PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES

Volume 38 June 15, 1952 Number 6

EXPERIMENTS ON THE EFFECTS IN HETEROZYGOUS CONDITION OF SECOND CHROMOSOMES FROM NATURAL POPULATIONS OF DROSOPHILA WILLISTONI

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Communicated by Th. Dobzhansky, March 31, 1952

The Problem.—Natural populations of all species of Drosophila so far studied in this respect carry a great amount of concealed genetic variability, consisting of recessive mutant genes and chromosomal aberrations of the inversion type. Many of the mutant genes are lethal or semilethal in homozygous condition.[1] The effects of these lethals on heterozygous flies are insufficiently known, and conflicting views about these effects have been expressed by several authors. Berg[2] showed that the lethals found in natural populations of *D. melanogaster* are deleterious in heterozygous condition, and Stern and Novitski[3] found the same to be true for lethals which arise in the laboratory. Dubinin[4] considered, however, the natural lethals in the same species to be completely recessive, but he quotes the results of Masing who regarded many of the natural lethals to be heterotic. In *D. pseudoobscura* Wright, Dobzhansky and Hovanitz[5] found that autosomal lethals are less frequent in natural populations than they would be if they were completely recessive and if the populations were wholly panmictic. The discrepancy may be due either to deleterious effects of most lethals in heterozygotes, or to local inbreeding in natural habitats, or to a combination of both causes. Muller[6] emphasized the importance of the viability effects of heterozygosis for deleterious recessive genes for an understanding of the dynamics of populations of sexual organisms, including man. He also referred to unpublished experiments on *D. melanogaster*, which indicated that mutants which are lethal when homozygous are deleterious in heterozygotes.

The experiments reported in the present article have been carried out to investigate the effects on the viability of the flies of heterozygosis for different chromosomes found in natural populations of *Drosophila willistoni*. Chromosomes which are lethal to homozygotes as well as chromosomes which permit the homozygotes to survive (the "Normals") have been studied. It appears that combinations of these chromosomes give rise to a variety of genotypes which differ in their viabilities under the conditions of the experiments.

Material and Methods.—Samples of wild populations of *D. willistoni* were collected at Quarai, Emboaba and Caxias, in the state of Rio Grande do Sul, and at Catuni, in the state of Bahia, in Brazil. The effects in homozygous condition of second chromosomes found in these samples were studied as described in a previous publication.[7] A total of 52 second chromosomes which were lethal in homozygous condition, and 60 second chromosomes which produced homozygotes close to normal viability were selected. All the lethals were known not to be allelic.

The lethal and the "Normal" chromosomes are maintained in separate strains in balanced condition. A chromosome which contains the dominant mutant Star, the recessives brown and abbreviated, and a pericentric inversion which suppresses crossing over in heterozygotes, is used as a balancer.[7]

Four series of experiments were arranged, as shown in table 1. In the first series, flies which carried Star (and the other genes) in one second chromosome, and a lethal in the homologous chromosome, were crossed to flies homozygous for a known Normal second chromosome. The offspring of this cross should consist of Star flies and wild-type flies (heterozygous for the lethal) in approximately equal numbers. In the second series of experiments, flies which carried one Normal second, and one Star second, chromosome were crossed to flies homozygous for a different Normal second chromosome. The offspring of this cross should, theoretically, consist also of Star and of wild-type flies in equal numbers. In the third series, flies heterozygous for the Star chromosome and for different (non-allelic) lethal chromosomes were intercrossed. Since the Star chromosome is lethal when homozygous, the offspring of this cross should consist of about two-thirds Star and one-third of wild-type flies. The fourth series of experiments differed from the third in that the chromosomes opposite to Star were known not to be lethal when homozygous. The offspring should, theoretically, consist of Star and wild-type flies in a ratio approaching 2:1.

A summary of the results is given in table 1. A total of 359,667 flies were counted. They came from 735 cultures, representing 198 different combinations of second chromosomes. The cultures were kept at 25°C. on banana agar medium; the flies that hatched were counted on alternate days until the ninth day after the beginning of the eclosion of adults in a given culture.

Incomplete Recessivity of the Lethals.—The theoretical ratios, of 1 Star: 1 wild-type, or 2 Star: 1 wild-type, expected in the two categories of the crosses can be realized only if the Star and the wild-type flies were equal in relative viability under the conditions which obtained in our experiments. In reality, the carriers of the Star chromosome were known to be less viable than their wild-type sibs.[7]

The ratios of Star: wild-type flies are, however, not uniform in the different crosses (table 1). It can be seen that the wild-type class is less frequent in the offspring of the Star/lethal to Normal crosses (55.20 per cent

TABLE 1

A SUMMARY OF THE EXPERIMENTAL DATA

TYPE OF CROSS	LOCALITY	COMBINATIONS OF CHROMOSOMES	NUMBER OF CULTURES	TOTAL FLIES COUNTED	PER CENT WILD IN TOTAL	MEAN FREQUENCY OF WILD-TYPE
Star/lethal × Normal/Normal	Quarai	32	102	54,724	60.42	60.94
	Catuni	37	135	100,292	55.33	54.91
	Emboaba	20	172	76,500	51.29	55.67
	Total	89	409	231,516	55.20	
Star/Normal × Normal/Normal	Quarai	16	42	19,979	60.33	64.70
	Catuni	2	29	14,876	56.16	56.80
	Emboaba	2	38	14,716	55.94	56.90
	Total	20	109	49,571	57.78	
Star/lethal × Star/lethal	Quarai	13	21	8,251	43.87	43.85
	Catuni	24	49	20,712	40.26	39.25
	Emboaba	3	7	5,068	44.87	43.53
	Caxias	18	106	33,471	41.10	41.31
	Total	58	183	67,502	41.47	
Star/Normal × Star/Normal	Quarai	5	6	1,610	44.35	45.46
	Catuni	19	19	4,760	45.29	46.73
	Emboaba	6	6	3,642	49.45	48.36
	Caxias	1	3	1,066	43.15	42.60
	Total	31	34	11,078	46.32	

in the total) than in the offspring of the Star/Normal to Normal crosses (57.78 per cent in the total). The obvious difference between these two categories of crosses is that in the first of them the wild-type flies are heterozygous for a chromosome which is known to be lethal to homozygotes. This suggests that, on the average, the lethal chromosomes used in our experiments are deleterious also in heterozygous condition. This suggestion is confirmed by comparison of the outcomes of the Star/lethal × Star/lethal crosses with those of the Star/Normal × Star/Normal crosses (table 1). The frequency of the wild-type class in the progeny of the former is lower (41.47 per cent) than in the latter (46.32 per cent). The wild-type flies in the progeny of Star/lethal × Star/lethal crosses are heterozygous for two (non-allelic) lethals, while their Star sibs are heterozygous for only one lethal (not counting Star itself).

The second-chromosome lethals used in our experiments seem, on the average, to reduce the viability of the heterozygotes. This inference can be tested further by examining the frequencies of the wild-type class separately in various series of experiments summarized in table 1. The lethal and the Normal chromosomes were derived from four different localities—Quarai, Catuni, Emboaba and Caxias. Seven possible comparisons can thus be made of the frequencies of the wild-type class in crosses which were free of lethals, and which contained lethals. These comparisons are as follows:

	STAR × NORMAL		STAR × STAR	
	LETHAL	LETHAL-FREE	LETHAL	LETHAL-FREE
Quarai	60.42	60.33	43.87	44.35
Catuni	55.33	56.16	40.26	45.29
Emboaba	51.29	55.94	44.87	49.45
Caxias	...	...	41.10	43.15

In six out of the seven comparisons, the lethal-containing wild-type class is less numerous than the lethal-free class in the corresponding crosses.

In the comparisons just considered, the percentages of wild-type flies were computed from the total yield of the flies obtained in all the crosses of a given type. It will, however, be shown below that the outcomes of the crosses involving different combinations of the chromosomes are significantly heterogeneous, as are different cultures involving the same combination of chromosomes. Mean frequencies of wild-type flies were therefore calculated, first averaging the frequencies for the different cultures of each combination of chromosomes, and then averaging the average frequencies for the different combinations of chromosomes. Since the numbers of cultures, and of flies within the cultures, obtained for different combinations of chromosomes were unequal, these mean frequencies differ somewhat from the frequencies weighted in accordance with the numbers of the flies counted. The mean frequencies are shown in the rightmost column in table 1. Mr. Allan Birnbaum and Prof. Howard Levene, of the Department of Mathematical Statistics, Columbia University, kindly devised a method for applying the analysis of variance to the data here discussed. Table 2 shows the differences between the mean frequencies of the wild-type flies in the lethal-bearing and in the lethal-free progenies of the various crosses. The table also shows the standard deviation of these differences, from which t values are derived. All the differences are negative, i.e., the lethal-bearing wild-type classes appear to survive less often than the corresponding lethal-free classes. The probability that all the differences would have the same sign by chance is 1/64. Considered separately, only two of the seven t values are significant; however, these are also the only two based on enough data to have adequate sensitivity.[9] Combined t values for all the Star × Normal and all the Star × Star crosses are significant, and the combined t value for all seven crosses, −3.96, is highly significant.

Estimation of the Magnitude of the Average Deleterious Effect Produced by the Lethals in Heterozygous Condition.—The difference between lethal-free and lethal-bearing classes of flies is not an adequate measure of the relative viability of these classes, since this difference depends on the frequencies of the Star and wild-type flies, and therefore on the viability of the carriers of the Star chromosome To measure the deleterious effect of heterozygosis for the lethals, a "coefficient of inviability"[8] is used. This coefficient is not identical with the selection coefficient (S); it measures the

TABLE 2

DIFFERENCE BETWEEN THE MEAN FREQUENCIES (IN PER CENT) OF THE WILD-TYPE CLASS IN THE LETHAL-CONTAINING AND THE LETHAL-FREE CROSSES

CROSS AND LOCALITY	DIFFERENCE	STANDARD DEVIATION	
Star × Normal			
Quarai	−3.76	1.52	−2.47[a]
Catuni	−1.89	3.61	−0.52
Emboaba	−1.23	3.68	−0.33
Combined Star × Normal	...	...	−1.92[a]
Star × Star			
Quarai	−1.61	2.60	−0.62
Catuni	−7.48	1.52	−4.90[b]
Emboaba	−4.83	3.51	−1.38
Caxias	−1.29	5.10	−0.25
Combined Star × Star	...	...	−3.57[b]
Combined all crosses	...	...	−3.96[b]

[a] Significant.
[b] Highly significant.

proportion of the lethal-bearing individuals which are eliminated due to differential mortality between the egg stage and the hatching of the adult insect, in relation to the lethal-free individuals which survive.

The coefficients of inviability were calculated only for the crosses Star/lethal × Normal/Normal and Star/Normal × Normal/Normal. The other crosses, which contain two lethals, raise the problem of the possible interactions of the deleterious effects of heterozygous lethals. The coefficient proved to be 14.8 per cent for Quarai, 6.1 per cent for Catuni and 0.8 per cent for Emboaba, giving a mean estimate of 7.2 per cent.

Heterogeneity of Effects of Different Chromosomes.—The data presented above show that, on the average, individuals heterozygous for lethals have a viability lower than individuals which are free of lethals. It is, however, probable that different lethals, as well as different chromosomes free of lethals, affect the viability to different extents. An analysis of variance has been carried out under the direction of Mr. Allan Birnbaum Its results are summarized in table 3.

As indicated above, different lethal-bearing and lethal-free (Normal) chromosomes have been utilized in the experiments. A total of 89 different combinations of chromosomes have been made in the crosses of the type Star/lethal × Normal/Normal (table 1). Some of these combinations have, however, been replicated in several culture bottles; a total of

TABLE 3

ANALYSIS OF VARIANCE OF FREQUENCIES OF WILD-TYPE FLIES OBSERVED IN DIFFERENT CROSSES

TYPE OF CROSS	SOURCE	SUMS OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARES	F
Star/lethal × Normal/Normal	Between chromosomes	13,132	86	152.7	
	Within chromosomes	11,246	320	35.1	4.345
	Total	24,378	406		
Star/Normal × Normal/Normal	Between chromosomes	1214	17	71.4	
	Within chromosomes	3010	89	33.8	2.112
	Total	4225	106		
Star/lethal × Star/lethal	Between chromosomes	1846	54	34.2	
	Within chromosomes	3461	125	27.7	1.234
	Total	5307	179		
Star/Normal × Star/Normal	Between chromosomes	1516	27	56.1	
	Within chromosomes	362	3	120.7	0.465
	Total	1878	30		

409 cultures were raised for crosses of this type. The analysis of variance shows that the variance between different combinations of chromosomes is significantly greater than that between cultures of the same combination of chromosomes. The F value equals 4.345, which is significant at a level below 0.5 per cent (table 3). Similarly, for the crosses of the type Star/-Normal × Normal/Normal, the F value is 2.112, which is significant at about the 2.5 per cent level (table 3). The crosses of the type Star/lethal × Star/lethal and Star/Normal × Star/Normal do not give significantly greater heterogeneity between combinations of chromosomes than be-

tween replicate cultures. This is probably due to the small number of replications made in this series of experiments.

This result is important. It shows that the so-called "Normal" flies in a natural population are in reality a diversified collection of genotypes having different reaction norms and different adaptive values. The chromosomes which carry recessive lethals, as well as chromosomes free of such lethals, contribute to the adaptive polymorphism of the normal species population. There is an indication in the data that different lethal-bearing chromosomes are more diversified with respect to their effects on heterozygotes than are the lethal-free chromosomes, but this point requires a more rigorous proof than can be adduced on the basis of the data so far available. There are also indications that while some lethal-bearing chromosomes are distinctly deleterious to heterozygotes, others are not deleterious, or even heterotic. It should also be noted that there exists a significant heterogeneity of the viability effects between the chromosomes derived from different localities (table 1).

Acknowledgments.—The writer wishes to express his appreciation to Mr. Allan Birnbaum, Prof. Howard Levene and Mr. Timothy Prout for advice in the statistical treatment of the data; to Prof. Th. Dobzhansky for his help in the preparation of the manuscript; to Miss F. Wolff and Mrs. Malvina Cordeiro for assistance in the execution of the experiments. A part of the work has been done during the tenure of a Fellowship provided by the Rockefeller Foundation.

Summary.—The effects in heterozygotes of lethals found in second chromosomes of natural populations of *Drosophila willistoni* have been studied. On the average, the viability of lethal heterozygotes is significantly lower than that of lethal-free individuals. The coefficient of inviability is of the order of 7 per cent. Both lethal-bearing and lethal-free chromosomes vary significantly with respect to their viability effects in combinations with other chromosomes.

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[7] Pavan, C., Cordeiro, A. R., Dobzhansky, N., Dobzhansky, Th., Malogolowkin, C., Spassky, B., and Wedel, M., *Genetics*, **36**, 13–30 (1951).

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[9] Footnote by Howard Levene. A preliminary Chi-square test showed significant heterogeneity between replicate cultures. Because of this the usual Chi-square tests and the formula pq/n for binomial variance are inapplicable and were never used. For all further work the percentage of wild flies per culture was treated as a normal variable with a variance to be found empirically. The analysis of variance in table 3 was an ordinary one-way analysis of variance with unequal numbers of replications, each

locality being treated separately and the sums of squares then combined. Since this analysis of variance showed significant differences between different combinations of chromosomes, for further work the average of the frequencies of wild-type flies for the different cultures of a single combination of chromosomes was considered as the basic variable. Then Star/Lethal × Normal/Normal at Emboaba, for example, might have a smaller frequency of wild type than Star/Normal × Normal/Normal at Emboaba simply because the chromosome combinations of the first type chosen by chance happened to give a smaller frequency of wild-type. We can say the difference is due to the presence of the lethal chromosome only if it is significantly larger than the variability between chromosome combinations. This can be tested by an ordinary two sample t test as in table 2. Because of small numbers in many of the fourteen groups an estimate of the error variance based on all the data was used. Then t is approximately normally distributed with unit variance, and the t values can be combined by adding them (taking account of signs) and dividing by the square root of the number of t's added. This combined value will again be approximately normal with unit variance. Returning to the example of Emboaba, Star/lethal × Normal/Normal is based on twenty chromosome combinations and has adequate accuracy. Unfortunately, Star/Normal × Normal/Normal is based on only two combinations and this is the limiting factor in the sensitivity of the comparison. Most of the labor in obtaining the twenty combinations is wasted; in fact, three combinations of each type would have given a more accurate comparison. A more delicate and complicated test, based on the analysis of variance, gave substantially the same results. Mr. Birnbaum and the author of this footnote hope to discuss the statistical aspects more fully elsewhere.

SELECTION AGAINST HETEROZYGOTES FOR AUTOSOMAL LETHALS IN NATURAL POPULATIONS OF DROSOPHILA WILLISTONI

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Communicated by Th. Dobzhansky, March 31, 1952

In an accompanying article, Cordeiro shows that the second chromosomes of *Drosophila willistoni* which are lethal when homozygous, produce a significant lowering of the viability of heterozygous flies as well. The method used in the experiments of Cordeiro is comparison of the ratios of flies heterozygous for lethals and of flies which are free of such lethals. Cordeiro estimates that, under the environmental conditions of his experiments, the average lowering of the viability produced by heterozygosis for a second chromosome lethal is approximately 7% (his coefficient of inviability). The present note reports an attempt to compare the average adaptive values of individuals heterozygous for second chromosome lethals and of individuals free of such lethals in natural populations of *D. willistoni*.

The genetic structure of natural populations of *D. willistoni* has been