THE EFFECTS OF HETEROZYGOSITY AND RECOMBINATION ON THE RELATIVE FITNESS OF EXPERIMENTAL POPULATIONS OF *DROSOPHILA MELANOGASTER*

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IN nature, evolution is most likely taking place on the interpopulational level. Certain populations are better able to adapt to environmental changes than others. By comparing populations as a whole, one could attempt to find out what makes one population more successful in the evolutionary sense than another. BUZZATI-TRAVERSO (1952) emphasized this interpopulational problem when he stated that the most fit population is one which can best exploit the available space and energy sources. The method was developed by CARSON (1958) on experimental populations. He used population size as a measure of the relative populational fitness by comparing genetically different populations exposed to uniformly controlled environmental conditions. "The population which maintains the larger relative size is judged to be performing better from the over-all biological point of view." (CARSON 1961).

The experiments of CARSON have shown that the introduction of a foreign genome into homozygous populations is followed by a very rapid enhancement of the relative population fitness; the populations trebled in size within three generations. Accompanying this change in population size was a change in the genetic composition of the population. A condition of balanced polymorphism ensued, apparently as a result of natural selection. The rapidity of the increase in fitness following the introduction of the foreign genome has led CARSON to the suggestion that the cause for the increase must be a simple heterosis requiring no coadaptive integration.

The experiments reported here have utilized *Drosophila melanogaster*. They were designed to probe further into the populational phenomena observed by CARSON. The first experiment consists of five populations founded with individuals from a second-chromosome mutant stock. Previously, all the experiments of CARSON had been performed with a particular stock marked by third-chromosome mutants. Thus, this second-chromosome mutant stock was chosen in order to ascertain whether there was something unique about the third chromosome or the particular stock which CARSON had used, which produced the rapid increase in fitness. The experimental procedure was identical to that of CARSON so that the performance of these second-chromosome mutant populations with the addition

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of the Oregon genome could be compared to the previous experiments using the third chromosome.

The second experiment was designed to determine whether the increase in fitness observed by CARSON was due to a simple heterosis or whether some coadaptation might have occurred. Since coadaptation may require free recombination to provide the many new gene combinations, one or more of which might be captured by selection to increase the population fitness, the possibility of the occurrence of coadaptation could be limited in a population by preventing crossing over. Accordingly, inversions extending over most of the second and third chromosomes were introduced into two homozygous third-chromosome mutant populations. These contained a system of balanced lethals which prevented crossing over and consequently limited coadaptation. They were compared to two similar populations which differed in that recombination was not prevented.

The third experiment was aimed at finding out what level of fitness could be maintained by homozygous third-chromosome mutant populations when the only chance for heterozygosity was the second chromosome. Here, two different foreign second chromosomes were introduced, one by one, into third-chromosome mutant populations.

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-TRAVERSO (1955) and modified by CARSON (1958). The chamber in which the populations are housed is similar to that described by CARSON (1961). It consists of (1) a 2.5×9.5 cm. glass shell vial, or food vial, and (2) a cellulose acetate tube, or supervial, twice the length of the shell vial. At regular intervals the food vial is removed from the supervial and is replaced by a fresh food vial. The used food vial is set aside among other such vials and now becomes part of what might be called the developing population. The developing population consists of food vials each of which has been exposed to the adult population for a two-day interval. Each population is run on a weekly cycle which is divided into the following periods: 2-day, 2-day, 2-day, and 1-day. The one-day vials are kept separate for further fitness tests. Flies which emerge from the developing population (the two-day vials) are added to the population chamber at each vial change. Competition, thus, does not occur between the developing flies and the adult individuals of a population, except for (1) the short interval after eggs are laid and the vial has not yet been replaced, and (2) the time before collection when there are newly emerged flies in the developing population. However, the adult individuals and the larvae, respectively, are exposed to stringent competition in most populations. Food, and frequently space, is in limited supply. This is felt most strongly among the developing stages of a large population, where the food mixture soon appears to be packed with larvae. In a small population, competition among larvae is less rigorous and there is ample food and space.

The temperature of the laboratory was maintained at $25^{\circ}\text{C} \pm 1^{\circ}$.

Measurements were taken at exact intervals to ascertain the fitness, or biological performance, of the populations. The adult population size was measured weekly by weighing the entire population under ether and then counting the living individuals. The weighings were made on a Roller-Smith microtorsion balance which has a capacity of 500 milligrams.

THE EXPERIMENTS

I. Introduction of Oregon genome into homozygous second-chromosome mutant (apl) populations: The stock used in these experiments is known as *apl*. It carries seven oligogenes, *aristaless* (*al*), *dumpy* (*dp*), *black* (*b*), *purple* (*pr*), *curved* (*c*), *plexus* (*px*), and *speck* (*sp*), in homozygous condition on the second chromosome. In the *apl* stock the genes on the other chromosomes are all wild-type. Five populations were started with 120 *apl* stock flies each on March 5, 1960. Sexes were not counted separately.

Six weeks later, when an apparent equilibrium in population size had been reached, single males heterozygous for the Oregon-R (from stock No. a4 of Indiana University) and *apl* genomes were introduced into three of these populations, namely, Numbers 1, 2, and 3. These males were F_1 progeny of an *apl* female and an Oregon male and had the chromosomal constitution: $X^{ap1} Y^{or}$, $II^{ap1} II^{or}$, $III^{ap1} III^{or}$, $IV^{ap1} IV^{or}$. Two *apl* populations, Numbers 4 and 5, were kept without introductions as homozygous controls.

The weekly measures of wet weight and of number of adults surviving in all five populations are recorded graphically in Figures 1 and 2. Arrows mark the time of introduction of the Oregon genome into populations 1, 2, and 3. For comparison, the measurements of an Oregon population, Number 24, which was running at this same time as a control for another experiment, is also recorded. This Oregon population was inbred and structurally homozygous and, therefore, may be presumed to be relatively homozygous genetically. A comparison of these graphs shows how closely wet weight is correlated with the number of individuals.

Within two weeks after the introductions, wild-type F_1 offspring from the single introduced males were observed in each of the hybrid populations. In four more weeks, the experimental populations began to increase rapidly in size while the controls showed no such change; in fact, a gradual decline was observed. The rapid mode of increase continued for a three-week period until a peak size was reached. In ensuing weeks, populations 2 and 3 fluctuated around this peak and even surpassed it but population 1 declined and remained close to the level of the homozygous Oregon population (number 24).

The mean and confidence intervals for each population, calculated on a 22-week period after the peaks were reached (weeks 14 through 22), are listed in Table 1. An analysis of paired *t*, performed by pairing the weight measurements during this 22-week period and calculating the *t* values of the mean of the differences between the weekly measurements, is shown in Table 2. The mean difference between each of these population pairs is significant at the one percent

FIGURE 1.—Size of five experimental populations as determined by milligrams wet weight over a 35-week period. The arrow indicates time of introduction of single heterozygous males. The homozygous apl populations are Nos. 4 and 5. The polymorphic populations are Nos. 1, 2, and 3. No. 24 is a homozygous Oregon population.

TABLE 1

Size of experimental populations during 22 consecutive weeks at equilibrium

Population	Mean of weekly number of individuals	95 percent confidence intervals	Mean of weekly population wet weight (mg)	95 percent confidence intervals
1	311.4	± 32.0	161.1	± 14.9
2	421.5	± 26.4	212.9	± 11.5
3	485.4	± 16.8	251.0	± 8.6
4	14.4	± 6.6	11.7	± 5.2
5	51.1	± 19.6	31.4	± 10.2
24	292.0	± 14.6	158.6	± 7.0

level, with the exception of populations 1 and 24. The differences between the hybridized populations and the homozygous apl populations are much greater than the differences between replicates. The mean difference in weight between the hybrid and the apl populations is at least 129.7 and at most 239.3 milligrams.

FIGURE 2.—Size of five experimental populations as determined by total numbers of flies surviving in the adult population each week. The arrow indicates time of introduction of single heterozygous males. The homozygous *apl* populations are Nos. 4 and 5. The polymorphic populations are Nos. 1, 2, and 3. No. 24 is a homozygous Oregon population.

TABLE 2

Comparison of mean wet weights and six populations, based on paired observations in seven population pairs

Pair	n	$\bar{d}$ (mg)	s_d	$t = \bar{d}/s_d$	P
2-1	22	51.7	7.18	7.209	<.001
3-2	22	38.0	5.35	7.113	<.001
3-1	22	89.9	9.45	9.513	<.001
5-4	22	19.7	6.56	3.002	<.01
1-5	22	129.7	9.13	14.211	<.001
3-4	22	239.3	5.90	40.537	<.001
1-24	21	3.0	7.49	0.407	>.6

n, number of observations; $\bar{d}$, mean difference in milligrams; s_d , standard deviation of mean difference; $\bar{d}/s_d$, the *t* value; P, the probability of observing so great a mean difference if the two mean wet weights are equal.

Although the controls dwindled to as low as two adult individuals, the populations did not become extinct.

The marker genes dropped in frequency following the male introductions. The gene frequencies of two of these genes, dumpy and curved, are plotted graphically for each polymorphic population in Figure 3. These frequencies were calculated on different data in the three successive time periods and hence are not strictly comparable from one period to the next. For weeks 12 to 18, the values are the square roots of the phenotypes observed in newly emerged flies. At week 32, the value is the square root of the phenotypes recorded surviving in the population during the weekly count. Following this, for a thirteen-week period, selection was made more rigorous by decreasing the amount of food and space. At week 45, all males were removed from both the developing population and the adult population, and were testcrossed. The gene frequencies calculated from these testcrosses constitute the last points on the graph in Figure 3. The gene frequencies for all seven oligogenes on the basis of these final testcrosses are shown in Table 3. All the oligogenes remained at substantial frequencies in the populations and not one oligogene was eliminated.

FIGURE 3.—Frequency of mutant genes, dumpy and curved, as measured by square root of phenotype frequencies observed at eclosion from the pupa (weeks 12 to 18), square root of phenotype frequencies observed in adult population (week 32) and absolute frequency based on test-cross results (week 45). The arrow indicates the time of the heterozygote introductions. Sample sizes ranged from 200 to 500 except for week 45.

TABLE 3

Gene frequencies calculated from genotypes observed in test crosses of all males emerging from the developing population and surviving in the adult population at week 45

Population Number	n	Gene frequency						
		<i>al</i>	<i>dp</i>	<i>b</i>	<i>pr</i>	<i>c</i>	<i>pz</i>	<i>sp</i>
1	26	0.154	0.154	0.250	0.269	0.116	0.135	0.269
2	28	0.162	0.268	0.358	0.275	0.107	0.090	0.178
3	26	0.132	0.254	0.424	0.462	0.116	0.250	0.308

At about the time (week 26) the rise in fitness was observed in population 5 (Figures 1 and 2), some flies were observed which were wild-type at the dumpy locus only. The adjacent loci *aristaless* and *black*, which lie 13 and 35.5 map units away respectively, were homozygous. Unfortunately, these populations were discarded before a progeny test could be done. However, the following postulations might be made about them. If these nondumpy flies were the result of a contamination, they would be the result of the union of gametes which carried homologous chromosomes both of which were the product of double crossovers in the same region. It seems unlikely that many such phenomena could have occurred in the 14 weeks the experimental populations had a chance to form recombinants, and that such an individual could have contaminated population 5 is also unlikely because extreme care was exercised to prevent any contamination. According to E. A. CARLSON (personal communication), the dumpy phenotype is not as pronounced and "overlaps" with wild type at room temperature (16 to 22°C), and also in overcrowding. However, population 5 was not overcrowded, and the laboratory temperature was 25°C. The optimum temperature for the expression of the dumpy phenotype is 28°C. Thus, temperature could have played a role in this phenomenon. Another possibility suggested by CARLSON is the accumulation of modifying genes which normalized the dumpy phenotype. This latter alternative seems most probable because no normalization of the dumpy phenotype occurred in the replicate population which was exposed to the same temperature and a similar degree of crowding. A unique genetic event must have occurred in the genetic background of one population which normalized the dumpy phenotype. This event appears to be correlated with a rise in population fitness.

In this experiment, although the fitness measure among replicates differed significantly, the differences between the hybrid populations and the *apl* controls were much greater. However, one hybrid population did not differ significantly from the homozygous Oregon population. A comparison of genotype frequencies among all the hybrid populations revealed a striking similarity in the genetic constitution of these populations on the basis of the seven oligogenes in testcross results.

II. Introduction of Curly and Payne inversions into homozygous third chromosome mutant (sesro) populations: The stock used for the base populations in these experiments is known as *sesro*. It carries five oligogenes, *sepia* (*se*), *spineless* (*ss*),

the sooty allele of ebony (e^s), kidney (k), and rough (ro) on the third chromosome.

Seven populations were established. Three of these populations, Numbers 6, 7, and 8, were begun on February 2, 1959 with 120 sesro flies each. These were not stock flies; they were sesro flies from experiments reported in CARSON (1961) and had consequently been exposed to the vial population system. A contamination was found in one of these (Number 8) on March 3, 1959 and the population was discarded. On this same day four more sesro populations, Numbers 9, 10, 11, and 12, were begun. These differed from the previous three in that sesro stock flies were used as the founders. These flies had not previously been exposed to the population system; they had been kept in stock bottles in the laboratory.

On March 10 and April 15, single males heterozygous for the Curly and Payne inversions and the sesro stock chromosomes were introduced into populations 6 and 10, respectively. These males were F_2 progeny from crosses of Curly females and Payne males. The particular Curly used was *Ins 05* from Bloomington, Indiana, Stock j80 which was supplied through the courtesy of Dr. H. J. Muller. It was produced through irradiations carried out by OSTER and contains at least three inversions, which effectively prevent crossing over throughout the second chromosome except for rare crossovers at the extreme right and left ends, beyond the limits of the left-arm and right-arm Curly inversions (MULLER, personal communication). The exact chromosomal constitution of the j80 Stock is *S Sp cn/dp^{ts1}Cy, Ins05 pr cn²; ru h InsCXF/Mé, Ins ri Sb¹*. The Payne male was from a Dichaete Stubble stock and its chromosomal constitution was $+/+; D_3Sb ca/Payne$. The Payne inversions are located on the right and left arms of the third chromosome. Crossing over is reduced in this chromosome to three in 30,000 (BRIDGES and BREHME 1944). Both the Curly and Payne inversions are homozygous lethal. Among the F_1 progeny of this cross, a Curly, Stubble, Moiré male was picked up. Care was taken to make sure that it was not Dichaete, assuring that it must carry the Payne inversion. This male was crossed to sesro virgin females and a Curly, not Stubble, Moiré male was used as the introduction. This series of crosses was made twice, once for each introduction.

At the same time the inversion introductions were made, single males heterozygous for the Oregon and sesro genomes were introduced into populations 7 and 11. These differed from the inversion introductions in that free recombination could occur, and consequently, populations 7 and 11 are referred to as the recombining populations. In contrast, the inversion populations are called nonrecombining populations, although it is only intrachromosomal, and not interchromosomal, recombination which is limited. These heterozygous males were the F_1 progeny of an Oregon male and a sesro female. Two sesro populations, Numbers 9 and 12, were kept homozygous without introductions as controls.

Population sizes in milligrams wet weight in adult populations 6, 7, and 9 are summarized graphically in Figure 4. The measurements for the replicate experiment, which was performed one month later, are summarized in Figure 5. Because these experiments were performed at different times and with different

FIGURE 4.—Size of three experimental populations as determined by milligrams wet weight over a 33-week period. The arrow indicates the time of introduction of single heterozygous males. No. 6 is the nonrecombining population, polymorphic for inversions Curly and Payne. No. 7 is the recombining population, polymorphic for the Oregon genome. No. 9 is the homozygous sesro population which was retained as a control.

FIGURE 5.—Size of three experimental populations as determined by milligrams wet weight over a 33-week period. The arrow indicates the time of introduction of single heterozygous males. No. 10 is the nonrecombining population, polymorphic for inversions Curly and Payne. No. 11 is the recombining population, polymorphic for the Oregon genome. No. 12 is the homozygous sesro population.

flies, population-exposed as opposed to stock flies, they are not strictly comparable and are dealt with separately.

For a period of seven weeks following the introductions, populations 6 and 7 increased in size until a peak size was reached. Although they decreased in size slightly following the peak, the cause for the decrease was probably environ-

mental as the control also was reduced in size at this time. Both the non-recombining (Number 6) and the recombining (Number 7) populations remained well above the control for the duration of the experiment.

At week 25, the Curly phenotype was no longer observed in the inversion population (Number 6). Thus, the second chromosome became homozygous for the sesro-stock chromosome, leaving the third chromosome as the main source of heterozygosity.

In the replicate experiment the size of the recombining population, Number 11, showed a rapid increase three weeks after the introduction. It reached a peak by seven weeks and then decreased in size. This decrease may have been a fluctuation which appears as a common characteristic of such populations because it increased again near the end of the experiment. The nonrecombining population (Number 10) never reached a very high peak in size, and it remained at this level throughout the experiment. At week 25, the Curly phenotype became extinct in this population, as it had in the replicate.

The mean and confidence intervals for each of these six populations, calculated on the 20-week period after the peaks were reached, are listed in Table 4. An analysis of paired *t*, performed by pairing the weekly wet weights in milligrams during the 20-week period, is shown in Table 5. The population pairs analyzed

TABLE 4

Size of six experimental populations during 20 consecutive weeks at equilibrium

Population Number	Mean of weekly number of individuals	95 percent confidence level	Mean of population wet weights (mg)	95 percent confidence level
6	221.4	± 13.5	105.3	± 6.3
7	225.2	± 12.0	118.2	± 6.1
9	89.8	± 6.4	57.2	± 4.1
10	190.4	± 8.4	89.7	± 4.0
11	251.2	± 25.4	133.2	± 12.2
12	99.2	± 8.7	61.0	± 3.9

TABLE 5

Comparison of weekly wet weights in six populations based on paired observations in four population pairs

Pair	n	$\bar{d}$	s_d	$t = \bar{d}/s_d$	P
7-6	20	12.9	3.26	3.962	$<.01$
6-9	20	48.2	3.35	14.366	$<.01$
11-10	20	43.6	6.15	7.086	$<.01$
10-12	20	28.7	2.66	10.778	$<.01$

n, number of observations; $\bar{d}$, mean difference in milligrams; s_d , standard deviation of mean difference; the *t* value equals $\bar{d}/s_d$; P, the probability of observing so great a mean difference if the two mean wet weights were equal.

are those which were running at the same time period (not replicate populations). The measurements of the nonrecombining populations (Numbers 6 and 10) fall between those of the recombining (Numbers 7 and 11) and the controls (Numbers 9 and 12). The difference between these pairs is significant at the one percent level.

Replicate experiments are compared by the *t* test between means for wet weights in Table 6. The means of the nonrecombining populations (Numbers 6 and 10) are significantly different from each other. The means of the recombining populations (Numbers 7 and 11) are significantly different from each other at the five percent level. The means of the control populations do not differ significantly.

The significant difference between experimental replicates in this experiment may be connected with the fact that they were constituted from different founders. Populations 6 and 7 were constituted from sesro flies which had been exposed to the rigorous population system in the experiments of CARSON. Populations 10 and 11 were formed from sesro stock flies. These had been kept in bottle cultures. The population environment may have imposed a higher degree of inbreeding and a different type of selection (one for the population system as compared to the bottle environment). Also these replicates were run at different times (one month apart).

In the nonrecombining populations, there was a rapid change in phenotype frequency following the male introductions. Figure 6 shows the rapid decrease in number of sesro individuals and the rise in frequency of individuals carrying the Payne inversion; these latter were phenotypically wild-type. Curly individuals were as frequent as 18 percent, and then were eliminated in both populations. The chromosome frequencies in these populations are shown in Figure 7. By 12 weeks after the introduction an equilibrium of the third chromosome had been established at about 52 percent sesro chromosomes in both populations. In spite of the lethality of the Payne chromosome when homozygous, the frequency of this chromosome is maintained at 48 percent owing to the superiority of the heterozygote. On the other hand, the second-chromosome inversion, Curly, appears not to have had advantage in the heterozygous condition, and it has been replaced by the second chromosome of the sesro stock.

TABLE 6

Comparison of mean wet weights in six populations based on the means

Pair	n	$\bar{X}-\bar{Y}$	$s_{\bar{X}-\bar{Y}}$	<i>t</i>	P
6-10	20	15.6	3.59	4.360	<.01
11-7	20	15.0	6.50	2.308	<.05
12-9	20	3.8	2.71	1.404	>.10

n, number of observations; $\bar{X}-\bar{Y}$, difference between the means in milligrams; $s_{\bar{X}-\bar{Y}}$, standard deviation of this difference; the *t* value equals $(\bar{X}-\bar{Y})/s_{\bar{X}-\bar{Y}}$; P, the probability of observing so great a difference if the two mean weights were equal.

FIGURE 6.—Phenotype frequencies in the nonrecombining adult populations (Nos. 6 and 10) and in the recombining adult populations (Nos. 7 and 11) over a 33 to 35-week period. The arrows indicate the time of introduction of single heterozygous males. Recombinants refer to those individuals which are homozygous for one or two, but not all, of the mutant genes, *sepia*, *spineless*, and *rough*. Sample sizes ranged from 150 to 350.

In the recombining populations (Numbers 7 and 11), the change in phenotype frequency resulting from the introduction is shown in Figure 6. Homozygous *sesro* individuals are rapidly replaced by individuals which are phenotypically wild-type. With time, a number of recombinants are formed and increase in frequency. Recombinants here refer to those individuals which are homozygous for any of the genes, *sepia*, *spineless*, or *rough*, but not all three of them. Individuals homozygous for all three genes fall into the *sesro* class.

In Figure 8, frequencies of the three oligogenes are summarized graphically. These were calculated by taking the square root of the phenotype frequencies observed in the total adult population. In both populations, *rough* is the most frequent at about 48 percent while *sepia* is around 25 percent. The *spineless* gene is the least frequent, and it appears to have been eliminated in population 11, but testcrosses showed that it had not been eliminated (CANNON 1962).

This experiment has shown that non-recombining populations display a similar (but somewhat lower) fitness pattern to freely recombining populations. Owing to the elimination of *Curly*, the second chromosome has become homozygous. In both nonrecombining and recombining populations, a balanced polymorphism was established with the *sesro* oligogenes stabilized at substantial frequencies.

FIGURE 7.—Frequencies of second chromosome (Curly) and third chromosome (sesro) in the nonrecombining populations over a 27-week period, calculated from the phenotypes observed in the adult populations. The arrows indicate the time of introduction of the single heterozygous males. Sample sizes ranged from 150 to 300.

III. *Introduction of apl and Oregon-second chromosomes into homozygous sesro (third chromosome) populations:* The stocks used in these experiments are sesro, apl, and Oregon. On January 24, 1960, four populations (Numbers 13, 14, 15, and 16) were formed, with 120 sesro stock flies each. At later dates three more sesro populations were founded. The last two of these (Numbers 18 and 19) were each composed of 180 flies, because this number was judged to be closer to equilibrium size.

When an apparent equilibrium size had been reached, approximately 16 males and females, each of which had a probability of 0.5 of being heterozygous for the apl second chromosome, were introduced into each of populations 13, 14, 15, 16, and 17, over a two-week period. These flies were obtained from a series of crosses performed in the following manner. First sesro virgin females were crossed to apl males. The F_1 males were backcrossed to sesro females. Among the backcross progeny sesro males were picked up. Each of these was crossed twice, first to apl virgin females and then, three days later, to sesro virgin females. If the first cross produced any apl progeny, then the progeny from the second cross for that particular male was used for introduction. Even then, there was only a probability of 0.5 that a single introduced fly would carry the apl chromosome; consequently several were introduced. Introductions were successful as indicated by the ap-

FIGURE 8.—Frequency of mutant genes *sepia* (*se*), *spineless* (*ss*), and *rough* (*ro*), as measured by square root of phenotypes observed in the adult population over a 20 to 23-week period. The arrows indicate time of introduction of the single heterozygous males. Sample sizes ranged from 200 to 350.

pearance of second-chromosome mutants in each population. The crosses were set up so that the *apl* chromosome could not have been broken by recombination before the male introductions were obtained; both the F_1 and F_2 heterozygotes were males, which are not subject to crossing over in *Drosophila*.

Twenty-three weeks after the first introduction, a second introduction was made into populations 14, 15, and 19. This was the introduction of a second chromosome from the Oregon stock. To obtain this introduction, *sesro* virgin females were crossed to Oregon males. Then the F_1 males were backcrossed to *sesro* females. *Sesro* males among the backcross progeny were picked up, and nine of these were added to each of the populations. Each male had a probability of 0.5 of carrying the Oregon chromosome, and the chance that not one of the introduced males per population carried the chromosome is $(1/2)^9$. Neither the *sesro* nor the Oregon second chromosome is marked by oligogenes, so it is impossible to distinguish between these two in the populations.

These introductions were arranged so that the third chromosome remained homozygous for *sesro* in all the populations. The fourth chromosome was uncontrolled. Both *apl* and Oregon Y-chromosomes were introduced, whereas the X chromosome was from the *sesro* stock only. The main source of genetic variability was the second chromosome. In contrast to the control population, Number 18, which was homozygous for the *sesro* second chromosome, all the other populations had at least two genetically different second chromosomes. Populations 13, 16, and 17 had *sesro* and *apl*; population 19 had *sesro* and Oregon; and populations 14 and 15 had all three: *sesro*, *apl*, and Oregon.

In Figure 9 the weights of these seven populations are shown in two separate graphs so that the populations can be distinguished more easily. Here again the populations are barely distinguishable, and it is not until well after the Oregon introductions that some differences appear. The similarity of these genetically differing populations is striking.

The means and confidence intervals for number and weight of these populations are listed in Table 7. They were calculated for two different periods. Period 1 begins nine weeks after the first introduction and extends over an interval of 15 weeks until the second introduction was made. The second period begins nine weeks after the second introduction and extends over an interval of 24 weeks, at which time the experiment was terminated. The periods were begun nine weeks after the introductions so that they would be comparable to the previous experiments, when the periods were begun after peak size had been reached. Peak size

FIGURE 9.—Size of seven experimental populations homozygous for the sesro third chromosome as determined by milligrams wet weight over a 69-week period. Arrows mark the successive sets of foreign second chromosome introductions: first *apl* and then Oregon. The homozygous sesro population is No. 18—|. The populations polymorphic for *apl* and sesro second chromosomes are No. 13—, No. 16—, and No. 17—|. The population polymorphic for Oregon and sesro second chromosomes is No. 19—|. The populations polymorphic for Oregon, *apl*, and sesro second chromosomes are No. 14— and No. 15—.

in those experiments was usually reached at eight weeks after the introductions were made.

The means of the weights of each population are compared from one period to the next by the *t* test of the means, shown in Table 8. The mean of the control sesro population dropped significantly from one period to the next. One of the populations with only the apl introductions (Number 17) differed in weight, while the other two replicate populations (Numbers 16 and 13) did not differ from one period to the next. The period means of the sesro population with only

TABLE 7

Size of seven experimental populations during two time periods at equilibrium

Population No. and description	Period	Mean of weekly number of individuals	95 percent confidence interval	Mean of weekly population wet weights (mg)	95 percent confidence interval
18 (....)	1*	158.3	± 19.9	95.5	± 10.6
(....)	2†	129.4	± 13.8	76.7	± 6.4
13 (apl)	1	184.3	± 15.4	103.6	± 7.0
(....)	2	196.0	± 14.5	107.9	± 6.8
16 (apl)	1	156.5	± 15.7	95.8	± 7.4
(....)	2	188.3	± 12.6	102.4	± 5.6
17 (apl)	1	129.0	± 13.3	82.9	± 6.4
(....)	2	182.5	± 13.4	106.5	± 6.0
19 (....)	1	154.9	± 13.5	91.5	± 6.2
(Oregon)	2	153.3	± 14.3	98.4	± 7.2
14 (apl)	1	193.3	± 7.1	105.1	± 3.2
(Oregon)	2	247.8	± 14.8	131.8	± 6.4
15 (apl)	1	197.5	± 7.5	111.1	± 3.7
(Oregon)	2	215.8	± 11.1	118.2	± 5.4

* Period 1 is 15 weeks.

† Period 2 is 20 weeks for number and 24 weeks for weights. (Number measurements were not taken for two 2-week intervals in period 2.) Second chromosomes superimposed on the base second chromosome (sesro) are given in parentheses. When no such chromosome was superimposed, dots are enclosed in parentheses.

TABLE 8

Comparison of means in two different time periods of each of seven populations

Population No.	$\bar{X}-\bar{Y}$	$s_{\bar{X}-\bar{Y}}$	<i>t</i>	P
WEIGHT				
18 (....)(....)	-17.2	5.53	3.116	<.01
13 (apl)(....)	4.3	4.93	0.872	>.30
16 (apl)(....)	6.6	4.35	1.518	>.10
17 (apl)(....)	23.6	4.35	5.425	<.001
19 (....)(Oregon)	6.9	5.00	1.380	>.10
14 (apl)(Oregon)	26.7	4.10	6.512	<.001
15 (apl)(Oregon)	7.1	3.60	1.970	>.05

$\bar{X}-\bar{Y}$, the mean of the second period minus the mean of the first period; $s_{\bar{X}-\bar{Y}}$, standard deviation of the difference of these means; the *t* value equals $(\bar{X}-\bar{Y})/s_{\bar{X}-\bar{Y}}$; P, the probability of observing so great a difference between the means if the two means were equal.

the Oregon introduction (Number 19) do not differ significantly. The periods of one of the populations with both introductions (Number 14) differ while that of its replicate (Number 15) do not.

The differences between each pair of these populations can be analyzed by the method of paired *t* for each time period. The populations can be paired in 20 different ways. Table 9 lists the paired *t* analyses of weight in five of these pairs for the two different time periods. Those particular pairs that were considered most informative were chosen for analyses.

Populations 19 and 18 which are both homozygous for *sesro* in period 1 are not significantly different. However, in period 2 when population 19 is heterozygous for the Oregon second chromosome, the two are significantly different at the five percent level. This appears to be mainly due to the decline in fitness of population 18.

Replicate populations 13 and 17, which are heterozygous for the *apl* second chromosome, diverge significantly in period 1, but in period 2 population 17 has increased in fitness to the extent that they no longer differ significantly. In fact, population 17 is significantly below the control (Number 18) in the first period and becomes significantly above it by the second period.

Population 14, which is polymorphic for all three chromosomes, is significantly below its replicate population (Number 15) in the first period. After the second introduction its fitness increases significantly more than that of the replicate. The second introduction apparently does not have much effect on population 15, as this population is already significantly above population 13 in the first period when they are both similar genetically.

Test crosses of samples of freshly eclosed flies were made in all five of the polymorphic populations at week 57. These were flies which emerged from the vials which were not included in the developing population (see MATERIALS and METHODS). The results of these testcrosses, and the gene frequencies calculated

TABLE 9

Comparison of mean wet weights in milligrams of seven populations in two different time periods based on paired observations in five population pairs

Pair	Period	n	$\bar{d}$	$\bar{s}_d$	$t = \bar{d}/\bar{s}_d$	P
19-18	1	15	— 4.0	4.04	0.990	>.30
	2	24	21.7	4.92	4.417	<.001
13-17	1	15	20.7	5.54	3.742	<.01
	2	24	1.3	3.61	0.369	>.70
17-18	1	15	—13.5	6.03	2.235	>.02
	2	24	29.9	3.24	9.216	<.001
14-15	1	15	— 6.1	1.80	3.366	<.01
	2	24	13.6	3.00	4.579	<.001
15-13	1	15	7.5	2.60	2.892	<.02
	2	14	10.4	6.30	1.648	>.10

n, number of observations; $\bar{d}$, mean difference; $\bar{s}_d$, standard deviation of mean difference, the *t* value $\bar{d}/\bar{s}_d$; the probability P of observing so great a mean difference if the two mean wet weights were equal.

from them, are recorded in Table 10. The Hardy-Weinberg test has not been performed on these samples because they are very small. These samples do show, however, that each of the seven oligogenes has become incorporated into the gene pools of all five populations at substantial frequencies. Since the Oregon second chromosome has no marker to distinguish it from the sesro-stock second chromosome, the extent of its incorporation into the gene pools of populations 14, 15, and 19 cannot be estimated.

This experiment shows that heterozygosity in the second chromosome alone causes a small but noticeable increase in fitness. The *apl* oligogenes were incorporated into the gene pools of the homozygous *sesro* populations at substantial frequencies, indicating that they, or the associated polygenes, serve some benefit to the populations; otherwise they would have been eliminated.

DISCUSSION

In the first experiment the fitnesses of two populations (the *apl* populations) carrying seven homozygous oligogenes on the second chromosome were compared to the fitnesses of *apl* populations to which a single Oregon genome had been introduced. Within seven weeks after the foreign genome was introduced into these populations, they had reached a fitness which surpassed that of the homozygous *apl* populations by a factor of 12. A homozygous Oregon population was likewise surpassed in fitness initially by all three of the heterozygous populations; the fitness of two of these remained permanently above the level and remained there. Although the improvement over the *apl* level was considerable, the increase over the Oregon level was not so great. It would appear that the *apl* populations were homozygous for so many deleterious genes that they had a considerably lower fitness. When the opportunity for overcoming the effects of these detrimental genes was presented by the Oregon genome additions, the fitness increased. However, the fact that the fitnesses of the polymorphic populations surpassed that of the homozygous Oregon population indicates that the heterozygous condition caused an increased vigor, a populational heterosis, and not a mere masking of deleterious effects of the loaded *apl* genome.

The heterosis is a simple one due at first to F_1 hybrid vigor because it occurred very rapidly; there was no gradual increase in vigor due to the building up of coadapted gene combinations. In the succeeding generations this hybrid vigor did not break down with accompanying production of inferior genotypes by recombination, but remained at a high level in two of the populations. Such a breakdown might be expected to occur if the population became characterized by inferior genotypes resulting from recombination (VETUKIV 1957). Such genotypes may have occurred, but it is felt that under these population conditions natural selection would have prevented them from becoming frequent in the population. In addition to the sustained high fitness, the mutant genes were maintained at a fairly high frequency in an approximate equilibrium, a condition of balanced polymorphism. Since the balanced polymorphism and the sustained high level of fitness occurred concurrently, the hypothesis suggests itself that they are directly

TABLE 10

Genotypes observed in test crosses of males emerging from vials which had been exposed to the population for 24 hours at week 57

Gene	Population No.	n	Genotypes observed in test crosses			Gene frequencies*
aristaless (<i>al</i>)			<i>+/+</i>	<i>+/al</i>	<i>al/al</i>	<i>al</i>
	13	19	12	7	0	.18
	16	14	4	10	0	.36
	17	15	7	7	1	.30
	14	19	14	5	0	.13
	15	18	12	6	0	.17
dumpy (<i>dp</i>)			<i>+/+</i>	<i>+/dp</i>	<i>dp/dp</i>	<i>dp</i>
	13	19	12	7	0	.18
	16	14	6	8	0	.29
	17	15	5	9	1	.37
	14	19	14	5	0	.13
	15	18	14	4	0	.11
black (<i>b</i>)			<i>+/+</i>	<i>+/b</i>	<i>b/b</i>	<i>b</i>
	13	19	9	9	1	.29
	16	14	4	9	1	.39
	17	15	9	6	0	.20
	14	19	9	9	1	.29
	15	18	11	6	1	.22
purple (<i>pr</i>)			<i>+/+</i>	<i>+/pr</i>	<i>pr/pr</i>	<i>pr</i>
	13	19	8	9	2	.34
	16	14	4	9	1	.39
	17	15	8	7	0	.23
	14	19	7	10	2	.37
	15	18	10	6	2	.28
curved (<i>c</i>)			<i>+/+</i>	<i>+/c</i>	<i>c/c</i>	<i>c</i>
	13	19	9	10	0	.26
	16	14	5	9	0	.32
	17	15	7	8	0	.27
	14	19	8	11	0	.29
	15	18	13	5	0	.14
plexus (<i>px</i>)			<i>+/+</i>	<i>+/px</i>	<i>px/px</i>	<i>px</i>
	13	19	10	8	1	.26
	16	14	6	8	0	.29
	17	15	9	6	0	.20
	14	19	10	9	0	.24
	15	18	16	2	0	.06
speck (<i>sp</i>)			<i>+/+</i>	<i>+/sp</i>	<i>sp/sp</i>	<i>sp</i>
	13	19	10	9	0	.24
	16	14	6	7	1	.32
	17	15	9	6	0	.20
	14	19	14	5	0	.13
	15	18	12	5	1	.19

* Gene frequencies are calculated on basis of each genotype shown.

associated. The heterozygous condition, which appears to underlie the initial increase of fitness, may also be responsible for the maintenance of the high level of fitness and for the associated balanced polymorphism. However, a balanced polymorphism can be produced by other methods, such as facilitation, a condition in which different genotypes are mutually beneficial to each other (LEWONTIN 1955), and a situation in which "the same gene substitution affects the fitness of the two sexes in a diploid in opposite directions" (HALDANE 1962). If there were an excess of heterozygotes above what was expected by the Hardy-Weinberg Law, there would be evidence of a possible heterozygous advantage. However, zygotic combinations in these experiments conformed to the Hardy-Weinberg equilibrium at the time the test crosses were made with one apparently insignificant exception (CANNON 1962). It seems impossible on the basis of this experiment to tell exactly what was preserving the polymorphism.

Natural selection favored those genotypes which collectively produced a large population size in this environment. Judging from CARSON's definition of fitness, the polymorphic populations are relatively more fit than the monomorphic ones.

Fitness attributes (specifically: viability, fertility, and longevity) of flies carrying these particular oligogenes, with the exception of aristaless, have been tested (KARP 1940). According to GILBERT (1960), who has analyzed KARP's data, the full heterozygote is not always superior. For example, when flies being tested were heterozygous for *dp b pr c px sp* and *++++ px sp* they were more viable and fertile than the *+++++ / dp b pr c px sp* combination. However, the mutant homozygote was lower than any of the heterozygous combinations in the fitness measures used. In the writer's polymorphic populations, the particular genotype which KARP showed to be superior in fitness may well have been favored by selection. Collectively, the superiority of these particular genotypes must have resulted in a balanced polymorphism.

The performance of populations polymorphic for the Curly and Payne inversions, which effectively prevented recombination on the two largest chromosomes, was compared to that of populations in which recombination occurred freely. The latter populations, *sesro* (Oregon) in structure, were a repeat of experiments of CARSON (1958). CARSON found that the polymorphic freely recombining population surpassed both donor populations in fitness in a rapid fashion and remained at this high level for many generations. These experiments confirm the results of CARSON in that the *sesro* base populations increased in fitness following the Oregon introduction and remained at a high level above the homozygous *sesro* population. No homozygous Oregon population was run concurrently in this experiment.

The high fitness of the nonrecombining populations above the homozygous *sesro* populations allows certain conclusions to be made about the nature of the heterosis produced in these laboratory populations. The high fitness is produced rapidly and would appear to be due to simple F_1 vigor. However, again it is sustained in one case for several generations. There is no period of coadaptation needed to produce the heterosis. In fact, any coadaptation requiring intrachromosomal recombination has been prevented. The Payne chromosome is maintained

in a condition of obligatory balanced polymorphism which appears to be directly associated with the high fitness. Heterosis has produced the balanced polymorphism on the third chromosome; otherwise the Payne inversions would have been eliminated as Curly was.

According to DOBZHANSKY (1955) this heterosis would be properly termed luxuriance, because there has been no process of coadaptation. The Payne and sesro genomes have had no common evolutionary history. Yet, if luxuriance in DOBZHANSKY's interpretation were responsible for the high fitness, fitness would be expected to suffer a breakdown. The present experiments and those of CARSON show that even when recombination is allowed to occur, the fitness does not break down for a long time, certainly not in the F_2 . Selection may be so strong that many of the array of genotypes produced in the F_2 are discriminated against and the resulting population consists of only a few favored, presumably heterozygous, genotypes.

This heterosis is a kind of captured luxuriance. It occurs rapidly, is non-coadaptive and prolonged, and is produced in a constant environment under strong selection. Obviously, it cannot be implied from these data that the heterozygous individuals would be at an advantage in other environments and indeed they may not be homeostatic.

The elimination of Curly in the nonrecombining populations is not surprising. TESSIER (1942) observed elimination of Curly in a population when the other homologue was wild-type but not when the other homologue was carrying the mutant vestigial; the frequency of Curly was stabilized at .332. TESSIER used a Curly stock which had one less inversion than the one used in the current experiments. When crossing over occurred on chromosome 2, both Curly and vestigial were eliminated. ERK (1955) got a decrease in Curly until the 13th generation, and then an increase. He used an artificial population system with no overlapping generations. He wished to protect the gene from the apparent selective disadvantage that flies of this genotype have; their wings get stuck in the food. The writer's experiments confirm the results of TESSIER and the suggestion of ERK; Curly is at a selective disadvantage and has been eliminated in populations which were polymorphic for Curly and chromosome 2 of the sesro stock.

When Curly was eliminated, the second chromosome was no longer heterozygous; it was homozygous for the second chromosome of the sesro stock. Consequently, any fitness beyond this time of elimination (and, in fact, before this time because Curly was always at a very low frequency) is due mainly to heterozygosity on the third chromosome alone. A small amount of the fitness may have been derived from heterozygosity of chromosome 4 which was always uncontrolled in these experiments. Most of the individuals were phenotypically Payne and thus obligatory heterozygotes. It appears that this third chromosome heterozygosity contributed almost all the fitness above the control level when populations 6 and 7 are compared, and about two thirds of the maximum when populations 10 and 11 are compared. This importance of chromosome 3 heterozygosity would tend to verify results of ROBERTSON (1955). He crossed inbred lines of *Drosophila melanogaster* and noted the effect of heterozygosity of each of the

three major chromosomes on such fitness characters as individual size and egg production. The relative effect of the three large chromosomes fell into the relationship: 3 = 100, 2 = 60, 1 = 20.

The contribution of heterozygosity in the second chromosome to a fitness increase can be estimated from the results of the performance of homozygous sesro third-chromosome populations which had opportunity to incorporate apl and Oregon second chromosomes into their gene pools. Here the fitness was increased at most by a factor of one, and usually less. Thus, from this experiment and the Curly-Payne results it might be concluded that both large chromosomes when heterozygous contribute to the heterosis effect, but that chromosome 3 is relatively more important. SMATHERS (1960) showed that it was not the sesro oligogenes on chromosome 3 that contributed to the fitness, but that it was polygenes or unknown oligogenes in the heterozygous condition.

There are several interpretations other than the small possible effect of second-chromosome heterozygosity that can be placed on the results of low fitness of the sesro populations which received foreign second chromosomes. (1) Since the fitness increase was gradual, there may have had to be a period of evolutionary coadaptation on chromosome 2 before an increased fitness could be attained. (2) The load of mutations and polygenes on the third chromosome may have counteracted most of the increased fitness which might have been produced by heterozygosity in chromosome 2. (3) There may have to be an interaction between heterozygous, nonhomologous chromosomes for the fitness increase to occur so rapidly. ROBERTSON (1954) has stressed the importance of chromosomal interactions as a causative factor of heterosis because he found that substituting a particular chromosome might increase, decrease, or have no effect on an individual's size, depending on the genetic background.

The slow improvement in fitness in the sesro populations, when the only heterozygosity is the second chromosome, is very similar to the slow increase in fitness in CARSON's Oregon populations when the intact sesro was introduced. These experiments both have in common the fact that the mutant genes were introduced in low frequency into populations which carried only the wild alleles of these particular oligogenes. The ultimate result was a gradual increase in frequency of the oligogenes accompanied by an increase in fitness. This result demonstrates that the polymorphisms observed in the reciprocal experiments are not fortuitous but represent conditions having positive selective value.

In contrast, the populations which responded with a rapid fitness increase had a different genetic system. These were homozygous mutant populations into which the wild alleles were introduced. Possibly the increase in fitness was rapid at first owing simply to the effects of dominance. The mutant genes and associated polygenes may indeed contribute such a heavy genetic load for a population that as soon as the opportunity to mask their effects by the wild alleles is offered, phenotypically wild individuals replace the homozygous mutant individuals as the most prevalent individuals in the population, and the fitness increases.

The rapid drop in frequency of the oligogenes after introduction of the wild allele is in accordance with this theory. That more than dominant effects on one

chromosome are involved eventually, is revealed by the superiority in fitness of the new hybrid populations over both donor populations and by the reciprocal experiment in which eventually the wild population increases in fitness as the mutant genes increase in frequency. The wild introductions do much more to improve the immediate fitness of the mutant populations than the mutant introductions do for the wild populations. The interpretation cannot be excluded that the slow increase in fitness of the wild populations with mutant introductions is due to a period of evolutionary coadaptation.

The study of population fitness in a controlled laboratory environment is far from the study of populations in their ability to adapt to different environments in nature. However, it does show what genetic aspects a more fit population in the laboratory has, at least for the species here studied. WRIGHT (1955) considers interpopulational selection a more favorable condition for evolution than intrapopulational selection. These experiments may help in the understanding of the importance of interpopulational selection by showing what makes one population more fit than another.

SUMMARY

When the Oregon genome was introduced into three homozygous *apl* populations (carrying oligogenes on chromosome 2), these populations increased rapidly in fitness by a factor of 12, and remained at a high level except for one population which dropped to the level of the homozygous Oregon population. In all three populations, the oligogenes were not eliminated during the course of the experiment, and maintained a balanced polymorphism with frequencies between 9 percent and 42 percent.

Nonrecombining populations formed by the introduction of Curly and Payne inversions into *sesro* populations were not far below the freely recombining populations in fitness, indicating that little if any coadaptation was involved in the production of population fitness, inasmuch as crossing over was effectively prevented. A heterozygous advantage of the Payne individuals held the Payne chromosome in a balanced polymorphism with the *sesro* chromosome, whereas the Curly heterozygotes were at a disadvantage and the Curly chromosome was eliminated. The relative homozygosity of the second chromosome made it possible to attribute most of the fitness above the control level to the heterozygosity on the third chromosome.

The failure of polymorphism on the second chromosome, in *sesro* populations homozygous for the third chromosome, to increase the fitness by more than a factor of one may be due to: (1) the heavy load of mutations and associated fitness genes in homozygous condition on the third chromosome, (2) the prevention of an interaction between heterozygous chromosomes, (3) prevention of a long period needed for coadaptation to occur, and (4) the small genetic load of *sesro* chromosome 2, indicating that additional polymorphism could do little to increase the fitness.

Fitness of populations, when the wild genome is introduced into mutant populations, as observed by CARSON and in these experiments, is due to a captured

luxuriance. It is rapidly produced, sustained over a period of several generations, and consequently does not involve any period of evolutionary coadaptation unless luxuriance is rapidly replaced by euheterosis before the breakdown is noticeable.

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