GENETIC LOAD IN NATURAL POPULATIONS: IS IT COMPATIBLE WITH THE HYPOTHESIS THAT MANY POLYMORPHISMS ARE MAINTAINED BY NATURAL SELECTION?[1]

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Manuscript received September 15, 1973
Revised copy received February 26, 1974

ABSTRACT

Recent studies of genetically controlled enzyme variation lead to an estimation that at least 30 to 60% of the structural genes are polymorphic in natural populations of many vertebrate and invertebrate species. Some authors have argued that a substantial proportion of these polymorphisms cannot be maintained by natural selection because this would result in an unbearable genetic load. If many polymorphisms are maintained by heterotic natural selection, individuals with much greater than average proportion of homozygous loci should have very low fitness. We have measured in *Drosophila melanogaster* the fitness of flies homozygous for a complete chromosome relative to normal wild flies. A total of 37 chromosomes from a natural population have been tested using 92 experimental populations. The mean fitness of homozygous flies is 0.12 for second chromosomes, and 0.13 for third chromosomes. These estimates are compatible with the hypothesis that many (more than one thousand) loci are maintained by heterotic selection in natural populations of *D. melanogaster*.

POPULATIONS of sexually reproducing organisms are genetically extremely polymorphic. The assay of genetically controlled protein variation by gel electrophoresis and other techniques permits estimating, to a first approximation, the proportion of structural loci that are segregating in a given population or that are heterozygous in an average individual. Fifteen or more loci have been studied in some 25 species of vertebrates and 26 species of invertebrates. The proportion of heterozygous loci per individual is, on the average, about 6% for vertebrates and 15% for invertebrates (AYALA *et al.* 1973; SELANDER and KAUFMAN 1973). The proportion of polymorphic loci is about 30% in vertebrates and 60% in invertebrates. These estimates are based on genetic variation detectable by gel electrophoresis. The true amount of variation is likely to be larger, perhaps much larger, than these estimates indicate, because they are based only on electrophoretically distinguishable variants.

What are the mechanisms by which genetic variation is maintained in natural populations? A possible answer is that the protein variation is adaptively neutral, i.e., that alternative genotypes have effectively identical fitnesses. If this were

[1] Work supported by grant AT(04-3)34 from the U.S. Atomic Energy Commission.
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the case, allelic frequencies would be determined exclusively by the random process of sampling from generation to generation. Evidence derived from the study of natural as well as of experimental populations seems, however, to indicate that genetically controlled protein variation is not adaptively neutral, but is rather maintained by balancing natural selection. (For Drosophila see, for instance, PRAKASH, LEWONTIN and HUBBY 1969; AYALA, POWELL and DOBZHANSKY 1971; AYALA, POWELL and TRACEY 1972; AYALA *et al.* 1972; AYALA 1972; AYALA and ANDERSON 1973; DOBZHANSKY and AYALA 1973; KOJIMA and YARBROUGH 1967; ZOUROS and KRIMBAS 1973).

Various forms of balancing selection have been shown to contribute to the maintenance of specific polymorphisms in natural populations. If heterosis is a mechanism by which a large number of polymorphisms are maintained, it follows that individuals homozygous for a much greater number of loci than wild individuals, should have much lower fitness than the latter. We report here a study of the fitness of *Drosophila melanogaster* flies homozygous for whole chromosomes. We have studied the effect on fitness of homozygosis for second and for third wild chromosomes. The fitness depression observed in homozygotes is compatible with the hypothesis that many natural polymorphisms are maintained by heterotic natural selection. Our results, however, are also compatible with other alternative hypotheses. Thus, we do not show that heterosis plays indeed a major role in the maintenance of the observed levels of genetic variation in natural populations, but rather that this is a tenable hypothesis.

MATERIALS AND METHODS

Collections of *Drosophila melanogaster* were made at MacDonald Ranch, Napa County, California, in October 1971. The technique used to obtain strains homozygous for wild chromosomes is diagramed in Figure 1.

Each wild male was individually crossed to each one of the balancer stocks. Use of a single male in the F_1 crosses insures that all wild chromosomes in a particular line are identical by descent. Two kinds of flies are expected in the F_3 generation: flies homozygous for a complete

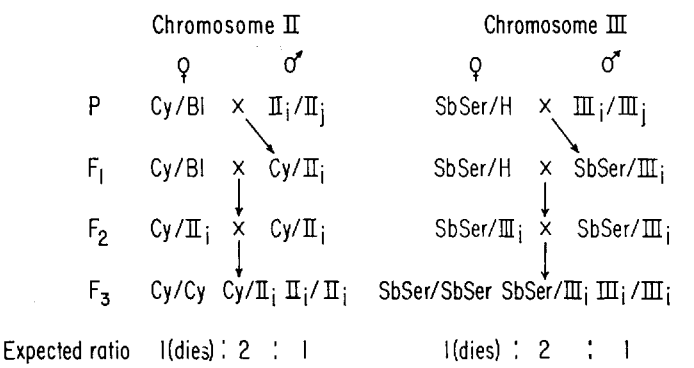

FIGURE 1.—The series of crosses made to obtain homo- and heterozygotes for wild chromosomes of *Drosophila melanogaster*. *Cy* and *SbSer* represent marker chromosomes which suppress recombination. *Bl* and *H* are marker chromosomes. The subscripts *i* and *j* refer to wild chromosomes.

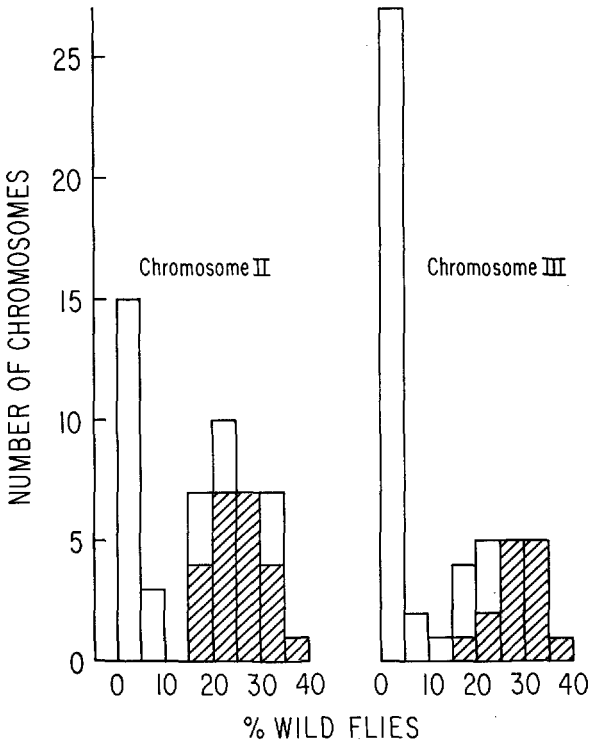

FIGURE 2.—Distribution of the number of wild chromosomes producing a given percentage of wild flies in the F_3 generation of the crosses depicted in Figure 1.

wild chromosome and flies heterozygous for that wild chromosome and the balancer in the ratio 1:2, respectively. The F_3 flies are progenies of flies heterozygous for a particular wild chromosome and the balancer. Since no meiotic drive is known to occur in these heterozygous flies, deviations in the F_3 progenies from the expected 1:2 ratios are due to viability differences between the homozygous and the heterozygous flies. In all, 50 second and 50 third wild chromosomes were examined. The distribution of these chromosomes with respect to the proportion of homozygous flies in the F_3 generation is depicted in Figure 2. As observed in other similar studies, the distribution of viabilities is bimodal, with one mode corresponding to lethal chromosomes, and the other mode somewhat below the expected frequency of 33%. Among the second chromosomes, 10 were completely lethal, and 8 others resulted in fewer than 10% wild flies; the other 32 chromosomes, or 64% of the total, are called "quasi-normals." Among the third chromosomes, 22 produced no homozygous wild flies, and 8 more resulted in less than 15% homozygous flies; 20 chromosomes, or 40% of the total have "quasi-normal" viability.

For our experimental studies we have selected 23 second and 14 third chromosomes, by choosing about 70% of all the chromosomes within the quasi-normal classes. The number of chromosomes selected from each viability class is indicated by the shaded areas in the histogram shown in Figure 2. The proportions of wild flies in the F_3 are given for each of these experimental chromosomes in Table 1 (columns 2 and 6). As a further measure of the viability of homozygous flies, and to test for their fertility, we have crossed flies homozygous for each wild chromosome to flies heterozygous for the same wild and the balancer chromosome in the two possible directions. The expected frequency of wild flies in the progenies of these crosses is 0.50; the observed frequencies for each experimental chromosome are given in Table 1 (columns 4 and 8).

TABLE 1

Percentage of wild flies in viability tests

	$Cy/II_i \times Cy/II_i$		$Cy/II_i \times II_i/II_i$		$SbSer/III_i \times SbSer/III_i$		$SbSer/III_i \times III_i/III_i$	
Chromosome	% wild	Total	% wild	Total	% wild	Total	% wild	Total
Expected	33	—	50	—	33	—	50	—
1	26	164	49	466	31	99	52	403
2	33	166	50	592	20	191	40	204
3	29	313	41	437	34	197	33	216
4	16	165	47	353	31	311	46	726
5	32	226	50	722	27	103	51	1006
6	23	150	47	555	27	290	32	526
7	26	384	43	472	29	264	47	569
8	26	227	41	1086	33	78	—	42
9	17	196	40	630	25	247	45	868
10	32	155	42	311	25	238	52	1312
11	30	372	43	683	28	432	50	923
12	31	161	36	358	40	139	47	866
13	25	284	45	420	29	173	47	988
14	32	479	50	518	33	123	46	834
15	20	153	36	213				
16	33	222	46	458				
17	25	415	44	554				
18	27	426	—	31				
19	23	167	36	778				
20	22	206	45	222				
21	22	210	42	146				
22	18	189	—	28				
23	23	117	39	158				
Mean	26±1	241±22	43±1	482±49	29±1	206±26	45±2	726±91
Controls	—	—	52	8,409	—	—	57	11,059

Among the second chromosomes, 3 and 12 produce homozygous males which are sterile; 13, 15, 18, 22 and 23 produce sterile homozygous females. Among the third chromosomes, 2 produces sterile homozygous males; 3 and 8 produce sterile homozygous females.

To compare the viability of flies heterozygous for random combinations of wild chromosomes with the viability of flies heterozygous for a wild and the balancer chromosome, we have made serial crosses of flies homozygous for a given wild chromosome with flies heterozygous for a different wild chromosome and the balancer. The proportions of wild flies in these crosses are given in the last row of Table 1. Progenies from these crosses were used to start the last row of Table 1. Progenies from these crosses were used to start the control population cages (see below).

The main part of our experiment was performed in population cages of the type described by AYALA (1968). A total of 92 laboratory populations were established, as follows. For each wild chromosome, two experimental populations were set up. One cage was started with 40% flies heterozygous for the wild and the balancer chromosome and 60% homozygous for the wild chromosome; the second cage was started with 90% heterozygous and 10% homozygous flies. These populations are called "experimentals". There are 46 and 28 experimental populations for second and third chromosomes, respectively. One hundred virgin females and 100 males of the appropriate genotypes were used to establish each experimental population. As "controls" we established 12 populations for the second and 6 populations for the third chromosome. As with the experimental populations, half of the control populations were started with 40% flies heterozygous for the balancer chromosome and 60% wild type; the other half were started

with 90% of the former and 10% of the latter. In the "controls" the wild flies were heterozygous for random combinations of wild chromosomes. All wild chromosomes were introduced in each control cage in equal numbers, both in the wild flies and in the flies heterozygous for the balancer chromosome.

Each population cage contains 10 one-ounce food cups with standard corn-meal-molasses-agar medium. The cups are changed in a rotating schedule so that any given cup remains in the cage for 21 days. The cages are maintained at 22 ± 1°. The flies used to establish the populations were removed and discarded after 8 days. Twenty-one days after the start, all adult flies in a cage were etherized, a random sample of 200–300 flies was counted, and all adults as well as the food cups containing eggs, larvae and pupae, were placed in a clean cage. This procedure was repeated thereafter every 21 days.

To estimate the relative fitnesses the zygotic frequencies must be known (PROUT, 1965; SVED and AYALA 1970). In all populations where the balancer and the wild chromosome reached stable equilibria, we obtained egg samples. This was done immediately after the 4th sample (on day 86) in the second-chromosome cages, and after the 5th sample (on day 107) in the third-chromosome cages.

RESULTS

The rationale for our experiment is a simple one. The two balancer chromosomes used, *SM1* and *TM3*, are known to inhibit crossing over in the second and in the third chromosome, respectively. In homozygous condition each one of the balancers is lethal. If we represent the balancer chromosome as B, the outcome of natural selection depends on the fitness of $B/+$ individuals relative to $+/+$ individuals. If these two genotypes have identical fitnesses, the B chromosome should be eliminated from the cages at a rate which is approximately given by:

$$t = 1/q_t - 1/q_o\,, \tag{1}$$

(FALCONER 1960), where t is the number of generations required for the balancer chromosome to change from frequency q_o to frequency q_t. The number of generations required to change the frequency of the balancer chromosome from q_o to $0.5q_o$ is simply $1/q_o$. Thus, if the $B/+$ and $+/+$ genotypes have the same fitness, the frequency of B should change from 0.20 (its initial frequency in half of the cages) to 0.10 in 5 generations, from 0.10 to 0.05 in 10 generations, and so on. If the $B/+$ genotypes have lower fitness than the $+/+$ genotypes, the B chromosome will be eliminated at a faster rate than that given by (1), and the rate of elimination can be used to determine the selective values of the two genotypes.

On the other hand, if the $+/+$ genotypes are at a disadvantage relative to $B/+$, then a heterotic equilibrium will be obtained, and the relative fitnesses can be directly estimated from the zygote equilibrium frequencies. If the fitnesses of $B/+$ and $+/+$ are $1{:}1{-}s$, the equilibrium frequency of B is given by

$$\hat{q} = s/(1+s) \tag{2}$$

The adaptive values can then be directly calculated from the equilibrium frequency; from (2):

$$s = \hat{q}/(1-\hat{q}) \tag{3}$$

It must be noted, however, that $\hat{q}$ in (2) and (3) refers to the equilibrium frequency of B at the time the zygotes are formed, and not to its frequency among adult flies.

Second chromosomes: The main results are given in Table 2 ("experimental"

TABLE 2

Percentages of Cy/II_i *flies in 46 experimental populations at various samplings*

Chromosome	Replicate	Days at time of sampling* 0	21	42	84	126	147	168	189	210	86 (Egg sample)†
II-1	(1)	40	64	42	46	70	54	..	..	..	66
	(2)	90	74	50	46	76	52	..	..	..	65
II-2	(1)	40	46	58	62	70	54	..	..	..	64
	(2)	90	70	48	64	72	52	..	..	..	58
II-3	(1)	40	76	72	66	78	70	..	..	..	74
	(2)	90	86	70	70	80	68	..	..	..	68
II-4	(1)	40	52	52	56	62	68	..	..	..	66
	(2)	90	76	48	62	66	56	..	..	..	68
II-5	(1)	40	48	62	62	68	66	..	..	..	69
	(2)	90	72	70	66	70	56	..	..	..	71
II-6	(1)	40	52	48	30	26	19	16	12	17	48
	(2)	90	80	60	48	36	28	18	19	17	55
II-7	(1)	40	72	58	50	60	54	..	..	..	67
	(2)	90	72	68	52	56	48	..	..	..	69
II-8	(1)	40	54	46	56	78	60	..	..	..	72
	(2)	90	72	46	58	70	50	..	..	..	74
II-9	(1)	40	59	42	46	38	46	..	..	..	59
	(2)	90	73	50	56	42	44	..	..	..	64
II-10	(1)	40	42	38	50	58	40	51	55	..	62
	(2)	90	72	66	64	54	54	48	51	..	66
II-11	(1)	40	68	70	56	62	34	19	18	..	69
	(2)	90	80	64	70	72	54	58	61	..	71
II-12	(1)	40	74	74	58	62	58	..	..	..	76
	(2)	90	78	74	72	74	64	..	..	..	73
II-13	(1)	40	82	78	70	68	62	..	..	..	73
	(2)	90	84	78	80	76	70	..	..	..	74
II-14	(1)	40	54	38	52	54	40	..	..	..	55
	(2)	90	82	54	56	50	42	..	..	..	57
II-15	(1)	40	94	92	88	86	82	..	..	..	89
	(2)	90	96	94	94	90	92	..	..	..	75
II-16	(1)	40	72	66	68	66	66	..	..	..	76
	(2)	90	76	62	60	66	58	..	..	..	75
II-17	(1)	40	90	52	62	46	22	20	23	18	77
	(2)	90	86	60	74	70	54	48	72	50	86
II-18	(1)	40	70	54	74	68	40	26	24	21	68
	(2)	90	68	56	64	44	26	22	38	44	59
II-19	(1)	40	68	64	66	72	60	..	..	..	68
	(2)	90	82	62	72	74	64	..	..	..	75
II-20	(1)	40	62	54	48	56	58	..	..	..	44
	(2)	90	66	54	62	70	68	..	..	..	40
II-21	(1)	40	48	38	46	42	28	..	..	..	46
	(2)	90	64	54	42	48	30	..	..	..	48
II-22	(1)	40	78	66	62	64	70	56	57	59	64
	(2)	90	74	58	62	58	38	34	26	23	77
II-23	(1)	40	84	62	58	70	48	44	55	..	79
	(2)	90	82	64	60	80	62	50	71	..	72

* The average number of flies counted per sample for each population is 280 ± 10.
† The average number of flies counted for each egg sample is 1585 ± 80.

populations) and Table 3 ("controls"). The values in the body of the tables are the percentages of Cy/II_i flies at various sampling times. The average number of flies counted per sample is 280 ± 10 and 312 ± 11 for the experimental and control populations, respectively.

The most remarkable result is that the Cy chromosome is not lost in any one of the experimental populations. The frequencies of the Cy/II_i heterozygotes remain approximately the same in most cages from day 42 on. Evidence that equilibria have been reached derives also from the reasonably good agreement between the frequencies of the two replicates started with 40 and 90% heterozygotes. After day 42 there are no significant differences between the replicates in most populations. The correlation between the replicates is $r = 0.81 \pm 0.13$ (with 21 degrees of freedom). Most populations were terminated after 147 days. When it was doubtful whether an equilibrium had been reached by that time, the populations were continued up to a maximum of 210 days.

The average equilibrium frequencies of the heterozygous flies are 0.56 ± 0.03 and 0.59 ± 0.03 for all experimental cages started at frequencies of 0.40 and 0.90, respectively. The range of the equilibrium frequencies extends from 0.24 (chromosome *II*-6) to 0.92 (chromosome *II*-15), but for most populations it falls within a narrow range around the mean equilibrium value of 0.56. Clearly, in the experimental cages the Cy/II_i genotypes have higher Darwinian fitness than the II_i/II_i homozygotes.

For the estimation of fitness and fitness components, an egg sample was taken from each cage on day 86, 48 hours after the fourth adult sample, when equilibrium frequencies had already been reached in most cages. Flies were allowed to develop from these eggs under near optimal conditions to minimize competition. The percentages of heterozygous flies in these samples are given in the last column of Table 2.

The control populations were started with random combinations of all the chromosomes used in the experiment, with each other and with the Cy chromosome. The phenotypically wild flies in these populations are nearly as heterozygous for the second chromosome as they are in nature. These populations allow to estimate the fitness of Cy/II_i flies relative to wild heterozygous flies. The initial frequency of Cy/II_i flies was 0.40 in six control populations, and 0.90 in the other six. The results are given in Table 3. Average frequencies for all cages with a given initial frequency are given in the last two rows of the Table. The Cy chromosome rapidly decreases in frequency in all populations, and has been eliminated in nearly all of them by the 231st day.

The rate of elimination indicates that the Cy/II_i flies have lower fitness than the wild flies. If the fitness of these two types of flies were the same, their frequency should, according to (1), take 65 generations to decrease from 40% to 2.5%. The generation time in our cages is probably between 3 and 4 weeks. Taking the lower estimate of 3 weeks, the frequency of Cy heterozygotes has decreased from 40 to about 2.5% in 10 generations in the first six replicates, and at about the same rate in the other six replicates started with 90% Cy flies.

All our censuses are taken by scoring separately males and females, although for simplicity the frequencies of each sex are not reported in the tables. A con-

TABLE 3

Percentages of Cy/II$_i$ *flies in 12 control populations at various samplings*

Replicate	Days at time of sampling*												
	0	21	42	84	126	147	168	189	210	231	252	273	86 (Egg sample)†
(1)	40	37	24	17	14	7	5	4	1	0	0	0	25
(2)	40	37	34	18	10	8	8	5	3	0	0	0	23
(3)	40	33	23	19	10	12	9	4	4	0	0	0	29
(4)	40	28	16	9	12	11	7	2	0	0	0	0	19
(5)	40	33	17	15	22	14	14	15	7	0	0	0	30
(6)	40	39	21	17	14	3	12	7	1	0	0	0	19
(7)	90	57	31	23	16	12	9	5	2	0	0	0	37
(8)	90	56	39	31	28	18	16	8	7	1	0.3	0	37
(9)	90	47	44	17	6	6	8	2	0	0	0	0	27
(10)	90	66	23	28	10	10	8	3	0	0	0	0	32
(11)	90	66	31	24	26	10	14	8	4	0	0	0	23
(12)	90	49	29	22	23	11	14	12	8	0.2	0	0	33
Averages:													
(1)–(6)	40	34.5±1.7	23.4±2.7	15.8±1.5	13.9±1.9	9.2±1.7	9.1±1.4	6.0±1.8	2.8±1.0	0	0	0	24
(7)–(12)	90	56.8±3.3	32.7±3.1	24.1±1.9	18.2±3.7	11.0±1.6	11.7±1.5	6.3±1.5	3.5±1.4	0.2±0.2	0.1±0.1	0	32

* The average number of flies counted per sample for each population is 312 ± 11.
† The average number of flies counted for each egg sample is 1736 ± 133.

sistent and statistically significant excess of females occurs among the *Cy* flies, but not among the wild-type ones. On day 147, when most cages were terminated, the average percentage of *Cy* flies in all 46 experimental populations was 45.5 ± 2.6 among males and 57.5 ± 2.8 among females. For all adult samples in the experimental cages, the ratio of males to females of *Cy* phenotype is 38 ± 1 to 62 ± 1, a significant departure from the expected 50:50 ratio. The ratio of males to females among the wild flies in the same cages is 46 ±3 to 54 ± 3.

There is, however, no significant deviation from the expected 50:50 ratio among the *Cy* flies in the egg samples taken on day 86, nor in the viability tests reported in Table 1. It seems, therefore, that in the cages the average viability or longevity of the *Cy* females is substantially greater than that of the *Cy* males.

Third chromosomes: The results for the 28 experimental and the six control populations are given in Tables 4 and 5, respectively. The results of the experimental populations fall into two classes. For chromosomes 1 through 8 the homozygous wild flies have lower fitness than the heterozygotes, *SbSer/III*$_i$; a

TABLE 4

Percentages of SbSer/III$_i$ *flies in 28 experimental populations at various samplings*

Chromosome	Replicate	Days at time of sampling* 0	21	42	63	84	105	126	147	168	189	107 (Egg sample)†
III-1	(1)	40	63	67	—	80	66	57	..	..	..	61
	(2)	90	70	63	—	79	70	65	..	..	..	56
III-2	(1)	40	74	66	—	86	66	64	..	..	..	66
	(2)	90	68	60	—	82	66	67	..	..	..	58
III-3	(1)	40	79	76	—	79	73	71	..	..	..	58
	(2)	90	70	79	—	77	74	75	..	..	..	52
III-4	(1)	40	36	12	—	45	23	11	..	..	..	12
	(2)	90	59	29	—	34	13	18	..	..	..	5
III-5	(1)	40	41	31	—	24	10	17	..	..	..	10
	(2)	90	56	30	—	28	17	17	..	..	..	9
III-6	(1)	40	54	71	—	81	68	63	..	..	..	62
	(2)	90	80	83	—	90	78	72	..	..	..	66
III-7	(1)	40	53	37	—	90	62	63	..	..	..	47
	(2)	90	55	47	—	53	7	3	..	..	..	7
III-8	(1)	40	76	73	—	79	77	78	..	..	..	73
	(2)	90	73	77	—	86	80	79	..	..	..	88
III-9	(1)	40	36	18	14	15	7	1	0	0	0	—
	(2)	90	56	30	21	16	13	2	3	0	0	—
III-10	(1)	40	28	11	5	6	4	2	1	0	0	—
	(2)	90	57	20	22	23	12	5	2	1	0	—
III-11	(1)	40	19	13	5	4	3	0	0	0	0	—
	(2)	90	61	43	37	12	7	1	0	0	0	—
III-12	(1)	40	26	11	6	3	1	0.3	0	0	0	—
	(2)	90	53	32	16	9	2	1	0	0	0	—
III-13	(1)	40	30	11	6	3	1	0	0	0	0	—
	(2)	90	45	18	8	5	3	0.3	0	0	0	—
III-14	(1)	40	17	5	4	0.2	0	0	0	0	0	—
	(2)	90	42	19	10	5	3	0.5	0	0	0	—

* The average number of flies counted per sample for each population is 279 ± 11.
† The average number of flies counted for each egg sample is 609 ± 95.

TABLE 5

Percentages of SbSer/III_1 *flies in 6 control populations at various samplings*

Replicate	Days at time of sampling* 0	21	42	63	84	105	126
(1)	40	17	6	3	0	0	0
(2)	40	13	7	1	0	0	0
(3)	40	6	1	0.3	0	0	0
(4)	90	39	17	3	1	0	0
(5)	90	38	12	5	0	0	0
(6)	90	26	4	1	0.3	0	0
Averages:							
(1)-(3)	40	12±3	5±2	1±1	0	0	0
(4)-(6)	90	34±4	11±4	3±1	0.4±0.3	0	0

* The average number of flies counted per sample for each population is 385 ± 33.

stable equilibrium between the marker and each wild chromosome has been reached in these cages. As with the second chromosomes, the agreement between the cages started with 40% and 90% *SbSer* flies is generally good (correlation coefficient, $r = 0.86 \pm 0.21$, with 6 degrees of freedom). The only exception occurs for chromosome 7, where the replicate cage started with 90% *SbSer* flies may have been contaminated.

The marker chromosome was eliminated in the experimental cages with chromosomes 9 through 14 and also in all the control populations. The rate of elimination of the marker chromosome is faster in the controls than in the experimentals.

To estimate fitness and fitness components in the populations where the *SbSer* chromosome reached a stable equilibrium, egg samples were taken 48 hours after the census taken on day 105. The percentages of *SbSer* flies emerging from these samples are given in the last column of Table 4. The estimation of fitness in the cages where the marker chromosome is eliminated is obtained from its rate of elimination.

No significant deviations from the expected 1:1 sex ratio were observed in the third-chromosome cages either among the *SbSer*, or among the wild flies.

ESTIMATION OF FITNESS

Theory: Parts of the theory for the estimation of fitness in experiments similar to the present one have been presented by POLIVANOV and ANDERSON (1969), SVED and AYALA (1970), SVED (1971) and MOURÃO, AYALA and ANDERSON (1972). First, we shall consider the estimation of fitness and fitness components in the populations where the marker chromosome is not eliminated. We shall designate the balancer chromosome as B.

At equilibrium, let p be the frequency of the balancer chromosome, let h' and $1-h'$ be the zygotic frequencies of the $B/+$ and $+/+$ genotypes; and let $0{:}1{:}w$ be the relative fitnesses of the $B/B : B/+ : +/+$ genotypes.

The relative contributions of the $B/+$ and $+/+$ genotypes to the next generation will be, by definition

$$h' : (1-h')w \qquad (4)$$

then,

$$p = \tfrac{1}{2}\, h'/[h'+(1-h')w] \tag{5}$$

The ratio of $B/+$ to $+/+$ among the zygotes of the next generation will be

$$2p(1-p) : (1-p)^2 \tag{6}$$

at equilibrium

$$2p(1-p) : (1-p)^2 = h' : (1-h') \tag{7}$$

or

$$2p(1-h') = h'(1-p) \tag{8}$$

Substituting the value of p from (5), and solving for w, we obtain

$$w = \frac{2-3h'}{2-2h'} \tag{9}$$

Tables 2 and 4 give in the last column estimates of the zygote frequencies of the $B/+$ genotypes in the cages. Although the eggs in these samples were developed under conditions which minimize competition, some differential mortality of the two genotypes may be expected. This effect can be corrected for, provided that frequency-dependent effects are not too strong, by using the results from the initial $B/+ \times B/+$ crosess (Table 1) made under similar conditions. If there are no differences in viability between the two genotypes in such crosses, the expected ratio of $B/+$ to $+/+$ among the adults is 2:1. Let the relative viabilities of these two genotypes be $1:v$, then the ratio of the two genotypes among the adult flies will be:

$$2:v \tag{10}$$

If the observed ratio of the two genotypes among the adults is $r:1-r$, we obtain, solving for v

$$v = \frac{2(1-r)}{r} \tag{11}$$

Let h and $1-h$ be the observed frequencies of the $B/+$ and $+/+$ genotypes among the adults developed from the egg samples (last column in Tables 2 and 4). Then

$$h' : (1-h')v = h : (1-h) \tag{12}$$

and, solving,

$$h' = \frac{vh}{1-h+vh} \tag{13}$$

Substituting (13) in (9) we obtain

$$w = \frac{2-2h-vh}{2-2h} \tag{14}$$

Substituting the value of v given by (11), and simplifying, we get

$$w = \frac{r-h}{r(1-h)} \tag{15}$$

Thus, we can estimate the relative fitness of the $+/+$ homozygous flies in the cages, from the frequency, h, of the $+/+$ adults developed from the egg samples (last column, Tables 2 and 4), and the frequency, r, of the heterozygous F_1 flies in the $B/+ \times B/+$ crosses (Table 1).

Since we know the frequencies, H and $1-H$, of the $B/+$ and $+/+$ genotypes among the adult flies in the cages, we can also estimate the fitness components,

V, for viability, and F, for fertility, under population conditions (similar to E and L in PROUT 1965).

Let the ratio of the relative viabilities *in the cages* be 1 and V for $B/+$ and $+/+$ respectively. Since the equilibrium zygotic frequencies of the two genotypes are h':$(1-h')$, then, among the adults, the ratio of $B/+$ to $+/+$ should be:

$$h' : (1-h')\ V \tag{16}$$

which, at equilibrium, should be equal to the ratio H:$(1-H)$ observed among the adults, and therefore

$$V = \frac{h'(1-H)}{H(1-h')} \tag{17}$$

Let the relative fertility of $B+$ to $+/+$ be 1:F. Then the gametic frequencies produced by the $B+$ and the $+/+$ adults (whose frequencies are H and $1-H$) are

$$\begin{aligned} B \text{ gametes} &= \frac{H}{2} \\ + \text{ gametes} &= \frac{H}{2} + (1-H)\ F \end{aligned} \tag{18}$$

and, therefore, the ratio of the $B/+$ to $+/+$ among zygotes will be

$$2\left(\frac{H}{2}\right)\left[\frac{H}{2} + (1-H)\ F\right] : \left[\frac{H}{2} + (1-H)\ F\right]^2 \tag{19}$$

which, at equilibrium, must be equal to h':$1-h'$. Solving for F we obtain

$$F = \frac{H(2-3h')}{2h'(1-H)} \tag{20}$$

The estimates given by equations (15), (17) and (20) measure the fitness, viability, and fertility of the homozygous wild flies relative to flies heterozygous for a wild and a marker chromosome. Our goal is to estimate the fitness of flies homozygous for complete chromosomes relative to flies heterozygous for random combinations of wild chromosomes. The control populations allow estimating the fitness of the $B/+_i$ flies relative to wild heterozygous flies, $+_i/+_j$. The fitness estimates obtained from (15) must then be multiplied by the fitness of the $B/+_i$ relative to $+_i/+_j$ flies.

Fitness in the control populations, and also in the third chromosome cages where a stable equilibrium was not reached, is estimated in two ways. First, we have used a measure called "net fitness" (MOURÃO, AYALA and ANDERSON 1973), which estimates the relative fitnesses that, if constant, would bring about the observed changes in chromosome frequencies in the observed time.

Let Q_T be the frequency of the B chromosome at generation T, in a population begun with frequency Q_0, and let W be the fitness of $B/+$ heterozygotes to $+/+$ flies (ANDERSON 1969).

Then,

$$Q_T = \frac{Q_0 W^T (W-1)}{W-1 + Q_0\ (2W^{T+1} - W^T - 2W + 1)} \tag{21}$$

which defines the following polynomial in W:

$$[Q_0(2Q_T-1)]W^{T+1} + [Q_0(1-Q_T)]W^T + [Q_T(1-2Q_0)]W + [Q_T(Q_0-1)] = 0 \tag{22}$$

This equation can be solved by Newton's iterative method. Although the equation may have many roots, only one will generally fall in the biologically meaningful range of W. The fitness, w, of $+/+$ flies relative to $B/+$ is, simply,

$$w = 1/W \tag{23}$$

We have estimated fitness assuming a 3-week generation interval. For Q_T we have used the appropriate last non-zero values and also the next-to-last non-zero values in the populations. These two sets of estimates give essentially the same results (correlation coefficient between the two sets, $r = 0.92 \pm 0.02$, with 28 degrees of freedom).

The second method used to estimate fitness in the 18 control populations, and in the 12 third-chromosome populations in which the balancer chromosome was eliminated, is the maximum likelihood method of ANDERSON (1969). This method assumes that relative fitnesses remain constant in each population throughout the experiment. Chi-square tests were made to test the goodness of fit of the frequencies observed in the various samplings with the expected frequencies calculated from the estimated fitness values. In 12 populations there was a satisfactory goodness of fit ($P>0.05$); selection was obviously not constant in the other 18 populations. Fluctuations in selection within experimental populations are not rare, and may be more the rule than the exception (POLIVANOV and ANDERSON 1969; MOURÃO, AYALA and ANDERSON 1970). Doubtless, selective values also fluctuate in nature as much as, or more than, in the carefully controlled laboratory environment. From the point of view of evolutionary dynamics, the ultimate result of the selection, and the time required to achieve that result are the most relevant parameters. In that sense, our results are very robust. The maximum likelihood estimates of fitness are essentially identical to the polynomial estimates of fitness (correlation coefficient, $r = 0.96 \pm 0.01$, with 28 degrees of freedom).

Second chromosomes: To estimate fitness we need to know the zygote frequencies at equilibrium. Egg samples were taken on day 86. Evidence that equilibrium frequencies had been reached by that time in most populations, derives from two two sources. First, genotype frequencies among the adults change very little in most cases from day 42 until the termination of the populations. Second, the two replicates started with widely divergent frequencies had already converged for most chromosomes. The exceptions are chromosome 6, for which the frequencies of homozygous flies decreased until day 168, and chromosomes 11, 17 and 18, for which there is no good agreement between the two replicates (Table 2).

The zygote frequencies as estimated from the egg samples, h, must be corrected for potential differences in viability under near-optimal conditions, using the results from the "ratio" tests (Table 1). Since we have two sets of crosses, $Cy/+ \times Cy/+$ and $Cy/+ \times +/+$, we have estimated v for each chromosome as its average value in both sets of crosses. We have then substituted h and v in equation (14) to estimate the relative fitness, w, of the homozygous wild flies relative to the balancer heterozygotes. The estimates of fitness are given separately for each replicate of the 23 chromosomes in columns 8 and 9 of Table 6.

There is reasonably good agreement between the replicates, considering the

TABLE 6

Fitness and fitness components of flies homozygous for wild second chromosomes

Chromosome	Equilibrium frequencies* 0.40	0.90	Fitness of +/+ relative to *Cy*/+ Viability (*V*) 0.40	0.90	Fertility (*F*) 0.40	0.90	Fitness (*w*) 0.40	0.90	Fitness of $+_i/+_i$ relative to $+_i/+_j$ 0.40	0.90
II-1	0.53±0.06	0.56±0.07	1.45	1.23	0.13	0.18	0.19	0.23	0.16	0.18
II-2	0.61±0.03	0.59±0.06	1.14	0.96	0.10	0.32	0.13	0.32	0.11	0.24
II-3	0.72±0.02	0.73±0.03	0.83	0.60	—0.08	0.31	—0.08	0.19	0	0.15
II-4	0.60±0.04	0.58±0.04	1.22	1.00	0.48	0.31	0.38	0.32	0.31	0.24
II-5	0.64±0.02	0.66±0.03	1.25	1.26	—0.09	—0.18	—0.11	—0.22	0	0
II-6	0.24±0.05	0.32±0.06	2.11	1.88	0.32	0.30	0.66	0.55	0.55	0.42
II-7	0.56±0.02	0.56±0.04	1.18	1.28	0.21	0.14	0.26	0.19	0.21	0.15
II-8	0.60±0.07	0.56±0.05	1.19	1.53	0.09	0.02	0.11	—0.03	0.09	0
II-9	0.43±0.02	0.48±0.03	0.79	1.04	0.58	0.50	0.60	0.51	0.50	0.39
II-10	0.49±0.03	0.56±0.03	1.44	1.28	0.22	0.14	0.31	0.18	0.26	0.14
II-11	0.56±0.08	0.65±0.04	1.40	1.05	0.08	0.03	0.10	—0.03	0.08	0
II-12	0.63±0.04	0.71±0.02	1.31	0.79	—0.09	0.04	—0.12	—0.03	0	0
II-13	0.68±0.04	0.76±0.02	0.96	0.67	—0.02	—0.09	—0.03	—0.07	0	0
II-14	0.46±0.04	0.50±0.03	1.38	1.27	0.30	0.29	0.40	0.35	0.33	0.27
II-15	0.87±0.02	0.92±0.01	0.64	0.91	—1.78	—1.92	—1.18	—0.53	0	0
II-16	0.66±0.01	0.62±0.02	1.47	1.65	—0.29	—0.21	—0.44	—0.36	0	0
II-17	0.35±0.07	0.61±0.04	4.55	2.91	—0.05	—0.44	—0.22	—1.24	0	0
II-18	0.44±0.08	0.42±0.06	2.08	1.50	0.09	0.31	0.19	0.45	0.16	0.34
II-19	0.66±0.02	0.68±0.03	0.63	0.80	0.62	0.19	0.39	0.14	0.35	0.11
II-20	0.54±0.02	0.64±0.04	0.46	0.26	1.59	2.89	0.73	0.77	0.60	0.59
II-21	0.38±0.04	0.44±0.05	0.92	0.78	0.78	0.89	0.72	0.70	0.60	0.53
II-22	0.62±0.02	0.43±0.06	0.54	2.16	1.02	0.62	0.56	0.18	0.46	0.14
II-23	0.56±0.04	0.63±0.05	1.83	0.92	—0.09	0.24	—0.17	0.20	0	0.15
Mean	0.56±0.03	0.59±0.03	1.34± 0.17	1.21± 0.12	0.179± 0.126	0.212± 0.163	0.147± 0.090	0.120± 0.090	0.207± 0.045	0.178± 0.037
Controls	0.00	0.00	—		—		1.21± 0.03	1.31± 0.03	1.00	1.00

* The frequencies of Cy/II_i flies at days 42, 84, 126 and 147 have been averaged to estimate equilibrium frequencies, except for chromosomes 6, 10, 11, 17, 18 and 22, for which all samples from day 42 to 210 have been used.

many possible sources of error. The correlation of fitness between replicates is 0.81 ± 0.12 with 21 degrees of freedom. Several estimates of fitness are negative. Clearly, fitness cannot be less than zero. There are two ways of handling these negative fitnesses. One is to assume that our estimates are as likely to be higher as to be lower than the true fitness values; the mean fitness value for all chromosomes should then be calculated using all fitness values as estimated. Another alternative is to change the negative fitnesses to zero. The mean fitnesses for all chromosomes are 0.15 ± 0.09 and 0.12 ± 0.09 if the negative fitnesses are used; but 0.25 ± 0.05 and 0.23 ± 0.05 if zeros rather than negative values are used. Averaging over replicates, the mean fitness for all chromosomes is 0.134 ± 0.063 when the negative values are used, and 0.239 ± 0.036 when zeros are used instead.

In the control populations, the fitness of the wild flies relative to the flies heterozygous for the balancer chromosome has been calculated according to the maximum likelihood method of ANDERSON (1969), and by estimating net fitness according to equations (21) to (23). The maximum likelihood method assumes that fitnesses remain constant from generation to generation; this was demonstrably not so in several populations. Yet, as pointed out earlier, there is good agreement between the estimates calculated by the two methods. (Correlation coefficient, $r = 0.96$, with 28 degrees of freedom). The mean net fitness of the wild flies are 1.21 ± 0.03 and 1.31 ± 0.03 respectively for the populations started with 0.40 and 0.90 frequency of heterozygotes for the balancer chromosome. The average net fitness for all 12 control populations is 1.26 ± 0.03. The mean fitness of all 12 populations as calculated by the maximum likelihood method is 1.33 ± 0.04.

To estimate the fitness of homozygous $+_i/+_i$ flies relative to random heterozygotes $+_i/+_j$, we have divided the fitness, w, obtained in each experimental population by the average net fitness of the six control populations started with the same initial frequency of $Cy/+$ flies. The results are given in the last two columns of Table 6. Negative values have been replaced by zeroes. The mean fitness of the homozygous flies is 0.207 ± 0.045 and 0.178 ± 0.037 for the populations started with frequencies 0.40 and 0.90 of $B/+$ flies, respectively. The grand mean is 0.182 ± 0.029. If negative values of fitness are used, the grand mean becomes 0.112 ± 0.050.

Viability, V, and fertility, F, can be calculated using formulas (17) and (20). The results are given in Table 6, columns 4 and 5 for viability, and 6 and 7 for fertility. V is greater than one in about two thirds of the populations; the mean value of V over all populations is 1.27. The ratio tests reported in Table 1 indicate, however, that under near-optimal conditions homozygous $+/+$ flies have, on the average, somewhat lower viability than $Cy/+$ flies. One possible explanation for this disagreement is to assume that under the crowded conditions of the experimental cages, the viability of the $+/+$ flies is, on the average, better than that of the $Cy/+$ flies. Another possibility, not mutually exclusive with the previous one, is that the longevity of the wild flies is greater than that of the $Cy/+$ flies. This difference would not influence the ratio of egg sample tests since

in these cases adults would be scored soon after emergence. The component of fitness, V, measures the difference between the zygotic frequencies and the adult frequencies; viability from egg-to-adult as well as longevity differences are included in V. Viability differences may be due, at least in part, to reduced longevity of $Cy/+$ males. We noticed earlier that the average ratio of males to females among the $Cy/+$ adult flies was, in the cages, 38 to 62, significantly lower than the 50:50 expected ratio.

The value of F is less than one for all but one chromosome, indicating that homozygous wild flies have much lower fertility than flies heterozygous for the balancer chromosome. The exceptional chromosome is 20, with an average F of 2.24 in the two replicate cages; although the viability of flies homozygous for this chromosome is low (average 0.36), their mean fitness, 0.74, is the highest for all second chromosomes tested. For all cages the average F, including the negative values, is 0.196 ± 0.102. As reported in Table 1, tests made outside the cages indicate that chromosomes 3 and 12 produce homozygous sterile males, and chromosomes 13, 15, 18, 22 and 23 produce homozygous sterile females. All these chromosomes, except 22, have F values close to zero in the cages. For chromosome 22, high fertility of males may have compensated in part for the sterility of the females.

Third chromosomes: The third chromosomes fall into two categories. For eight chromosomes an equilibrium was reached between the wild and the balancer chromosome, indicating that the wild homozygotes have lower fitness than the flies heterozygous for the balancer chromosome. With the other six chromosomes, the balancer was eliminated; balancer heterozygotes have in these cases lower fitness than homozygotes. The agreement between the two replicate cages started with 0.40 and 0.90 $B/+$ flies is generally good, with the only exception of chromosome 7 (Table 4).

Fitness and fitness components for the first eight chromosomes were calculated as for the second chromosomes (Table 7). The average fitness for all 16 experimental populations is 0.623 ± 0.063. The viability, V, of the homozygous flies is for these third chromosomes always lower than that of the balancer heterozygotes. Fertility, F, is lower in five out of 16 cages. This situation contrasts with the results obtained with the second chromosomes, where V was lower than 1 in about one third of the chromosomes, but F was lower than 1 for 22 out of 23 chromosomes (Table 7). The mean V for the third chromosomes is 0.411 ± 0.055; the mean F is 1.95 ± 0.51. Among these third chromosomes, chromosome 2 produces sterile homozygous males, and chromosomes 3 and 8 produce sterile homozygous females.

Fitness was calculated for the third chromosomes in which the balancer was eliminated, according to the "net fitness" and maximum likelihood methods. The net fitness estimates are given in Table 7; their average value is 1.95 ± 0.51. As stated earlier the estimates of fitness obtained by the two methods agree well with each other. Maximum likelihood and net fitness were also estimated for the six control populations. The mean net fitness for all six controls is 3.77 ± 0.55. The estimates of w obtained for each experimental population have been divided by

TABLE 7

Fitness and fitness components of flies homozygous for wild third chromosomes

Chromosome	Equilibrium frequencies* 0.40	0.90	Fitness of +/+ relative to *SbSer*/+ Viability (*V*) 0.40	0.90	Fertility (*F*) 0.40	0.90	Fitness (w) 0.40	0.90	Fitness of $+_i/+_i$ relative to $+_i/+_j$ 0.40	0.90
III-1	0.68±0.05	0.69±0.04	0.74	0.57	0.30	0.64	0.23	0.37	0.06	0.10
III-2	0.70±0.05	0.69±0.05	0.48	0.35	0.90	1.72	0.44	0.60	0.12	0.16
III-3	0.75±0.02	0.76±0.01	0.35	0.26	1.38	2.29	0.48	0.59	0.13	0.16
III-4	0.23±0.03	0.23±0.05	0.19	0.07	2.27	7.02	0.94	0.98	0.25	0.26
III-5	0.20±0.04	0.23±0.04	0.40	0.29	2.40	3.29	0.95	0.96	0.25	0.26
III-6	0.71±0.04	0.80±0.04	0.39	0.29	1.32	1.41	0.51	0.42	0.13	0.11
III-7	0.63±0.11	0.28±0.13	0.44	0.16	1.41	5.90	0.62	0.97	0.16	0.26
III-8	0.76±0.01	0.80±0.02	0.85	0.75	—0.41	—0.67	0.41	0.50	0.11	0.13
III-9	21 wks.	24 wks.					1.64	1.69	0.43	0.45
III-10	24 wks.	27 wks.					1.67	1.69	0.44	0.45
III-11	18 wks.	21 wks.					1.69	1.82	0.45	0.50
III-12	21 wks.	21 wks.					2.08	2.13	0.54	0.57
III-13	18 wks.	21 wks.					1.96	2.22	0.51	0.60
III-14	15 wks.	21 wks.					2.78	2.17	0.73	0.58
Mean (1–8)	0.58±0.08	0.56±0.09	0.48± 0.08	0.34± 0.08	1.20± 0.33	2.70± 0.92	0.57± 0.09	0.67± 0.09	0.15± 0.02	0.18± 0.02
(9–14)	19.5 wks.	22.5 wks.					1.97± 0.18	1.95± 0.10	0.45± 0.05	0.52± 0.03
Controls	12 wks.	14 wks.					3.82± 1.04	3.72± 0.64	1.00	1.00

* The frequencies of $SbSer/III_i$ flies at days 42, 84, 105 and 126 have been averaged to estimate equilibrium frequencies. Where equilibria did not occur, the number of weeks to elimination of the marker chromosome is recorded.

the mean w of the control populations to estimate the fitness of the homozygous wild flies relative to flies heterozygotes for random combinations of wild third chromosomes. These estimates range from 0.06 to 0.73; their mean value for all 28 experimental cages is 0.318 ± 0.037.

DISCUSSION

SVED, REED and BODMER (1967), KING (1967) and others have suggested that an efficient method for testing whether heterosis plays a major role in natural populations is to compare the fitness of ordinary outbred individuals with the fitness of individuals homozygous for a larger-than-average proportion of loci. This method permits us to ascertain whether heterozygotes are at an overall advantage over homozygotes. However, the superiority of the heterozygotes may be due to dominance in fitness at individual or linked loci, as well as to overdominance.

Numerous experiments, particularly in Drosophila, have shown that an increase in homozygosity results in a decrease in fitness. The experiments published before 1970 were, in general, carried out by measuring particular components of fitness, mostly viability (for instance, DOBZHANSKY and SPASSKY 1963) and fertility (GOWEN 1952; MARINKOVIC 1967), and were not, in any case, performed under population conditions (LATTER and ROBERTSON 1962). SVED and AYALA (1970) devised a method by which the fitness of homozygotes can be measured under population conditions, where equilibrium population density and a stable age distribution obtain. They found that *D. pseudoobscura* flies homozygous for non-lethal second chromosomes have a mean fitness of about 0.30. Similar techniques have been used by SVED (1971), who showed that the mean fitness of *D. melanogaster* flies homozygous for second chromosomes is about 0.15; and by MOURÃO, AYALA and ANDERSON (1972) who found that *D. willistoni* flies homozygous for second chromosomes have a mean fitness of about 0.33 (see also SPERLICH and KARLIK 1970).

We have now measured in experimental populations of *D. melanogaster* the fitness of flies homozygous for a second or a third wild chromosome relative to heterozygous wild flies. The chromosomes were extracted from a natural population just before the beginning of the experiment. In this natural population, 36% of the second chromosomes and 60% of the third chromosomes were "lethal" or "semilethal"; that is, flies homozygous for them have zero or very low viability under near-optimal conditions. The experimental populations were established with "quasi-normal" chromosomes; i.e., chromosomes which in homozygous condition resulted in flies with near-normal viability under favorable conditions. Twenty-three second and 14 third "quasi-normal" chromosomes were tested in experimental population cages. The mean fitness of flies homozygous for these chromosomes relative to random heterozygotes under population conditions is 0.192 ± 0.029 for the second, and 0.318 ± 0.037 for the third chromosomes. If the "lethal" chromosomes are taken into account, the mean fitness of flies homozygous for randomly chosen wild second chromosomes is 0.12 ($0.19 \times$

0.64) and for randomly chosen wild third chromosomes is 0.13 (0.32×0.40). (If the negative estimates of fitness are used, the mean fitness of flies homozygous for a wild second chromosome becomes $0.112 \times 0.64 = 0.072$, when the lethal chromosomes are taken into consideration). On the average, homozygosity for a whole second or third chromosome decreases fitness by at least 87 to 88%.

Our results agree with, and confirm, previous studies of fitness of flies homozygous for "quasi-normal" chromosomes relative to wild heterozygotes under population conditions. The mean fitness for 18 quasi-normal second chromosomes of *D. pseudoobscura* was 0.037 ± 0.03 (SVED and AYALA 1970); for 24 second chromosomes of *D. melanogaster*, it was 0.20 ± 0.03 (SVED 1971); for 15 second chromosomes of *D. willistoni*, it was 0.34 ± 0.03 (MOURÃO, AYALA and ANDERSON 1973). There seems to be little doubt that individuals completely homozygous for a chromosome are at a considerable disadvantage relative to heterozygous individuals.

Recent studies of protein polymorphisms in natural populations of Drosophila lead us to estimate that the proportion of polymorphic loci in a population ranges from 30 to 60% and that the average proportion of loci that are heterozygous in an individual ranges from 7 to 20% (AYALA *et al.* 1973; SELANDER and KAUFMAN 1973). The nature of the mechanisms maintaining all this genetic variation remains a controversial issue. LEWONTIN and HUBBY (1966) pointed out that if a large proportion of the polymorphisms were maintained by selection in favor of heterozygotes, an enormous genetic load would be imposed upon the population. But our results indicate that many polymorphisms could indeed be maintained by natural selection.

Assume, as LEWONTIN and HUBBY (1966) did in their calculations, that selective interactions between loci are multiplicative and that there is no linkage disequilibrium. If at each locus maintained by heterosis the heterozygote has a 0.01 selective advantage over either homozygote, then the fitness of a homozygous individual relative to an individual heterozygous at 210 loci would be $(0.99)^{210} \approx 0.12$. This is the mean fitness of individuals homozygous for a complete second or third chromosome, as estimated in our experiment. Since under our assumptions an individual would be heterozygous on the average at 50% of the heterotic loci, the total number of polymorphic loci maintained by heterosis in each chromosome could be 420. The second and third chromosomes of *D. melanogaster* are estimated to contain together about 75% of the genome. Therefore, the number of polymorphic loci that could be maintained by heterosis in the whole genome could be, approximately $(420 + 420)/0.75 = 1120$. (Using the negative estimates of fitness for the second chromosome, this value becomes $(524 + 420)/0.75 = 1258$ loci).

These calculations are based on assumptions which are unlikely to obtain in nature. But some more realistic assumptions could permit the maintenance of an even greater number of heterotic polymorphisms (SVED and AYALA 1970). Our experiments do not demonstrate that the decrease in fitness of the homozygous flies is due to homozygosis for heterotic loci. It is equally possible that the decrease in fitness is due to homozygosis for deleterious alleles present in all wild chromo-

somes. Nevertheless, we have shown that arguments of genetic load cannot be used against the hypothesis that many natural polymorphisms are maintained by heterosis. Moreover, other forms of balancing selection may also contribute to the maintenance of genetic polymorphisms. HEDRICK (1972) has shown that frequency-dependent selection may be a more effective mechanism to maintain genetic polymorphisms than heterosis.

MUKAI and CARDELLINO (1973) have estimated that the effective number of overdominant loci in wild second chromosomes of *D. melanogaster* is about ten (see also other reports by MUKAI and collaborators). But this estimate is based on differences between homozygotes and heterozygotes in *viability* under conditions similar to those existing in our ratio tests reported in Table 1. Homozygotes for the 23 second chromosomes used in our experiment have, under such conditions, fitnesses which are also only slightly lower than those of heterozygotes for random combinations of wild chromosomes (43% homozygous flies *versus* 52% heterozygous flies in the ratio tests; mean homozygous fitness for viability under these conditions, 0.83). However, Table 6 shows that homozygosity for second chromosomes has a much greater effect on fertility than on viability. When all fitness components are included under population conditions the mean fitness of homozygotes relative to random heterozygotes is, even for the quasi-normal chromosomes, very low: 0.192 (or 0.112 if negative fitness estimates are included). When all fitness components are included, the number of polymorphic loci that can be maintained by heterosis is much greater than was estimated by MUKAI and CARDELLINO (1973).

We are grateful to DR. M. M. GREEN, who provided the stocks with the balancer chromosomes, and to PROFESSORS T. DOBZHANSKY and W. W. ANDERSON for reading and criticizing the manuscript. We are also greatly indebted to W. W. ANDERSON for the calculation of maximum likelihood and "net" fitness estimates.

LITERATURE CITED

ANDERSON, W. W., 1969 Selection in experimental populations. I. Lethal Genes. Genetics **62**: 653–672.

AYALA, F. J., 1968 An inexpensive and versatile population cage. Drosophila Inform. Serv. **43**: 185. ——, 1972 Darwinian versus non-Darwinian evolution in natural populations of *Drosophila*. Proc. Sixth Berkeley Symp. Math. Stat. Prob. **V**: 211–236.

AYALA, F. J. and W. W. ANDERSON, 1973 Evidence of natural selection in molecular evolution. Nature New Biology **241**: 274–276.

AYALA, F. J., D. HEDGECOCK, G. S. ZUMWALT and J. W. VALENTINE, 1973 Genetic variation in *Tridacna maxima*, an ecological analog of some unsuccessful evolutionary lineages. Evolution **27**: 177–191.

AYALA, F. J., J. R. POWELL and T. DOBZHANSKY, 1971 Enzyme variability in the *Drosophila willistoni* group. II. Polymorphisms in continental and island populations of *Drosophila willistoni*. Proc. Natl. Acad. Sci. U.S. **68**: 2480–2483.

AYALA, F. J., J. R. POWELL and M. L. TRACEY, 1972 Enzyme variability in the *Drosophila willistoni* group. V. Genic variation in natural populations of *Drosophila equinoxialis*. Genet. Res. **20**: 19–42.

AYALA, F. J., J. R. POWELL, M. L. TRACEY, C. A. MOURÃO and S. PÉREZ-SALAS, 1972 Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. Genetics **70**: 113–139.

DOBZHANSKY, T. and F. J. AYALA, 1973 Temporal frequency changes of enzyme and chromosomal polymorphsms in natural populations of *Drosophila*. Proc. Natl. Acad. Sci. U.S. **70**: 680–683.

DOBZHANSKY, T. and B. SPASSKY, 1963 Genetics of natural populations. XXXIV. Adaptive norm, genetic load and genetic elite in *Drosophila pseudoobscura*. Genetics **48**: 1467–1485.

FALCONER, D. S., 1960 *Introduction to Quantitative Genetics*. Ronald Press Co., New York.

GOWEN, J. W., 1952 *Heterosis*. Hafner Publ. Co., New York.

HEDRICK, P. W., 1972 Maintenance of genetic variation with a frequency-dependent selection model as compared to the overdominant model. Genetics **72**: 771–775.

KING, J. L., 1967 Continuously distributed factors affecting fitness. Genetics **55**: 403–413.

KOJIMA, K. and K. YARBROUGH, 1967 The mode of selection at the polymorphic esterase-6 locus in cage populations of *Drosophila melanogaster*. Genetics **57**: 677–686.

LATTER, B. D. H. and A. ROBERTSON, 1962 The effects of inbreeding and artificial selection on reproductive fitness. Genet. Res. **3**: 110–138.

LEWONTIN, R. C. and J. L. HUBBY, 1966 A molecular approach to the study of genic heterozygosity in natural populations. II. Amounts of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. Genetics **54**: 595–609.

MARINKOVIC, D., 1967 Genetic loads affecting fertility in natural populations of *Drosophila pseudoobscura*. Genetics **57**: 701–709.

MOURÃO, C. A., F. J. AYALA and W. W. ANDERSON, 1972 Darwinian fitness and adaptedness in experimental populations of *Drosophila willistoni*. Genetica **43**: 552–574.

MUKAI, T. and R. C. CARDELLINO, 1973 Overdominance and protein polymorphisms in *Drosophila melanogaster*. Genetics **74**: s187.

POLIVANOV, S. and W. W. ANDERSON, 1969 Selection in experimental populations. II. Components of selection and their fluctuations in two populations of *Drosophila melanogaster*. Genetics **63**: 919–932.

PRAKASH, S., R. C. LEWONTIN and J. L. HUBBY, 1969 A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations of *Drosophila pseudoobscura*. Genetics **61**: 841–858.

PROUT, T., 1965 The estimation of fitness from genotypic frequencies. Evolution **19**: 546–551.

SELANDER, R. K. and D. W. KAUFMAN, 1973 Genic variability and strategies of adaptation in animals. Proc. Natl. Acad. Sci. U.S. **70**: 1875–1877.

SPERLICH, D. and A. KARLIK, 1970 The genetic conditions in heterozygous and homozygous populations of *Drosophila*. I. The fate of alien chromosomes. Genetica **42**: 265–304.

SVED, J. A., 1971 An estimate of heterosis in *Drosophila melanogaster*. Genet. Res. **18**: 97–105.

SVED, J. A. and F. J. AYALA, 1970 A population cage test for heterosis in *Drosophila pseudoobscura*. Genetics **66**: 97–113.

SVED, J. A., T. E. REED and W. F. BODMER, 1967 The number of balanced polymorphisms that can be maintained in a natural population. Genetics **55**: 469–481.

ZOUROS, E. and C. B. KRIMBAS, 1973 Evidence for linkage disequilibrium maintained by selection in two natural populations of *Drosophila pseudoobscura*. Genetics **73**: 659–674.

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