THE EFFECTS OF NATURAL SELECTION ON LINKAGE DISEQUILIBRIUM AND RELATIVE FITNESS IN EXPERIMENTAL POPULATIONS OF *DROSOPHILA MELANOGASTER*

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THE process of natural selection may be studied in laboratory populations in two ways. First, the genetic changes which occur during the course of microevolutionary change can be followed and, second, accompanying this, the size of the populations can be measured. CARSON (1961) considers the relative size of a population to be an important measure of relative population fitness when comparing genetically different populations of the same species under uniform environmental conditions over a period of time.

In the present experiment, the experimental procedure of CARSON was utilized to study the effects of selection on certain gene combinations and to measure the level of relative population fitness reached during the microevolutionary process. Experimental populations were constructed with certain oligogenes in low frequency and in certain associations in order to provide a situation likely to be changed by natural selection.

Specifically, oligogenes on the third chromosome were allowed to recombine freely with the homologous Oregon chromosome so that three separate blocks could be selected for introduction into homozygous Oregon populations. The introduction contained all five of the oligogenes. At intervals samples were removed from the populations and testcrossed to determine whether selection had favored the coupling or repulsion phases of these blocks. In addition, the fitness of the populations was measured.

This paper will show first the changes in frequency of the various gene combinations which occurred in the three experimental populations. Secondly, the fitness of these polymorphic populations will be compared to Oregon monomorphic populations, and finally to similar populations of CARSON which differed from the present ones only in that all oligogenes were introduced in the coupling phase rather than as repulsion blocks.

MATERIALS AND METHODS

All populations were composed of flies from laboratory stocks of *Drosophila melanogaster*. The population system used is one originally devised by BUZZATI-

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TRAVERSO (1955) and modified by CARSON (1958). It is identical to that described by CANNON (1963).

Base populations were founded with flies from Oregon-R (Stock No. a4 of Indiana University). Four populations were formed with 180 Oregon stock flies, each on January 6, 1960. Another was formed on February 3, 1960.

The stock used for the oligogene introductions is known as *sesro*. It carries five oligogenes, *sepia* (*se*), *spineless* (*ss*), *kidney* (*k*), the sooty allele of *ebony* (*e^s*), and *rough* (*ro*). The map locations are *se*-26.0, *ss*-58.5, *k*-64.0, *e^s*-70.7, and *ro*-91.1 (BRIDGES and BREHME 1944). The oligogene blocks which were introduced into three of these Oregon populations, Numbers 20, 21, and 22, were obtained through a series of crosses such as that shown in Figure 1. First Oregon females were crossed to *sesro* males. Then *F*₁ females were crossed to *sesro* males. The crossover progeny of the *F*₁ female were carrying the desired blocks. The way in which one of the blocks (*se* + + + +) was obtained is shown in Figure 1. The other two blocks which were picked up in a similar manner were + *ss k e^s* + and + + + + *ro*. Each block-carrying male was crossed to a virgin Oregon female. The *F*₃ male was crossed first to virgin Oregon females and then to *sesro* virgin females. If the latter cross showed that the *F*₃ male carried the block rather than

FIGURE 1.—Diagram of cross of Oregon female (solid black) and *sesro* male (cross hatched). Recombinant progeny of the *F*₁ female, in this case *sepia*, were picked up. In order to be sure that the *F*₃ male was heterozygous for a block and not the whole chromosome, he was crossed (1) to Oregon and then (2) to *sesro*. If (2) produced some block progeny, then males from (1) were added to Populations 20, 21, and 22. The chromosomes designated by open bars were uncontrolled.

the intact sesro chromosome, then male progeny from the former cross were added to the populations. Because there was still a one to one chance that each introduced male did not carry the block, a number were introduced. In fact, ten males from the *se* + + + + bearing father and ten males from the + + + + *ro* bearing father were introduced on April 9 and 16 males from the + *ss* *k* *e*^s + bearer were introduced on April 18. All three introductions were made into each of Populations 20, 21, and 22; that is, each population received 36 males.

RESULTS

The genetic situation: The gene frequencies of the introduced oligogenes were calculated at three different times during the experiment. These data are given in Table 1. At Week 15, these frequencies were calculated on the basis of the introductions which were made 11 and two days prior to this time; the finite size of the population at Week 15 was known. The gene frequencies were then the quotient of one half the number of introductions for each gene and twice the number of individuals in the population. For example, the size of Population 20 at Week 15 was 347, and the number of sepia introductions made 11 days prior to this was ten. So the estimated frequency of sepia was $5/(2 \times 347)$. It should be pointed out that these frequencies are merely an estimate because the exact number of introduced chromosomes is unknown.

Another estimate of gene frequency was made at Week 43, when 38 to 48 males from the vials which had been exposed to the population for 24 hours were testcrossed. The frequencies calculated on the basis of the testcrosses are recorded in Table 1. Indications previous to this measure were that the oligogenes were being incorporated into the gene pools at a very low rate. Testcrosses performed on 32 males from Population 20 after eight weeks revealed that only four of these were heterozygous, two for sepia, one for rough, and one for *ss k e*^s. It was not until 26 weeks after the introductions that flies homozygous for all the blocks had appeared in all three populations.

At Week 65, the experiment was terminated. One hundred males were taken at random from each population and were testcrossed. These were from both the developing and the adult populations. Genotypes of the testcrossed males which

TABLE 1

Gene frequencies of five oligogenes calculated on the basis of number of introductions at Week 15 and on the testcross results at Weeks 43 and 65

Gene	Population 20			Population 21			Population 22		
	Week 15	Week 43	Week 65	Week 15	Week 43	Week 65	Week 15	Week 43	Week 65
<i>se</i>	.007	.102	.058	.007	.044	.073	.005	.026	.037
<i>ss</i>	.012	.052	.216	.012	.078	.203	.009	.106	.186
<i>k</i>	.012	.026	.200	.012	.100	.177	.009	.092	.175
<i>e</i> ^s	.012	.013	.174	.012	.133	.219	.009	.106	.181
<i>ro</i>	.007	.064	.084	.007	.066	.094	.005	.026	.048

produced offspring are shown in Table 2. These were distributed in accordance with the HARDY-WEINBERG equilibrium; tests of goodness of fit of the number observed at each locus to those expected give chi-square values with $P > .05$ (Table 3). The gene frequencies calculated from these observed genotypes are recorded in Table 1.

The chromosome frequencies during the course of the experiment can be com-

TABLE 2

Genotypes observed in testcrosses of males removed from the developing and adult populations at the termination of the experiment (Week 65)

Genotype										Population No.		
										20	21	22
+	+	+	+	+	/	+	+	+	+	35	33	42
+	+	+	+	+	/	+	+	+	ro	5	6	4
+	+	+	+	+	/	+	+	e	+	2	7	6
+	+	+	+	+	/	+	+	k	+	1	0	0
+	+	+	+	+	/	+	+	k	e	2	2	1
+	+	+	+	+	/	+	+	e	ro	0	1	0
+	+	+	+	+	/	+	ss	+	+	3	5	4
+	+	+	+	+	/	+	ss	+	ro	0	1	0
+	+	+	+	+	/	+	ss	+	e	0	1	0
+	+	+	+	+	/	+	ss	+	e	2	0	0
+	+	+	+	+	/	+	ss	k	+	6	1	5
+	+	+	+	+	/	+	ss	k	e	19	15	21
+	+	+	+	+	/	+	ss	k	e	3	1	0
+	ss	k	e	+	/	+	ss	k	e	0	1	0
+	ss	k	e	+	/	se	+	+	+	1	3	0
+	ss	k	e	+	/	+	+	+	ro	1	1	3
+	ss	k	e	+	/	+	ss	+	+	1	0	0
+	ss	k	e	+	/	+	ss	k	+	1	1	0
+	ss	k	e	+	/	+	+	k	e	0	1	0
+	ss	k	e	+	/	+	ss	+	ro	0	1	0
+	ss	k	e	+	/	+	ss	k	ro	1	0	0
+	ss	k	e	+	/	+	ss	k	e	0	0	1
+	+	+	+	ro	/	+	+	+	ro	2	0	0
+	+	+	+	ro	/	+	+	k	e	0	1	0
+	+	+	+	ro	/	se	+	+	+	0	2	0
+	+	+	+	ro	/	+	ss	k	ro	0	1	0
+	+	+	+	ro	/	+	+	+	e	0	1	0
se	+	+	+	+	/	+	+	+	e	0	1	0
se	+	+	+	+	/	+	ss	+	+	0	1	0
+	ss	+	+	+	/	+	+	k	e	0	1	0
+	+	+	+	+	/	se	+	+	+	10	4	5
+	+	+	+	+	/	se	+	+	ro	0	1	1
+	+	+	+	+	/	se	+	k	e	0	1	1
+	+	+	+	+	/	se	ss	+	+	0	1	0
										95	96	94

TABLE 3

Chi-square and P values of goodness of fit of genotypes at each locus observed in final testcrosses (Week 65) to those expected, assuming the three genotypes are distributed in accordance with the Hardy-Weinberg law

Locus	Population					
	20		21		22	
	χ^2	P	χ^2	P	χ^2	P
<i>se</i>	.3616	> .5	.5898	> .3	.1424	> .7
<i>ss</i>	.3776	> .5	.1240	> .7	1.5667	> .2
<i>k</i>	1.2796	> .2	.0000	> .99	1.1088	> .2
<i>e^s</i>	3.0132	> .05	1.3598	> .2	1.3247	> .2
<i>ro</i>	.4153	> .5	.0389	> .8	.2366	> .5

Yates correction was used whenever applicable.

pared in order to determine whether the unions of any of the oligogenes from separate blocks have been favored by selection. In Table 4 the chromosome or gamete frequencies for each pair of genes are shown at three different times during the experiment. These times correspond to those given above for the gene frequency and are based on the same data: at Week 15 on the introductions and at Weeks 43 and 65 on the testcross data. These frequencies will be analyzed in the DISCUSSION to show whether any significant change has occurred in the populations.

The fitness measures: Fitness measures were taken on these populations during the course of the experiment. Figures 2 and 3 are graphic representations of the number and weight of adult individuals in the populations each week. The arrow marks the time of the introduction of the blocks. The huge initial increase of these populations in size is a common characteristic of many populations. They increase at a rapid rate until density dependent factors such as competition for food and space prevent overpopulation and are responsible for achievement of a steady state—an equilibrium (ODUM 1959). This huge initial burst in population size can be avoided by starting the populations with numbers closer to the apparent equilibrium size. At 11 weeks after the introductions, the fitness of the polymorphic populations becomes different from that of the homozygous controls. The increase in fitness is gradual and no dramatic peak is reached. After Week 47 the control populations differ and Population 23 becomes as high in fitness as the polymorphic populations, while the other control, Number 24, drops in fitness.

Because of this increase in fitness, Population 23 was considered a suspect for contamination by an individual from one of the polymorphic populations. However, none of the oligogene markers was ever seen in the developing population. Mass F_2 's of the flies emerging from vials which had been exposed to the population for one day were observed, and here again no evidence of contamination by the oligogene markers was observed.

The means and confidence intervals for number and weight are recorded in Table 5. These were calculated in two periods, each 18 weeks in duration. Period

TABLE 4

Chromosome (or gamete) frequencies of oligogene pairs calculated from one half the number of introductions at Week 15 and from testcross results at Weeks 43 and 65

Gene pair	Population 20			Population 21			Population 22		
	Week 15	Week 43	Week 65	Week 15	Week 43	Week 65	Week 15	Week 43	Week 65
+	.981	.897	.732	.981	.867	.724	.986	.868	.771
ss	.012	.039	.184	.012	.067	.182	.009	.105	.181
+	.007	.051	.052	.007	.067	.073	.005	.026	.043
ss	.000	.013	.032	.000	.000	.021	.000	.000	.005
+	.986	.833	.858	.985	.889	.839	.989	.947	.906
se	.007	.103	.058	.007	.045	.068	.005	.026	.032
+	.007	.064	.084	.007	.067	.088	.005	.026	.043
se	.000	.000	.000	.000	.000	.005	.000	.000	.005
+	.981	.872	.742	.981	.878	.755	.986	.895	.793
se	.007	.103	.058	.007	.033	.068	.005	.013	.032
+	.012	.026	.200	.012	.078	.172	.009	.079	.170
se	.000	.000	.000	.000	.011	.005	.000	.013	.005
+	.981	.885	.768	.981	.856	.714	.986	.895	.787
se	.007	.103	.058	.007	.022	.068	.005	.013	.032
+	.012	.013	.174	.012	.100	.214	.009	.092	.176
se	.000	.000	.000	.000	.022	.005	.000	.013	.005
+	.981	.936	.768	.981	.811	.698	.986	.868	.777
e ^s	.012	.000	.147	.012	.122	.208	.009	.105	.176
+	.007	.051	.058	.007	.067	.083	.005	.026	.043
e ^s	.000	.013	.026	.000	.000	.001	.000	.000	.005
+	.981	.846	.727	.981	.900	.729	.986	.868	.777
se	.007	.103	.058	.012	.045	.068	.005	.026	.037
+	.012	.051	.216	.007	.056	.198	.009	.105	.186
se	.000	.000	.000	.000	.000	.005	.000	.000	.000
+	.981	.910	.737	.981	.844	.740	.986	.882	.782
k	.012	.026	.179	.012	.089	.167	.009	.092	.170
+	.007	.064	.063	.007	.067	.083	.005	.026	.043
k	.000	.000	.022	.000	.000	.001	.000	.000	.005
+	.988	.962	.779	.988	.878	.766	.991	.895	.793
k	.000	.026	.047	.000	.000	.016	.000	.000	.027
+	.000	.013	.022	.000	.033	.057	.000	.013	.032
k	.012	.000	.153	.012	.089	.162	.009	.092	.149
+	.988	.949	.768	.988	.911	.766	.991	.882	.803
ss	.000	.026	.032	.000	.000	.057	.000	.026	.021
+	.000	.000	.016	.000	.022	.031	.000	.013	.011
ss	.012	.026	.184	.012	.067	.146	.009	.079	.165
+	.988	.949	.762	.988	.878	.714	.991	.882	.771
ss	.000	.039	.063	.000	.000	.068	.000	.013	.048
+	.000	.000	.022	.000	.056	.083	.000	.013	.043
ss	.012	.013	.153	.012	.067	.135	.009	.092	.138

FIGURE 2.—Size of five experimental populations of *Drosophila melanogaster* as determined by number of individuals in the adult population over a 59-week period. The arrow marks the time of introduction of the sesro blocks into these Oregon populations. The homozygous Oregon populations are Nos. 23 and 24. The populations polymorphic for the sesro blocks are Nos. 20, 21, and 22.

TABLE 5

Size of five experimental populations of Drosophila melanogaster during two time periods

Population no.	Period	Mean no. individuals per week	95 percent confidence interval	Mean wet weight (mg per week)	95 percent confidence interval
20	1	405.1	± 28.1	212.9	± 15.1
	2	430.2	± 21.7	232.7	± 10.5
21	1	380.9	± 33.0	194.6	± 15.1
	2	429.4	± 27.3	225.9	± 13.8
22	1	387.8	± 19.2	201.6	± 9.7
	2	452.5	± 33.3	233.1	± 14.6
23	1	291.0	± 17.8	155.4	± 9.2
	2	405.6	± 38.9	208.9	± 17.5
24	1	288.3	± 16.1	156.2	± 7.1
	2	258.8	± 15.0	139.2	± 7.0

Period 1 extends from Week 24 to Week 42. Period 2 begins at Week 42 and extends to Week 59.

FIGURE 3.—Size of five experimental populations of *Drosophila melanogaster* as determined by milligrams wet weight over a 59-week period. The arrow marks the time of introduction of the sesro blocks into these Oregon populations. The homozygous Oregon populations are Nos. 20 and 24. The populations polymorphic for the sesro blocks are Nos. 20, 21, and 22.

1 begins nine weeks after the introduction at Week 24 and extends to Week 42. Period 2 begins at Week 42 and extends to Week 59. At Week 34, no measurements were taken and at certain other weeks the numbers were not counted, but these means are based on at least 16 measurements. The means of each population are compared from one period to the next by the *t*-test of means in Table 6. The means of weight differ significantly from one period to the next in all five populations. All showed an increase with the exception of the control (No. 24), which decreased. Although these differences also were reflected in mean number, they were not significant in this measurement in Populations 20 and 21.

Pairs of these populations have been compared by the method of paired *t* for each time period. Out of the ten possible pairs, those six pairs considered to be most informative were chosen for analyses. These are listed in Table 7.

The experimental populations are not significantly different from each other at the one percent level in either period. However, in the first period Populations 20 and 22 differ in weight at the five percent level. The controls are similar in the first period but become significantly different during the second period. The increase of Control 23 is such that it does not differ significantly from experi-

TABLE 6

Comparison of means in two different time periods of each of five populations of Drosophila melanogaster

Population no.	$\bar{X}-\bar{Y}$	$s_{\bar{X}-\bar{Y}}$	t	P
Number				
20	25.1	16.66	1.508	> .1
21	48.4	20.28	2.388	> .02
22	64.7	17.65	3.665	< .001
23	114.6	19.71	5.816	< .001
24	29.5	10.41	2.832	< .01
Weight				
20	19.8	8.50	2.328	> .02
21	31.4	9.67	3.244	< .01
22	31.5	8.43	3.734	< .001
23	53.5	9.51	5.628	< .001
24	17.0	4.72	3.600	< .01

$\bar{X}-\bar{Y}$: Mean of the second period minus the mean of the first period.

$s_{\bar{X}-\bar{Y}}$: Standard deviation of the differences of these means.

t value: $(\bar{X}-\bar{Y})/s_{\bar{X}-\bar{Y}}$.

P: The probability of observing so great a difference between the means if the two means are equal.

mental Population 21 in the final period. However, Population 21 does differ from control Population 24 in both periods at the one percent level.

DISCUSSION

Certain investigators have dealt with the segregation of two pairs of genes. Among these are LI (1955) and LEWONTIN and KOJIMA (1960). The method which these investigators have used will be described and applied to the present data in the following paragraphs.

After many generations of random mating a state of linkage equilibrium should be reached so that one gene is random with respect to another. The state of linkage equilibrium can be described mathematically. For example, for the *ss* and *ro* pair, if the gamete frequencies are $++ = a$, $ss+ = b$, $+ro = c$, $ssro = d$, and the frequencies of the genes are $+^{ss} = p$, $ss = 1-p$, $+^{ro} = q$, $ro = 1-q$, then at linkage equilibrium certain conditions will be satisfied. The frequencies of each gamete will be equal to the product of the respective gene frequencies for that gamete such that $a = pq$, $b = (1-p)q$, $c = p(1-q)$, $d = (1-p)(1-q)$. It follows that the gamete frequencies will satisfy the equation such that $ad = bc$. In other words, the product of the frequencies of the gametes with the genes in the coupling phase will equal the product of the frequencies of the gametes with the genes in repulsion phase. This relationship may be shown also in the form of a gametic matrix set up in the following manner:

$$\begin{vmatrix} a & b \\ c & d \end{vmatrix} = ad - cb = D.$$

TABLE 7

Comparison of mean number and wet weight of adult individuals in five populations in two different time periods based on paired observation in six population pairs

Pair	Period	<i>n</i>	$\bar{d}$	$\bar{s}_d$	$\bar{d}/\bar{s}_d$	P
Number						
20-21	1	16	24.3	14.33	1.697	> .1
	2	16	.9	14.00	.062	> .9
22-21	1	17	6.9	11.25	.612	> .5
	2	16	26.2	15.81	1.660	> .1
20-22	1	16	18.2	8.76	2.077	> .05
	2	16	-22.2	13.36	1.665	> .1
21-24	1	17	92.6	14.05	6.594	< .001
	2	16	170.6	15.5	10.976	< .001
23-24	1	17	2.5	7.77	.318	> .7
	2	16	146.8	22.08	6.649	< .001
21-23	1	17	89.9	9.31	9.656	< .001
	2	16	23.6	20.02	1.180	> .2
Weight						
20-21	1	16	18.3	5.74	3.189	< .01
	2	18	6.8	6.02	1.126	> .2
22-21	1	17	7.1	4.55	1.550	> .1
	2	18	7.2	6.03	1.188	> .2
20-22	1	16	11.2	3.82	2.926	> .01
	2	18	-4	5.37	.072	> .9
21-24	1	17	38.4	5.48	7.009	< .001
	2	18	86.8	7.37	11.778	< .001
23-24	1	17	-9	3.40	.277	> .7
	2	18	70.3	9.52	7.385	< .001
21-23	1	17	39.2	3.55	11.046	< .001
	2	18	17.1	9.23	1.849	> .05

n: The number of observations.

$\bar{d}$: Mean difference.

$\bar{s}_d$: Standard deviation of mean difference.

t value: $\bar{d}/\bar{s}_d$.

P: The probability of observing so great a mean difference if the two means are equal.

When the genes are in linkage equilibrium the gametic determinant, D , will equal 0 and $ad = bc$.

Obviously, when the conditions of the above equation are not satisfied, the population is not in a state of equilibrium with respect to the particular pair of genes under consideration. The gametic frequencies will be different from those at equilibrium such that

$$D = \begin{vmatrix} a - D & b + D \\ c + D & d - D \end{vmatrix} = 0.$$

D has been defined by LEWONTIN and WHITE (1960) as a measure of linkage disequilibrium, and by LI (1955) as "an index of divergence of an arbitrary population from equilibrium." From the equation $ad - bc = D$, the sign of the D values can be explained. If D is positive, the product of a and d (the coupling

gamete frequencies) is greater than the product of c and b (the repulsion gamete frequencies). Conversely, if both the repulsion gamete frequencies are in excess, the D value will be negative. The maximum numerical value for D is .25. This would be obtained if, for example, $a = .5$, $b = 0$, $c = 0$, $d = .5$. If any of the values, a , b , c , or d , is equal to 0, then obviously the product of this value and its diagonal factor will be 0, no matter how high in value the diagonal factor may be. For example, see the $se\ k$ combination at Week 65 in Population 20 (Table 4). The $se\ k$ gamete frequency is 0, so the D value is negative even though there is a high frequency of the $++$ gamete.

When there is no evolutionary pressure such as selection, the D value will approach 0 with each generation. The number of generations which it will take for linkage equilibrium to be reached is dependent on the recombination frequency between the particular genes in question. If the genes are not linked, the D value will be halved each generation (LI 1955). If they are linked and R is the recombination value between them, then D_1 , the D value of the following generation, will be $(1-R)D_0$ where D_0 is the present D value. After n generations D_n , the D value at the n th generation will be equal to $(1-R)^n D_0$. Consequently, if two genes are closely linked it will take longer for them to reach equilibrium than two genes which are not as closely linked. For example, if $D_0 = .1$ and $R = .1$, then $D_1 = .09$, but if $D_0 = .1$ and $R = .5$, then $D_1 = .05$.

When there is selection, the D values will not necessarily approach 0 in the same way that they would if there were no selection. Predictions have been made about how the D values will respond to selection by LEWONTIN and KOJIMA (1960) and by KOJIMA and KELLEHER (1961). In order to apply these predictions, the adaptive values of the various genotypes must be known. The adaptive value is the relative probability that a zygote of a certain genotype will leave a zygote in the next generation (LEWONTIN and KOJIMA 1960). The experiments reported here were not set up in a manner that the adaptive value could be determined, and no additional experiments were performed to estimate it because it was unknown at the time of the experiment that these values would be of any use for the purpose of the experiment. In view of the possibility that these data may be further analyzed by some investigator working on the theoretical aspects of linkage disequilibrium, they are presented as completely as possible.

The chromosome frequencies can be looked at in another way—that is, to see whether one gene is random with respect to another. In STERN (1960), it is shown that the expected chromosome frequencies in respect to two genes on the same chromosome are equal to the product of the gene frequencies. This relationship is exactly what was stated above as one of the conditions which reveals a state of linkage equilibrium. According to STERN's method, data can be judged as to whether the genes are random or nonrandom on the basis of the chi-square test of goodness of fit. By comparing the observed with the expected chromosome frequencies based on the gene frequencies, the chi-square for each array of two genes can be calculated. In Table 8 these chi-square values are listed for each array at Week 65. According to this test for randomness, all the genes are random with respect to each other ($P > .1$) in all populations, with the exception of the

TABLE 8

Chi-square and P values of tests of goodness of fit of chromosome frequencies observed in the final testcrosses to those expected based on the gene frequencies at that time

Gene combination	Population					
	20		21		22	
	χ^2	P	χ^2	P	χ^2	P
<i>ss ro</i>	1.7	> .1	.04	> .8	.02	> .8
<i>se ro</i>	.2	> .5	.1	> .3	.1	> .3
<i>se k</i>	.2	> .1	.5	> .3	.1	> .8
<i>se e^s</i>	1.3	> .2	1.1	> .2	.1	> .7
<i>e^s ro</i>	1.4	> .2	.7	> .3	.3	> .5
<i>se ss</i>	2.0	> .1	.9	> .3	.6	> .3
<i>k ro</i>	.4	> .8	.2	> .5	.01	> .9
<i>k e^s</i>	109.8	≤ .0001	111.2	≤ .0001	115.1	≤ .0001
<i>ss k</i>	134.3	≤ .0001	93.7	≤ .0001	144.0	≤ .0001
<i>ss e^s</i>	98.9	≤ .0001	54.3	≤ .0001	86.9	≤ .0001

three genes in the central block, which are not random with respect to each other. As can be seen in Table 9, these same arrays also have much higher *D* values at this time. The *D* values for the random genes range from +.013 to −.017; while the *D* values for the nonrandom genes range from +.09 to +.14. On the basis of the chi-square results, the former *D* values can be considered to be insignificant values of linkage disequilibrium.

The *D* values from period to period cannot be compared. Selection has been acting; the gene frequencies have changed, and the genetic composition of the population has become different. Predictions about what the *D* value would be expected to become in this new situation cannot be made because the adaptive values are unknown. However, if the *D* values are considered as a point from

TABLE 9

D values, measures of linkage disequilibrium, calculated from the chromosome frequencies at three different time periods. See text

Genes	Population								
	20			21			22		
	Wk. 15	Wk. 43	Wk. 65	Wk. 15	Wk. 43	Wk. 65	Wk. 15	Wk. 43	Wk. 65
<i>ss ro</i>	−.00008	+.0097	+.0139	−.00008	−.0045	+.0018	−.00004	−.0027	−.0039
<i>se ro</i>	−.00005	−.0067	−.0049	−.00005	−.0030	−.0018	−.00002	−.0007	+.0032
<i>se k</i>	−.00008	−.0027	−.0116	−.00008	+.0071	−.0079	−.00004	+.0106	−.0015
<i>se e^s</i>	−.00008	−.0013	−.0101	−.00008	+.0166	−.0110	−.00004	+.0104	−.0017
<i>e^s ro</i>	−.00008	+.0122	+.0117	−.00008	−.0082	−.0166	−.00004	−.0027	−.0037
<i>se ss</i>	−.00008	−.0052	−.0125	−.00008	−.0025	−.0098	−.00004	−.0027	−.0069
<i>k ro</i>	−.00008	−.0167	+.0049	−.00008	−.0060	−.0131	−.00004	−.0024	−.0034
<i>k e^s</i>	+.01186	−.0003	+.1182	+.01186	+.0781	+.1231	+.00892	+.0823	+.1173
<i>ss k</i>	+.01186	+.0247	+.1408	+.01186	+.0610	+.1166	+.00892	+.0693	+.1328
<i>ss e^s</i>	+.01186	+.0123	+.1154	+.01186	+.0588	+.0907	+.00892	+.0810	+.1039

which equilibrium would be reached if there were no selection, certain statements might be made. The number of generations for each array to reach equilibrium can be calculated. This calculation is a refinement of the formula given above, $D_n = D_0(1-R)^n$, because there is no crossing over in male *Drosophila*. R is replaced by $(e + f)/2$, where e is the crossover value for females and f the value for males. In this case R will be replaced by one half its former value, as $f = 0$. The number of generations needed for the closely linked genes, spineless, kidney, and ebony, to reach equilibrium in the beginning of the experiment is less than at its termination. For example, at the time of the introductions in Population 20, the disequilibrium is such that it would take ss and k eight generations to reach an approximate state of equilibrium ($D = .009$). At the experiment's termination the linkage disequilibrium is so extensive in Population 20 for ss and k that it would take approximately 88 generations for them to reach linkage equilibrium. Merely because the D values have become significant, it cannot be concluded that the association has become tighter. There has been an increase in frequency of the repulsion chromosome of the central block genes. Obviously, the linkage is breaking down. The writer knows of no way to predict in how many generations from the beginning of the experiment these genes would be expected to reach equilibrium in this complicated situation of changing gene frequencies.

Although the meaning of the change in D values cannot be dealt with here because of the changing gene frequencies, certain characteristics about the D value can be pointed out by considering these data. By referring to the chromosome frequencies of the $ss\ k$ array in Population 20 at Week 15 and Week 65 (Table 4), it would appear that the increase in disequilibrium has occurred because selection favored the increase of the $ss\ k$ block. It increased from $+.012$ to $+.184$. Accompanying the increase of the $ss\ k$ block was a decrease in frequency of $++$ so that the product of the coupled chromosomes would be higher as their values approached $.5$ than if they remained at extremes. This effect was not counteracted by the increase of the repulsion chromosomes. Although the number of repulsion chromosomes was increasing, the D value is not much affected by these increases. The D value is much more affected by the approach to equality of the coupling chromosomes. The increase in frequency of the central block genes has caused the disequilibrium as measured by D to increase. Selection has favored the central block genes. The breakdown of the coupling unions of these genes is occurring, but the magnitude of this effect as compared to the great increase in frequency of the coupled phase is very small when the D value is used. It may be suggested that in this case the D value is not a good measure of linkage disequilibrium because its magnitude is so strongly influenced by the increase in frequency of the central block genes.

These data might be compared to the results of others on the association of inversions in natural populations. CARSON and STALKER (1949), LEVITAN and SALZANO (1959), and STALKER (1960) have shown that certain inversions on the same chromosome are nonrandomly associated in nature. This phenomenon has been attributed to the nonadaptedness of certain combinations. Selection has

favored certain combinations and not others. With respect to the terminal oligogenes, sepia and rough, and the association between them or associations between them and oligogenes of the central block, there is no evidence that selection has favored certain combinations. The insignificant *P* values of the chi-square results and the low *D* values verify this. However, the genes of the central block are associated nonrandomly with each other.

The nonrandomness of the central block genes may be due to one of two factors. The tight linkage of these genes as compared to the loose linkage of the terminal genes might be such that there has not been enough time for sufficient crossing over to occur and result in their becoming random with respect to each other in this complicated situation of changing gene frequencies. On the other hand, there may be an epistatic interaction which holds these central genes together. LEWONTIN and KOJIMA (1960) have dealt with a hypothetical relationship of two genes and have concluded that "if linkage is tighter than the value demanded by the magnitude of epistatic deviation there may be permanent linkage disequilibrium and the gene frequencies may be affected." The linkage of the central genes may be tight enough to be affected by the epistasis; whereas, the magnitude of the epistasis may not be great enough to hold the loosely linked terminal genes in a nonrandom association. The possible epistasis may have been effective in producing an overdominance of the tightly linked central block genes, and this may have caused them to increase in frequency in accordance with the theory of BODMER and PARSONS (1962). Unfortunately, the writer's data are not extensive enough to conclude just which of these alternatives is responsible for the nonrandom association of these central genes.

Since the whole sesro genome was probably introduced into these populations (Figure 1), the fitness pattern might be compared to results of CARSON (1961) where he introduced sesro genomes into Oregon populations. In his populations, the sesro chromosome was left intact; the genes were introduced in coupling. In these experiments, three separate blocks of oligogenes were introduced in repulsion.

In two of CARSON's populations, mutant individuals had appeared within three weeks and the fitness increased at a rapid rate, while in one population there was a lag in incorporation of the oligogenes into the gene pool which was accompanied by a lag in the fitness increase. In these block populations, the incorporation of the oligogenes was very slow, and it was not until six months after the introductions that homozygotes for all the mutant genes were observed in all three populations. Gene frequencies calculated from testcross analyses also show that the incorporation of the oligogenes was very slow. Here the rise in fitness was a gradual one—beginning 11 weeks after the introduction and continuing for approximately 26 weeks. Apparently, the intact chromosome once accepted by the population produced a more immediate rise in fitness than the blocks.

The gene frequencies observed at the end of the experiment at about 50 weeks after the introductions can be compared to those of CARSON for the same duration of time. He observed a low frequency of spineless, ten percent, while the terminal

genes, sepia and rough, were at higher frequencies, 25 percent and 43 percent respectively. In the block experiments, the terminal genes never reached a frequency of more than ten percent while the three genes of the central block, spineless, kidney, and ebony, ranged from 17 to 22 percent.

When the chromosome was introduced in a different state, all coupling versus some repulsion and some coupling, the results of selection were different. The terminal genes were selected in the former, and the central block genes in the latter. Thus, although the initial genetic raw material in these two sets of experiments and the end results as revealed by these fitness measurements are similar, selection has produced populations of different genetic compositions which brought about the end result, the fitness increase.

SUMMARY

When a multiply marked third chromosome (sesro) is introduced into Oregon populations in three separate blocks, a random association of these blocks ensues. However, selection favors the increase in frequency of the central block genes which are nonrandom with respect to each other. The nonrandom association may be due to an epistatic relationship between these three central genes or to the tenacity of close linkage in holding on to the combinations.

The fitness of the polymorphic populations possessing the oligogene block introductions increases at a slow rate and seems to follow a slow rate of incorporation of the oligogenes into the populations.

The frequency at which the sesro genes have become balanced is different when they are introduced as blocks in the repulsion phase than when they are introduced in the coupling phase into Oregon populations.

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