MUTATION RATES FOR AUTOSOMAL LETHALS IN *DROSOPHILA MELANOGASTER**

BRUCE WALLACE

Cornell University, Ithaca, N.Y.

Received March 7, 1968

THE degree of recessivity of lethal chromosomes in *Drosophila* can be estimated by comparing their relative rates of origin and elimination from randomly mating populations. The parameters required for this purpose are the probability that two lethal chromosomes fail to complement one another, the frequency of individuals that carry two homologous lethal chromosomes, and the rate at which lethal chromosomes arise by mutation.

In a recent study (WALLACE 1966), the allelism of second and third chromosome lethals from a Colombian population of *D. melanogaster* was measured. At the time, no published data on lethal mutation rates for the third chromosome were available; in order to compare the estimated elimination rate of lethals with mutation rate, it was necessary to assume that mutation rates for the second and third chromosomes are about the same. The present paper presents data on the comparative spontaneous mutation rates of these two chromosomes.

At the time of the earlier study, the bulk of all information concerning mutation rates in *Drosophila* (especially on mutation rates typical of wild caught flies) was based on mutation rates in males. Since females captured in nature are nearly always inseminated, the procedures for studying mutation are simplified by initiating analyses by crossing wild caught males with virgin females from laboratory tester strains. Although the mutation rate in males may be similar to that in females (see CROW and TEMIN 1964), there have been reports that the mutation rate in females is substantially lower (AUERBACH 1941; GLASS and RITTERHOFF 1956). The present paper contains data on the comparative mutation rates for autosomal lethals in the two sexes.

MATERIALS AND METHODS

The mutation "rate" of the present study equals the proportion of lethal-bearing gametes produced by individuals (male or female) that were themselves lethal-free. This point is stated explicitly in order to avoid problems related to relative rates of mutation at various stages of gametogenesis; these problems, interesting as they may be, are not dealt with here.

Wild-type flies from a number of different geographical strains of *D. melanogaster* were used in this study. Some strains (those from Bermuda, Barcelona, and Riverside, California) have been in the laboratory for nearly fifteen years; others (from Madeira and Bogotá) were captured during 1965; the remainder (strains numbered 1 through 11) were captured at various localities in the eastern United States during the summer of 1966 by DR. and MRS. PETER CHABORA.

* The work reported here has been done under contract No. AT-(30-1)-2139, U.S. Atomic Energy Commission.

Males and virgin females (aged for several days after being collected) were mated individually with *CyL/Pm* or *Ubx/Sb* flies of the opposite sex. *CyL* is the tester chromosome for the second chromosome; it carries the dominant marker genes *Curly* (wing) and *Lobe* (eye) as well as crossover suppressors. *Ubx* is the tester chromosome for the third chromosome; it carries *Ultrabithorax* (a dominant haltere mutation) together with an array of inversions and transpositions that effectively suppresses crossing-over.

27 *CyL/+* (or *Ubx/+*) sons of each of several parents were mated individually once more to *CyL/Pm* (or *Ubx/Sb*) females. *CyL/+* F_2 brothers and sisters were mated to yield F_3 test cultures in the case of chromosome 2; *Ubx/+* F_2 brothers and sisters, in the case of chromosome 3.

Among the approximately 27 F_3 cultures representing a single original wild-type fly, one might or might not encounter lethals (no wild-type F_3 flies). Four possible outcomes can be listed: (1) None of the approximately 27 F_3 cultures are lethal; the original fly, then, was lethal-free in respect to the chromosome being tested. (2) One, two, or three of the 27 F_3 cultures are lethal; the original fly in this case was also lethal-free and the few lethals observed represent new mutations. (3) About one half of the 27 cultures are lethal; the original fly was heterozygous for a lethal already present in the stock culture. (4) All 27 cultures are lethal; the original fly carried two different lethal chromosomes both of which were present in the wild-type laboratory strain. Data obtained from cultures of the first and second sorts can be used to estimate mutation rates since the original fly in each case was lethal-free; data obtained from cultures of the third and fourth sorts are of no use in estimating mutation rates but are interesting in respect to the frequency of lethals preexisting in the wild-type strains.

Relatively few cultures in these tests failed to yield offspring so that the number of F_3 tests per original fly is very nearly a constant (the average number of tests per group of 27 matings is about 26.4). Because of this uniformity, two techniques for estimating mutation rates are available. One consists of the ratio of lethal tests to the total number of tests completed. The second consists of equating the proportion of lethal-free groups (number of groups corresponding to outcome 1 described above divided by the total number of groups falling within outcomes 1 and 2) to e^{-m} , the zero term of the Poisson distribution. The mean number of lethals per group (m) can be divided by the average number of tests per group ($\bar{n}$) in order to estimate the mutation rate (u) per chromosome tested.

RESULTS

The mutation rates observed for second and third chromosomes and for males and females are listed separately in Table 1. A total of eighty lethals were recovered among 11,655 chromosomes obtained from lethal-free parents; the overall frequency equals 0.0069. It is quite clear from the direct estimates listed in Table 1 that none of the four values differs from the overall average.

The indirect estimates of mutation rate shown in Table 1 agree well with the direct estimates but are systematically lower. This consistent bias suggests that some of the lethals recovered in these tests were members of clusters and were not independent in origin; lethals of gonial origin might possibly cause such clusters. Unfortunately, lethals were not retained in stock cultures as they were encountered and so the allelism of clustered lethals was not tested.

Table 2 lists data that have been combined either by sex or by chromosome. There is no suggestion that the mutation rates differ for either the two autosomes or the two sexes. As in the earlier table, the mutation rates estimated by the indirect method are consistently, but not significantly, lower than those estimated directly.

Table 3 lists the frequencies of lethal second chromosomes among those carried

TABLE 1

Mutation rates for second and third chromosomes and for males and females

Direct estimate:					
Chromosome	Sex	Lethals	Total	<i>u</i>	
II	Male	22	3454	0.0064	
II	Female	20	2337	0.0086	
III	Male	16	2797	0.0057	
III	Female	22	3067	0.0072	
Indirect estimate:					
Chromosome	Sex	<i>e^{-m}</i>	<i>m=nu</i>	$\bar{n}$	<i>u</i>
II	Male	0.865	0.145	26.0	0.0056
II	Female	0.831	0.185	26.3	0.0070
III	Male	0.876	0.132	26.6	0.0050
III	Female	0.852	0.161	26.7	0.0060

Mutation rates have been estimated (1) directly as the proportion of lethals among chromosomes tested and (2) indirectly by means of the zero-class of the Poisson distribution.

by flies taken from the wild-type strains to initiate the mutation studies. These are the lethals that were revealed when half (or, in some instances, all) of the 27 tests originating from a single fly were lethal. The original flies, in these cases, were heterozygous for lethals already present in the wild-type stock cultures. There is considerable variation from strain to strain but on the average it seems that one second chromosome in five or six obtained from the wild-type strains is lethal. (The average frequency of preexisting lethals calculated from these data is too low for the following reason: strains with high lethal frequencies

TABLE 2

Mutation rates summarized by chromosome (sexes combined) and sex (chromosomes combined)

Direct estimate:					
			<u>Lethals</u>	<u>Total</u>	<u>u</u>
Chromosome	II		42	5791	0.0073
	III		38	5864	0.0065
Sex	Male		38	6251	0.0061
	Female		42	5404	0.0078

Indirect estimate:					
		<u>e^{-m}</u>	<u>m=nu</u>	<u>$\bar{n}$</u>	<u>u</u>
Chromosome	II	0.851	0.162	26.09	0.0062
	III	0.864	0.146	26.65	0.0055
Sex	Male	0.870	0.139	26.26	0.0053
	Female	0.843	0.171	26.49	0.0065

TABLE 3

Frequency of lethal second chromosomes among those carried by flies tested

	Lethals	Total	Percent
Old strains:			
Bermuda	9	32	28.1
Barcelona	5	34	14.7
Riverside, Calif.	2	38	5.3
Total	16	104	15.4
1965 strains:			
Madeira	15	96	15.6
Bogotá	6	16	37.5
Total	21	112	18.8
1966 strains (state and strain number):			
New York (1)	10	60	16.7
Ohio (2)	10	160	6.3
Ohio (3)	7	40	17.5
Kentucky (4)	27	62	43.5
Georgia (5)	4	24	16.7
Georgia (9)	0	24	0
S. Carolina (10)	4	14	28.6
N. Carolina (11)	2	24	8.3
Total	64	408	15.7

yielded little data relevant to mutation rates; rather, results obtained from them tended to fall within outcomes 3 and 4 described earlier. Consequently, such high-lethal strains were generally passed over in subsequent tests.)

Table 4 lists the frequencies of third chromosome lethals observed among those carried by flies from the various wild-type strains. Only the recently collected strains were used in the studies on chromosome 3. Again, it appears that about

TABLE 4

Frequency of lethal third chromosomes among those carried by flies tested

	Lethals	Total	Percent
1966 strains:			
New York (1)	16	72	22.2
Ohio (2)	17	120	14.2
Ohio (3)	32	128	25.0
Kentucky (4)	18	126	14.3
Georgia (5)	23	96	24.0
Georgia (9)	13	24	54.2
S. Carolina (10)	7	24	29.2
N. Carolina (11)	29	120	24.2
Total	155	710	21.8

one chromosome in five is lethal. A comparison of the frequencies of second and third chromosome lethals in the 1966 strains reveals that, with one exception, the frequencies for chromosome 3 are higher. The probability for a consistent difference of this sort were the lethal frequencies actually identical is very nearly 7%. Although the observed bias is not statistically significant, it agrees with the generally higher frequencies of third chromosome lethals observed by others (see WALLACE, ZOUROS and KRIMBAS 1966).

DISCUSSION

The studies reported here were carried out for two purposes: (1) To obtain an estimate of the lethal mutation rate for the third chromosome of *D. melanogaster*. (2) To determine whether the rates of mutation are strikingly different for the two sexes.

The data have yielded clear answers in respect to both questions. (1) Lethal third chromosomes arise at a frequency very nearly identical to that of second chromosome lethals; results based on nearly six thousand chromosomes of each sort do not differ significantly. (2) There is no indication in the results of the present test that the mutation rate is lower for females than for males; this conclusion is based on tests of five or six thousand chromosomes obtained from each sex.

SUMMARY

Comparative studies were made of spontaneous lethal mutation rates (1) for second and third chromosomes and (2) in the two sexes of *D. melanogaster*. No significant differences were found between chromosomes or between sexes in tests of 11,655 chromosomes. The overall average mutation rate based on a direct estimate (80 lethals/11,655 tests) equals 0.0069; the overall average calculated by means of the Poisson distribution equals 0.0059.

LITERATURE CITED

- AUERBACH, C., 1941 The effect of sex on the spontaneous mutation rate in *Drosophila melanogaster*. *J. Genet.* **41**: 255-265.
- CROW, J. F., and R. G. TEMIN, 1964 Evidence for partial dominance of recessive lethal genes in natural populations of *Drosophila*. *Am. Naturalist* **98**: 21-33.
- GLASS, B., and R. K. RITTERHOFF, 1956 Spontaneous-mutation rates at specific loci in *Drosophila* males and females. *Science* **124**: 314-315.
- WALLACE, B., 1966 Distance and the allelism of lethals in a tropical population of *Drosophila melanogaster*. *Am. Naturalist* **100**: 565-578.
- WALLACE, B., E. ZOUROS, and C. KRIMBAS, 1966 Frequencies of second and third chromosome lethals in a tropical population of *Drosophila melanogaster*. *Am. Naturalist* **100**: 245-251.