural populations of D. melanogaster from four locations in Lubbock, Texas, were collected, and the second chromosome was tested for lethality, allelism, and viability. Diallelic crosses were performed for within and between location groups. Results revealed no significant difference in the degree of allelism for within and between groups although there was a trend toward increased allelism within groups. Viability counts for flies not demonstrating balanced lethals showed higher viability for heterozygotes bearing two lethals rather than those bearing one lethal. The increase was not significant, but there appears to be some heterosis due to the lethals in this population. Local conditions appear to be responsible for this populations' high percentage of allelism and increased viability of its lethal carriers in the heterozygous condition.

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APPENDIX

TABLE 4

VIABILITY COUNTS FOR INTERLOCALITY CROSSES

	A-9	A-15	A-8	A-18	Total	
A-9	-	66-28	59-36	78-45	203-109	Tot.
A-15		-	86-34	45-3*	152-62	
A-8			-	75-34	153-79	364-177
A-18				-	-	

	B-15	B-14	B-23	B-2	B-19	B-4	Tot.
B-15	-	79-39	49-28	40-18	60-40	53-33	281-158
B-14		-	69-33	65-25	48-30	72-39	312-160
B-23			-	81-48	30-0*	66-38	263-133
B-2				-	44-24	54-29	278-155
B-19					-	61-38	200-112
B-4						-	290-166
							812-442

	C-24	C-10	Tot
C-34	-	60-31	60-31
C-10	-	-	

	D-15	D-24	D-27	D-7	D-3	Tot	
D-15	-	67-35	73-42	55-25	76-36	271-138	
D-24		-	60-28	62-29	44-24	233-116	Tot.
D-27			-	66-29	62-36	261-135	598-302
D-7				-	33-18	216-101	
D-3					-	215-114	

*Lethals and semilethals

TABLE 5

VIABILITY COUNTS FOR INTRALOCALITY CROSSES

	B-15	B-14	B-23	B-2	B-19	B-4	Totals
A-9	58-31	53-31	39-21	76-0*	46-27	63-35	259-145
A-15	60-32	59-24	50-25	20-6	47-13	50-28	219-109
A-8	61-27	78-35	52-30	64-25	68-19	38-23	293-140
A-18	72-40	51-28	38-15	44-8*	22-12	44-26	228-121
Total	251-130	241-118	180-91	64-25	68-39	195-112	999-515

	B-15	B-14	B-23	B-2	B-19	B-4	
D-15	49-30	45-29	76-32	36-20	41-27	43-26	290-164
D-24	53-31	46-20	70-33	63-34	58-34	38-28	328-180
D-27	48-22	73-40	66-32	30-14	67-33	52-30	336-171
D-7	70-38	53-30	34-15	39-20	67-37	56-36	319-176
D-3	68-34	71-34	56-31	49-30	38-31	67-40	349-200
Total	288-155	288-153	302-143	217-118	271-162	256-160	1622-891

	B-15	B-14	B-23	B-2	B-19	B-4	
C-10	77-31	50-26	38-23	46-26	43-42	38-24	292-172
C-24	41-0*	49-20	47-14	33-15	75-43	41-20	198-98
Total	77-31	99-46	38-23	79-41	118-85	79-44	490-270

	A-9	A-15	A-8	A-18	Totals
D-15	60-36	40-23	60-31	20-8	180-98
D-24	39-17	69-23	66-35	69-31	174-83
D-27	77-37	66-38	60-90	65-30	268-145
D-7	65-31	50-24	41-24	66-30	222-109
D-3	28-12	62-28	58-30	69-33	217-103
Total	269-133	218-113	231-160	289-132	1061-538

TABLE 5--Continued

	A-9	A-15	A-8	A-18	Totals
C-10	46-28	63-34	47-12	36-19	145-81
C-24	40-18	56-26	40-4*	33-3*	96-44
Total	86-96	119-60	-	36-19	241-125

	C-10	C-24	Totals
D-15	72-42	70-30	142-72
D-24	48-25	65-32	113-57
D-27	67-30	74-35	141-75
D-7	53-23	48-16	101-39
D-3	40-21	65-30	105-51
Total	280-141	274-127	602-294

*Lethals and semilethals