EVOLUTIONARY CHANGES IN COMPONENTS OF FITNESS
AND OTHER POLYGENIC TRAITS IN *DROSOPHILA*
MELANOGASTER POPULATIONS

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I. INTRODUCTION

CHARLES DARWIN's theory and current genetic interpretations regard natural selection as the main determinant of evolutionary events. In the course of succeeding generations, a population copes with a given environment through a process of trial and error. The errors, that is, the variations which are less fitted to the environment, are lost, successful trials or variations better fitted are preserved; the process responsible for the fate of different variations is based on the fact that unlike types leave different average numbers of adult progeny in the next generation. If such unlikeness is at least partly hereditary the variations responsible for it persist for longer or shorter periods. In the course of time the population thus becomes better adapted to its surroundings, and this adaptation is made apparent by a series of changes in the morphology and physiology of the organism concerned. These changes in time are generally referred to as evolution.

Morphological and physiological characters which may undergo change fall under the control of genetic determinants which, for the sake of simplicity, have been classified by Mather (1943) under two main categories: unifactorial (or oligogenic) and multifactorial (or polygenic). Evolutionary change may thus be described either (1) in terms of variations in frequency of those genes that primarily affect single traits, or (2) in terms of variations in the mean values of morphological and physiological characters that show continuous variability and are controlled by polygenes.

While most of our genetic knowledge on evolutionary processes is derived from the study of variations in the frequency of oligogenes and chromosomal types, the significance of changes of the type indicated under (2) needs be stressed because of the following facts. In the first place, most of the paleontological evidence on the historical process of evolution is inferred from successions of fossils differing in shape and size, and the traits concerned were most likely controlled by polygenic systems. In the second place, zoo- and phyto-geographic data indicate that living organisms may differ, over their distribution

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range, in morphological and physiological traits which are considered to be adaptive and are known to be mostly of polygenic type. In the third place, the components of fitness, such as fecundity, fertility and the like, fall under the control of polygenes in the majority of the studied forms.

In spite of the importance of polygenic characters in evolution, however, no experiments have been reported thus far, which might show how natural selection operates on polygenic systems over a number of generations. The work reported here is an attempt to analyse the changes in such systems which natural selection can produce in experimental populations.

2. TECHNIQUES FOR BREEDING POPULATIONS

The classic paper of L'Héritier and Teissier (1934) was the first to demonstrate the effects of natural selection on single gene differences present in artificial populations of *Drosophila melanogaster*. Since that time variations in the frequencies of genes and of chromosome types, produced by natural selection, have been described in many *Drosophila* species kept in population cages. These are small boxes in which the amount of food available to the population is kept relatively constant; that is, every day or every few days fresh food is introduced and the worked-out food is removed. The population of the cage will reach an approximately stationary number, regardless of the original number of flies introduced, provided that the supply of fresh food as well as other environmental factors, such as temperature, remain constant. The amount of available food at any particular time determines the number of flies that reach the adult stage. This number is smaller than the number of zygotes represented by the fertilised eggs because of mortality in the larval and pupal stages. The struggle for existence and reproduction may also occur in the adult stage because of high population density. The genotypes better adapted to the prevailing conditions will accordingly become more frequent than those less well adapted.

Different experimental techniques have been used by other authors. In order to evaluate the relative selective values of eleven mutants of *D. melanogaster*, Wiedemann (1936) crossed them with wild-type flies. In the F_1 , F_2 and succeeding generations the first 50 adults to emerge were used as parents of the following generation. A comparison between the observed frequencies of various phenotypes and the ones calculated for Mendelian ratios in succeeding generations, using Jennings' formulas, provided a measure of the relative viability of the various mutants. Nikoro and Gusev (1938), with a similar technique, kept populations of flies with various genotypes in large bottles and collected at random in each generation 100 pupæ, which were transferred to a new bottle where they could hatch and breed. Kalmus (1945) started an experiment with five homozygous wild-type females and five homozygous *ebony* males, in one breeding bottle. The progeny was counted and 150 flies were chosen at random and subdivided into 5 groups of thirty flies each to become parents of the following generation in five separate bottles. Records were kept of the numbers of wild-type and *ebony* flies appearing in each generation. This procedure was repeated for several generations in eight parallel series kept at different temperature and humidity conditions. Reed and Reed (1948) used the "population bottle," consisting of two half-pint milk bottles, the mouths of which were connected by rubber tubing. Each milk bottle had a paper cap perforated with two or three quarter-inch holes which allowed passage of the flies but prevented food and debris from being pushed from one bottle to the other by larvæ. The experiments were started with five pairs of flies in one

bottle, the second bottle being added after two months. Two months later the first bottle was removed and a fresh one substituted. By repeating this operation the population can be kept going at will. Techniques such as these seem to be less reliable for a thorough study of natural selection phenomena than the population cages of L'Héritier and Teissier because : (a) the population number is smaller and thereby random fluctuations in the frequency of a gene may give a biased picture of the selection process ; (b) a periodically changing environment (such as that of the "population bottle") or arbitrary choice of parents in each generation may emphasise some and neglect other of the viability factors which are of significance for the competitive success of a given genotype.

The technique used in the work to be reported here is more laborious than those previously described, but provides a wider range of information on the dynamics

FIG. 1.—Schematic representation of the breeding technique used for the experimental populations. The vials are indicated with successive Arabic numbers ; the arrows protruding from them indicate the transfer of the adult individuals from one vial to the next, which occurred every three days (indicated in Roman numbers). The lines originating from the vials and the downward arrows indicate that the adult progeny of each vial was added to the population ; there are three arrows for each vial to show that the adults hatching from each vial were collected and added to the population over a period of 15 days (3×5) ; after that the vial was discarded.

of the populations under study. This method, already described in an earlier paper (Buzzati-Traverso, 1947a), takes advantage of the knowledge derived by Pearl (1927) while studying the growth of *Drosophila* populations. The experiment is started by introducing a certain number of adult flies of the kinds desired into a vial with food. After one, two or three days, according to the type of experiment one wishes to carry out, the flies are transferred to a new vial of the same size and with the same amount of fresh food. The transfer is repeated to new vials with the chosen constant periodicity. With a three day periodicity the number of flies in the first three vials of the series will be the same, unless some die in the meantime. At 25° C., adults of *Drosophila melanogaster* emerge on the ninth day after eggs have been laid, and therefore, some young flies will be found in the first vial on the tenth day from the beginning of the experiment. These are counted, and added to the original group of flies, which, by this time, will be in the fourth vial.

Every day the flies emerging in the earlier vials are transferred to the last one, which, as time passes, will have an increasing number of adult individuals (fig. 1). Such increase will continue up to a point when an equilibrium is reached between the amount of available food and space and the number of individuals which can live under such conditions. The equilibrium will be an approximately stationary one under constant conditions, although, as it will be shown later, this is not always true.

By means of this procedure, one can obtain : (1) data on the number, sex and phenotypic ratios of the flies present in the population at the time of each transfer to a new vial ; (2) data on the birth rates of the two sexes and of the phenotypes in each vial. This information makes it possible to evaluate at any particular time : (a) variations in the population size ; (b) variations in the sex ratio of the population as a whole or of its constituent phenotypes ; (c) variation in the gene frequencies ;

FIG. 2.—Elimination of the mutant *white* in the four experimental populations (P_1 , P_2 , P_3 , P_4).

(d) the mean life length of adult individuals ; (e) selection differentials for the developmental stages and for imagoes ; (f) the total productivity of the population at any given time.

3. THE EXPERIMENT

In the experiment to be described here, four populations of *Drosophila melanogaster* were kept at 25° C., on the standard corn-meal, agar, molasses medium seeded with a constant amount of baker's yeast. Two of these (P_1 and P_2) were started about one year earlier than the other two (P_3 and P_4). The foundation stock of all four populations consisted of 10 virgin females and 10 males of wild-type Oregon-R strain, and 10 virgin females and 10 males of another strain homozygous for the sex linked genes *white* and *Bar*. The populations were bred in glass vials 12 cm. high and 3.5 cm. in diameter for about 100 generations over a period of four years. After

equilibrium was reached the population numbers fluctuated around 700 flies at first, but increased later. A total of about 2,000,000 flies were counted and classified for sex and phenotype.

Figs. 2 and 3 summarise the elimination of the mutant genes in competition with their wild-type alleles. As it will be seen, the mutants *white* and *Bar* were completely replaced by their normal alleles in the course of approximately 30 generations. The selection coefficients of the two genes were very similar throughout the experiment. As has been previously pointed out (Buzzati-Traverso, 1947), the selective value of these genes remains the same irrespective of its absolute frequency in the population. This conclusion is based on a plot of

FIG. 3.—Elimination of the mutant *Bar* in the four experimental populations (P_1 , P_2 , P_3 , P_4).

the logarithms of observed gene frequencies against time expressed in generations.

At their face value, the results obtained with the four populations showed no more than what might have been expected from similar data published by other authors. (L'Héritier and Teissier, 1934 and 1937; Reed and Reed, 1948 and 1950; Merrell, 1933*a* and *b*). The technique employed in this experiment, however, makes possible, as it has been pointed out before, to get more detailed information on the selection mechanism responsible for eliminating the mutant alleles. This aspect of the experiment will be analysed in a separate paper. Here only two facts brought out by the detailed analysis need to be mentioned in view of the discussion to follow: (1) the average generation time was 15 days; (2) the mean life of the adult flies in the experimental populations averaged 8.82 days.

Disregarding for the time being the obvious changes which occurred, when in thirty generations two genes, originally present in half the individuals, completely disappeared, one can still raise the question whether natural selection also brought about other genetic changes not so easily detectable but perhaps of even greater significance for adapting the fly populations to the conditions described. In other words, were the wild-type flies constituting the population after the disappearance of the mutant types genetically identical to the Oregon-R and *white-Bar* strains? These certainly differed from each other not only in the characters of the eye which made them easily distinguishable, but also in an indefinite number of genetic factors affecting other morphological and physiological characters. Now, did natural selection also affect this part of the genotype of the two original strains? Is it possible to find whether any genetic changes were produced in this respect and if so, of what sort? And, if any such changes did occur during the process of eliminating the *white* and *Bar* alleles, did the populations undergo similar changes in later generations, when their members had become all wild type? How different and how much better adapted to the cultural conditions might the flies be after natural selection had operated for many generations?

In order to find the answers to these and to similar questions, the populations were continued for another seventy generations, and were subjected to tests which will be analysed in the following sections.

4. CHANGES IN COMPONENTS OF FITNESS

The characters which most likely have been influenced by the process of natural selection are those directly responsible for the fitness of the flies. It is clear that the individuals having greater viability under the chosen experimental conditions had a better chance of contributing their genetic material to the following generations. In an experiment of this type viability can accordingly be defined in terms of relative survival value in competition. Various factors may contribute directly or indirectly to the production of a greater survival value, and it is possible to devise various tests to measure some of them. If we observe that one strain has a higher survival value than another, we may try to relate this difference to different values of some measurable characters such as fecundity, fertility, rate of development, etc. (see below). If such factors are inherited at all, one should be able to find whether their values in the experimental populations were greater than in the two original strains. It is known that in *Drosophila melanogaster* such characters are indeed heritable largely on the basis of a multifactorial or polygenic scheme. With this idea in mind samples of the two original strains and of the four populations were tested at different times. The following characters were measured: (a) percentage of sterile females; (b) fecundity of single females, measured by the number of eggs laid per day; (c) fertility, in the sense of Pearl (1932), *i.e.* the percentage of adults emerging from a

known number of eggs ; (d) rate of development, or the average time between the laying of the egg and the emergence of the imago ; (e) adult longevity, or the average time between emergence of the adult and its death.

(a) Sterility

Approximately 20-30 individuals of both sexes were taken at random from the Oregon-R, the *white-Bar* strains and from the four populations and were transferred to 300 c.c. culture bottles. Female offspring from these matings were cultured singly with three brothers each in 3.5×12 cm. vials. Every four days the flies were transferred to a new vial with fresh food, such transfer being repeated five times. If by the 24th day no larvæ were observed on the medium, the female was considered to be sterile. The number of sterile females was recorded, after due allowance for accidental deaths. Table 1 shows clearly that the incidence of sterile females in the original stocks was reduced to zero in the experimental populations. If one takes

TABLE 1
Sterility of females of original strains and of experimental populations

Strain or population	No. ♀♀ tested	No. ♀♀ sterile	Percentage sterility
Oregon-R	252	4	1.58
<i>white-Bar</i>	252	32	12.69
P ₁	250	0	0
P ₂	248	0	0
P ₃	251	0	0
P ₄	252	0	0

the average viability as measured in terms of sterility of the two parental strains as being equal 1.00, the viability of the four populations improved by a factor of 1.07.

(b) Fecundity

Samples of about 20-30 individuals of both sexes were taken at random from the two original strains and from the four populations and were cultured in 300 c.c. culture bottles (only in the case of tests performed in May 1948, see table 2, were the flies directly derived from the population bottles.) Virgin female progeny were isolated and mated singly within 24 hours from their emergence to three brothers in 3.5×12 cm. vials. The surface of the medium in these was covered with a disc of blue absorbent paper soaked in a suspension of yeast. The flies were transferred every day to a new vial of this type and the number of eggs laid on the blue paper during the previous 24 hours was recorded. Egg laying took place at 25° C., and the recording was continued to the 20th day of life of the female or to the time of her death. For each test and day the quantity and quality of food and laying medium was the same for both strains and all experimental populations.

Table 2 gives the results of various tests for fecundity at different times. Data are presented separately for the first ten days and for the first fifteen days of life of the females, because some of the females died before the fifteenth day. The mean life of flies in the populations being approximately nine days, it did not seem worthwhile to extend egg counts beyond the 15th day. Because of the laboriousness of egg counting over a period of several days, no counts were made for the two original strains in the May 1948 test. The relatively low figures for the four populations in this test are probably due to the fact that the females were sampled directly from the population bottles. They

TABLE 2
*Fecundity of females of original strains and of experimental
populations at various times*

Strain or population	Date	Generation tested	10-day period		15-day period		Total no. eggs
			No. ♀♀ tested	Mean no. eggs/day	No. ♀♀ tested	Mean no. eggs/day	
Oregon-R .	2/48	...	6	11.50	4	10.50	1330
	1/49	...	13	11.74	10	13.58	2683
<i>w-B</i> . .	2/48	...	6	6.45	6	9.53	1107
	1/49	...	11	15.00	9	16.63	2397
P ₁ . . .	2/48	59	6	32.45	6	29.46	4297
	5/48	65	11	22.34*	11	29.85*	5940
	1/49	82	12	37.36	12	35.70	7748
P ₂ . . .	2/48	59	6	31.90	6	30.30	5104
	5/48	65	12	25.38*	11	29.72*	6140
	1/49	82	12	31.31	12	37.09	6843
P ₃ . . .	2/48	32	6	27.46	6	26.26	4318
	5/48	38	11	22.03*	11	26.00*	4925
	1/49	56	12	28.70	12	28.64	6063
P ₄ . . .	2/48	32	6	33.43	6	34.33	6537
	5/48	38	12	16.55*	11	20.65*	4232
	1/49	56	12	34.83	12	34.17	7296

* All the values of the May 1948 test are systematically lower for reasons explained in text.

were thus therefore certainly less well fed than those in the other tests, a factor of significance in egg production, since it is known that culture conditions during larval development affect fecundity (Hadorn and Zeller, 1943). Table 3 shows the results of the analysis of variance of the square roots of the actual numbers of eggs counted. The comparison between the mean number of eggs of the two original strains and those of the four populations gives a value of F which is significant beyond the $P = 0.01$ value. Differences between various samples of the same strain or population, on the other hand, are not significant, with the exception of the *white-Bar* strain in which the

average number of eggs per day changed from 9.53 to 16.63 in the course of about one year. Even though the means of the populations

TABLE 3
*Analysis of variance of transformed (square root) values of
egg production for 15 days*

	February 1948				January 1949		
	DF	SSQ	MS		DF	SSQ	MS
Total Populations .	33 5	1039 795	159	Total . Populations .	66 5	1797 632	126.4
	28	244	8.71		57	1165	20.4
	F = 18.25**				F = 6.19**		
	Oregon-R				white-Bar		
	DF	SSQ	MS		DF	SSQ	MS
Total Samples .	13 1	167 10	10	Total . Samples .	14 1	214 120	120
	12	157	13		13	94	7.23
	F = < 1				F = 16.56**		
	Population 1				Population 2		
	DF	SSQ	MS		DF	SSQ	MS
Total Samples .	28 2	559 12	6	Total . Samples .	28 2	891 12	6
	26	547	21		26	879	33.8
	F = < 1				F = < 1		
	Population 3				Population 4		
	DF	SSQ	MS		DF	SSQ	MS
Total Samples .	28 2	330 4	2	Total . Samples .	28 2	1078 169	84.5
	26	326	12.5		26	909	34.9
	F = < 1				F = 2.42		

for the February 1948 and January 1949 tests (table 2) are markedly different, especially in the case of P₁ and P₂, their F values are not

significant due to the wide range in the number of eggs of different females of the same population. Fig. 4 gives the mean cumulative fecundity of the two strains and the four populations over a period of 15 days and brings out in a very clear way the considerable difference in fecundity at the time of the last test between the original strains and the experimental population, derived from them. In spite of the already mentioned lack of significance between samples within populations, and disregarding the May 1948 test there seems to have been a trend toward higher values in each population as the selection experiment proceeded. The May 1948 test had smaller values than

FIG. 4.—Comparison between the cumulative fecundities of the two original strains (O-B, *w-B*) and of the four experimental populations.

either of the others, but they were highly significant with respect to both tests on the original strains. Calculating the average means for Populations 1 and 2 on the one hand, and those of Populations 3 and 4 on the other, one can arrange the fecundity of females with respect to the number of generations as follows :

Generation	32	30.44	eggs per day
„	56	31.76	„
„	59	32.17	„
„	82	37.33	„

This pooling of data of the various populations seems justified by the remarkably parallel behaviour of the populations in every other respect, which makes the trend toward a continuous improvement in the fecundity very clear. If we take now the average viability as measured in terms of fecundity of the two parental strains at the first test as equal to 1.00, the average viability of Populations 1 and 2 at the 82nd generation had improved by a factor of 3.69.

(c) Fertility

Fertility, or the percentage of imagoes emerging from a known number of eggs, is influenced by the age of the mother, as shown by Hadorn and Zeller (1943). As a consequence, it was not possible

TABLE 4
Fertility of original strains and of populations at various times during the selection experiment

		Oregon		<i>w-B</i>	
		Egg no.	Per cent. emergence	Egg no.	Per cent. emergence
June 1947	. . .	200	61.5	200	66.5
Nov. 1947	. . .	200	62.0	200	50.0
Jan. 1949	. . .	3320	64.51	3320	60.18
Mar. 1949	. . .	1800	79.00	2040	61.42

Date	Generation	P ₁		P ₂		P ₃		P ₄	
		Egg no.	Per cent. emergence	Egg no.	Per cent. emergence	Egg no.	Per cent. emergence	Egg no.	Per cent. emergence
4/48	35	...	...	...	...	200	73.5	200	81.00
6/47	40	200	90	...	...	...	...	...	...
1/48	51	200	81.5	200	93.5	...	...	...	...
12/48	55	...	...	...	...	2430	88.35	2430	92.75
3/49	60	...	...	...	...	1360	84.26	1320	89.92
12/48	81	2430	88.97	2430	86.62	...	...	...	...
2/49	86	1020	91.17	1400	84.28	...	...	...	...

to use egg samples taken directly from a strain or population because of the uncertain age of the females concerned. Young daughters of flies sampled from the Oregon-R and the *white-Bar* strains as well as from the experimental populations were mated to several brothers and used as egg producers for the determination of fertility. Two tests were performed in 1947, for the sole purpose of determining fertility. In this case, groups of 20 eggs were transferred to 3.5 × 12 cm. vials; after developing at 25° C. the number of adults present in the vials was recorded. For any particular test special care was taken to have cultural conditions of the six experimental series as homogeneous as possible. The two sets of data of the 1949 tests, on the

other hand, are taken from an experiment in which the main purpose was to ascertain the weight of emerging flies. For reasons to be discussed below, the number of eggs per vial had to be different in each strain and population. The numbers of eggs per vial for the January 1949 experiment are to be found in table 10; the numbers of eggs per vial in the March 1949 experiment were as follows: Oregon-R: 45; *w-B*: 51; Population 1: 34; Population 2: 35; Population 3: 34; Population 4: 33.

Table 4 shows the results of fertility measurements on a total of 26,900 eggs. While in the Oregon-R strain a noticeable improvement took place in the course of almost two years, the fertility of the *white-Bar* strain remained stationary. All of the populations tested were clearly superior to both parental strains. No clear cut trend in the improvement in fertility with the progress of the selection is noticeable. This, however, can be related to the fact that the conditions of measuring

TABLE 5
*Analysis of variance of fertility data. Transformed $p = \sin^2 \phi$.
Data of June and November 1947 and of March 1949*

	d.f.	Sum of squares	Mean squares
Total	31	2218	...
Tests	2	365	182.5**
Populations	5	1532	306.4**
Error	24	321	13.37

fertility were not identical in successive tests. In order to test the significance of differences in fertility, two separate analyses of variance were made on the transformed values. The first (table 5) refers to the data of June and November 1947 and of March 1949, derived from vials not dissimilar in the number of eggs; the second (table 6) to the January 1949 data, for which a preliminary analysis of covariance had to be made in order to eliminate variability due to considerable differences in egg densities within each strain or population. In every case the data have been transformed to the $\sin^2$ of the observed percentage. This statistical treatment confirmed the conclusions already noted regarding the differences between the fertilities of the two strains and of the four populations. If we now take the average viability as measured in terms of fertility of the two original strains at their first test as equal to 1.00, the average viability of the four populations at their last test has improved by a factor of 1.37.

(d) Developmental rate

Groups of 10 females and 20 males taken from strains and populations were transferred to 300 c.c. bottles having a paper spoon medium heavily seeded with baker's yeast. The size of the spoon was such

that it could be easily removed from and introduced back into the bottle without disturbing the flies. Collection of eggs for determining the developmental rate was made only on the second day after the flies had been transferred to the egg laying vials when the females had recovered from the shock of transfer and were laying eggs regularly. This technique made certain that the fertilised eggs had not stayed for a long period in the oviduct, and had therefore just started their development at the time of laying. The eggs were collected every four hours and transferred in groups of 20 into vials with equal amounts of food. The collecting took place at the same time for the strains and populations in order to insure the maximum of homogeneity in feeding and environmental conditions. Development took place at $25^{\circ} \pm 0.5^{\circ}\text{C}$. After pupæ had been formed in the vials they were

TABLE 6

Analysis of covariance and test of significance of differences between adjusted means in January 1949 data of fertility. Transformed values $p = \sin^2 \phi$

Source of variation	Sums of squares and products				Errors of estimate		
	d.f.	Sx^2	Sxy	Sy^2	Sum of squares	d.f.	Mean square
Total . .	35	1,280,889	240,336	67,769	22,676	34	...
Populations .	5	176,002	236,344	67,341			
Error . .	30	1,104,867	4,092	428	413	29	14.2
					22,263	5	4452

For test of significance of adjusted means

$$Sy^2 - \frac{(Sxy)^2}{Sx^2} = 413 \quad F = 4452/14.2 = 313 **$$

removed and placed singly in 30 c.c. glass test tubes containing a strip of filter paper which had been soaked in water to provide high humidity. Water was added each day to the test tubes until the flies emerged. Beginning with the seventh day after the eggs had been collected, the test tubes were inspected at 2.00 a.m., 8.00 a.m., noon, 4.00 p.m., 8.00 p.m. and midnight, and the number of emerged flies recorded. Thus the time of egg laying was known within ± 2 hours, and the time of emergence of the imago was determined within ± 1 , 2 or 3 hours. The schedule chosen was not the best possible one because the time intervals were not constant, but in view of the large number of records to be kept at the same time for the six experimental series, no other choice was available. The test was performed on Populations 1 and 2 at their 53rd generation and on Populations 3 and 4 at their 27th generation. A total of 862 flies was used. The mean times of development expressed in days and hours, together

with the number of flies in each sample are given in table 7. In view of the fact that the time intervals were not constant no standard errors are given. In order to reach a more complete evaluation of the data, they are presented in graphical form in fig. 5, with time intervals of one day.

TABLE 7
Average time of development of samples of flies taken from the two strains and the four populations

Strain or population	Days	Hours	No. of flies
Oregon-R	10	6	123
white-Bar	9	11	100
Population no. 1	9	0	163
Population no. 2	8	19	170
Population no. 3	8	19	147
Population no. 4	9	12	159

A comparison of the distributions of developmental times of the four populations with those of the two strains indicates that the former are skewed towards lower values and that this is more marked in the

FIG. 5.—Distributions of the times of development for the two original strains and for the four populations. On the abscissa the days, on the ordinates the number of flies.

older populations (1 and 2) than in the younger ones (3 and 4). Furthermore, it is worthwhile pointing out that the extreme values of the distribution ranges shown on the figure are smaller in three

out of four populations. A comparison between the six distributions carried out with the chi-square test shows that they are highly statistically different ($\chi^2 = 245.91$; d.f. = 23; $P \ll 0.001$). Also, a chi-square test between the pooled values of the two original strains on the one hand and of the four populations on the other hand shows that they are different ($\chi^2 = 109.76$; d.f. = 7; $P \ll 0.001$), and even a comparison of the developmental rates of the fastest growing of the two strains, *white-Bar*, with the population having the skewest distribution, P_2 , shows that the lower values of the latter are significantly different from the former ($\chi^2 = 50.39$; d.f. = 7; $P \ll 0.001$). It therefore seems fair to conclude that in the experimental populations natural selection favoured those genotypes which led to a faster rate of development. If one compares the mean time of development of the two original strains (9 days, $20\frac{1}{2}$ hours) with that of the four

TABLE 8
Mean life of adult flies (in days)

	Oregon	<i>w-B</i>	P ₁	P ₂	P ₃	P ₄
No. of flies .	466	473	443	464	462	448
Average life .	30.37	22.50	36.90	30.24	37.81	40.41
Analysis of variance						
	DF	SS		MS		
Total	2755	506,025				
Populations	5	98,278		19,655		
	2750	407,747		148		
F = 132.5**						

populations (9 days, 3 hours), one finds a difference of 17.5 hours, or about 7.4 per cent. of the whole period of development. Thus, assuming that under the optimal conditions of this test time of development equals generation length, it is possible to compute that over a period of 100 days the two original strains would have produced 10.15 generations as against 10.96 generations produced by the average population. Thus, if we take the average viability as measured in terms of developmental rate of the two strains as equal to 1.00, the average viability of the four populations at the time of the test has improved by a factor of 1.08.

(e) *Adult longevity*

Ten groups of 50 newly emerged flies which were the progeny of samples taken from the strains and populations were placed in

3.5 × 12 cm. vials with standard food. They were transferred every five days without etherisation into a new vial with fresh food and record was kept of the deaths which had occurred during the interval. The flies were kept at $25^{\circ} \pm 0.5^{\circ}$ C. throughout the experiment.

Populations 1 and 2 had reached the 73rd generations, and Populations 3 and 4 had reached the 47th when the samples were taken. Table 8 summarises the observed results. The actual numbers of flies of each group were somewhat less than the original 500 because some escaped in the process of transfer and some died of accident. The length of life has a normal distribution and the analysis of variance can accordingly be calculated on the actual figures. The differences between the observed means are highly significant, as shown in the table. Populations 1, 3 and 4 show mean life length values which are considerably greater than either of the two parental strains; only Population 2 has a length of life equal to that of the Oregon-R strain.

As has been noted earlier, the mean life of the flies in the population under experimental conditions was about nine days. It seems hard to explain how a life span beyond the 22nd or 30th day under optimal environment could be of advantage under conditions of very great overcrowding. The data concerning fecundity and longevity in *Drosophila melanogaster* appear to be contradictory. Alpatov (1932) found that they are negatively correlated, and Hadorn and Zeller (1943) found that they are positively correlated. Our data indicate that there is no particular parallelism between them. Thus, it may be that they are products of independent genetic and physiological processes.

Longevity does not therefore appear to be selected for either directly or as a correlated response of the improvement in fecundity. It seems likely that it is the product of some general factor for increased resistance and hardiness which has been favoured during the period of selection. If this is the case, and if we take the average viability as measured in terms of longevity of the two original strains as equal to 1.00, the average viability of the four populations improved by a factor of 1.37.

5. CHANGE IN MORPHOLOGICAL CHARACTERS

The evidence so far discussed indicates that natural selection has operated at the level of quantitative physiological characters and that the genotypes of the four populations after several generations differ from those of the two parental strains. In view of these facts a new question can be raised: do these quantitative physiological changes find a counterpart in some quantitative change at the morphological level? If this is the case, we might understand better the changes in size and shape which have occurred in many organisms in the course of evolution. Two sets of experiments were started in order to bring some evidence on this point; the object of the first was to ascertain whether any changes had occurred in the size

and shape of the wing, and of the second to see whether the body weight and size had changed appreciably.

(a) *Wing size*

In order to make sure that larval competition did not influence the size of the characters to be measured, batches of 20 eggs, obtained from females hatched from samples taken from the strains and populations, were placed in 3.5×12 cm. vials with a constant amount of food and kept at $25^\circ \pm 0.5^\circ$ C. The left wings of about 50 males were removed and dry mounted between two glass slides. They were then enlarged 133 times by projection with a Bush Metaphot microscope and measured on millimetre paper.

FIG. 6.—The two characters measured on the wings of the flies.

Table 9 records the results of the measurements and of the analysis of variance. The differences between the six means are highly significant, and the values of t for the differences between means taken two at a time show that (a) Oregon-R has wings longer than those of any other group; the statistical significance is at the 0.05 level for Population 4 and at the 0.01 level for the other four; (b) only Populations 2 and 3 have wings shorter than the *white-Bar* strain; (c) Oregon-R and *white-Bar* both have wider wings than any of the populations, but only Populations 2 and 4 show a statistically significant difference from the latter strain.

On the basis of such data we reach the general conclusion that under the experimental conditions genotypes with shorter and narrower wings were favoured and that the intensity of size decrease does not seem to be strictly correlated with the number of generations. Smaller wings were probably advantageous for the flies which lived under the more crowded conditions of the populations but the response to selection was not equally rapid in parallel populations.

(b) Body weight

It seemed particularly interesting to find out whether the action of natural selection for several generations had had any effect on body size. To measure this with satisfactory precision in *Drosophila melanogaster* is difficult, but an indirect estimate could be obtained from body weight.

In order to make significant comparisons between the body weights of samples of adult flies derived from the strains and populations it seemed necessary to make the feeding conditions as similar

TABLE 9
Values of wing characters no. 1 and 2 (wing length and width)

Strain	No. wings	$\bar{x} \pm e_s$	Analysis of variance
Character no. 1 (wing length)			
+Oregon-R	37	163.6 $\pm$ 0.594	F = 6.38** d.f. = 270 and 5
w-B	44	159.8 $\pm$ 0.787	
P ₁	45	161.0 $\pm$ 1.012	$t_{.05} = 1.720$ $t_{.01} = 2.268$
P ₂	50	157.7 $\pm$ 0.726	
P ₃	50	158.0 $\pm$ 0.903	
P ₄	50	161.6 $\pm$ 1.126	
Character no. 2 (wing width)			
+Oregon-R	37	123.7 $\pm$ 0.575	F = 28.55**
w-B	44	118.8 $\pm$ 0.571	
P ₁	45	118.0 $\pm$ 0.706	$t_{.05} = 1.108$ $t_{.01} = 1.146$
P ₂	50	117.3 $\pm$ 0.584	
P ₃	50	116.7 $\pm$ 0.828	
P ₄	50	117.9 $\pm$ 0.592	
Wing length		Wing width	
Oregon	163.6	Oregon	123.7
P ₄	161.6	w-B	118.8
P ₁	161.0	P ₁	118.0
w-B	159.8	P ₄	117.9
P ₃	158.0	P ₂	117.3
P ₂	157.7	P ₃	116.7

as possible. In view of the different degrees of fertility shown by the strains and populations (see section 4c) it did not seem valid to let equal numbers of eggs develop on a constant amount of food. In order to take this factor into account, a first experiment was carried out, by using six different egg densities for each strain and population. The actual densities were calculated on the basis of the fertility data in such a way as to produce similar larval and pupal densities. It was hoped that by this procedure one could perform the analysis of covariance of the observed data, and calculate the F on the adjusted

means after elimination of the density variable. The general results, which can be seen in table 10, however, were not suitable for this treatment, because it turned out that the regression between egg density and weight was linear for the Oregon-R and the *white-Bar*

TABLE 10

Mean living and dry weight of samples of 100 adults (50 ♀♀ and 50 ♂♂) expressed in 10⁻² gr., hatching from different egg densities (indicated in parentheses)

	Oregon	<i>white-Bar</i>	P ₁	P ₂	P ₃	P ₄
Adults and eggs per vial	256 (40)	160 (40)	204 (30)	217 (30)	212 (30)	213 (30)
Samples weighed	2	1	2	2	2	2
Mean living weight ($\bar{x}$)	9.19	9.79	11.42	11.78	11.63	11.14
Mean dry weight ($\bar{x}$)	2.48	2.56	2.91	3.17	3.02	2.92
Adults and eggs per vial	240 (120)	200 (120)	246 (90)	230 (90)	244 (90)	241 (90)
Samples weighed	2	2	2	2	2	2
Mean living weight ($\bar{x}$)	8.75	9.23	8.45	8.45	9.52	8.82
Mean dry weight ($\bar{x}$)	2.37	2.52	2.19	2.30	2.68	2.41
Adults and eggs per vial	261 (200)	266 (200)	260 (150)	244 (150)	259 (150)	282 (150)
Samples weighed	2	2	2	2	2	2
Mean living weight ($\bar{x}$)	7.46	8.30	7.80	7.92	8.70	7.70
Mean dry weight ($\bar{x}$)	1.85	2.31	2.10	2.17	2.41	1.95
Adults and eggs per vial	411 (300)	361 (300)	382 (210)	344 (210)	395 (210)	392 (210)
Samples weighed	3	2	3	3	3	3
Mean living weight ($\bar{x}$)	6.47	7.27	6.72	7.33	7.38	7.54
Mean dry weight ($\bar{x}$)	1.86	2.08	1.78	1.88	1.97	1.96
Adults and eggs per vial	463 (370)	459 (370)	482 (270)	477 (270)	472 (270)	509 (270)
Samples weighed	4	4	4	4	4	4
Mean living weight ($\bar{x}$)	6.66	7.82	8.36	8.17	8.24	8.09
Mean dry weight ($\bar{x}$)	1.75	2.35	2.55	2.52	2.52	2.58
Adults and eggs per vial	511 (450)	552 (450)	588 (330)	593 (330)	565 (330)	617 (330)
Samples weighed	5	5	5	5	5	5
Mean living weight ($\bar{x}$)	5.70	7.14	6.68	6.71	6.93	6.72
Mean dry weight ($\bar{x}$)	1.55	1.95	1.71	1.77	1.74	1.68
Total adults	933	903	963	931	951	1005
Mean living weight (100 flies)	7.371	8.258	8.201	8.393	8.733	8.335
Mean dry weight (100 flies)	1.976	2.295	2.206	2.302	2.381	2.250

strains, but not for the four populations. If a transformation was used for all the values which made the regression of the populations linear, then the regression of the two strains became curvilinear. From a direct inspection of the data, however, it appears again that every population gave weight values which were greater than those of the Oregon-R strain and equal or slightly superior to those of the *white-Bar* stock.

In view of this difficulty, a second experiment was carried out using just one egg density per strain or population, as indicated by the numbers in parentheses in table 11 (experiment 2), which also summarises the data of the first experiment. A total of 8940 eggs was used, and besides weighing the sample groups of males and 50 females and comparing their mean weights, the total weight of adults produced in an average culture of each strain and population was calculated, by multiplying the mean weight by the actual mean number of adults obtained per culture. The two sets of data are in good agreement, in that they both show that the flies derived from

TABLE 11
Weight of Drosophila expressed in 10^{-2} gr. For explanation see text

	Oregon-R	white-Bar	P ₁	P ₂	P ₃	P ₄
<i>Experiment 1</i>						
Mean weight (100 flies)—						
Fresh	7.371	8.258	8.201	8.393	8.733	8.335
Dry	1.976	2.295	2.206	2.302	2.381	2.250
Total weight—						
Fresh	4.127	4.743	4.739	4.688	4.983	5.026
Dry	1.106	1.243	1.275	1.285	1.359	1.357
<i>Experiment 2</i>						
Mean weight (100 flies)—	(45)	(51)	(34)	(35)	(34)	(33)
Fresh	11.111	12.550	12.585	12.841	12.533	12.344
Total weight—						
Fresh	3.270	3.488	4.019	3.794	3.598	3.667
	♀ Oregon-R × ♂ white-Bar			♀ white-Bar × ♂ Oregon-R		
Mean weight (100 flies)	(33)			(33)		
Fresh	12.643			12.688		
Total weight—						
Fresh	3.709			3.869		

the four populations have produced more "Drosophila material" per culture than flies obtained from either of the parental strains. The actual values are lower in the second experiment than in the first because of the smaller number of eggs present in the culture vials. The data from the crosses between the strains in table 11 will be considered later.

It seems therefore safe to conclude that under the experimental conditions those genotypes were favoured that allowed the flies to make a better use of the available food, assimilating it and transforming it into "Drosophila material," and thereby reaching a greater weight and thus, presumably, a larger size.

6. THE NEW GENOTYPE

The evidence thus far presented shows that in the course of these experiments every tested character in each of the four populations

has undergone a certain change. The degree of change was different in each population, but the trend was the same in all. For some characters, as was the case for the fecundity of females, the values finally reached were much higher than those of either of the two parental strains, while in others, such as wing length, they were very close to one or the other of the two original stocks. Furthermore it has been shown that these changes continued after the mutant genes had disappeared, and the members of the populations all had a wild-type phenotype.

The physiological characters tested can be regarded as measures of fitness and because of this consistent change toward a greater total fitness the inference seems justified that the observed changes clearly had a selective value and were the product of natural selection. The viability factors which were calculated with respect to the average values of both parental strains for each tested character, and were presented at the close of subsections of section 4, can be used to estimate the total improvement in fitness of the populations. If the factors computed for sterility, fecundity, fertility and developmental rate are multiplied together one gets a compound factor of 5.84. And if the improvement in longevity can also be considered, as an index of an increase in viability, then the total factor of fitness increase becomes equal to 8.00. In other words, the total fitness improved in the course of about 80 generations by about 800 per cent. This value may represent a potential which would not actually be realised in the population bottles because all of the measurements were made on flies which developed under optimal conditions, which certainly did not exist in the population bottles. But since the improvement in each of the tested factors was certainly of an hereditary nature, the factor of 8 may be viewed as a valid estimate of the amount of evolutionary change in fitness between the beginning and the end of the experiment. The change in wing size was also probably adaptive, since it can be surmised that under conditions of extreme overcrowding such as prevailed in the population bottles, smaller wings must have favoured survival. It is not as easy, at first, to account for the associated increase of body weight and therefore presumably of size, because smaller individuals might be expected to have a better chance of survival in competition, under conditions of great density. But this change was accompanied by, or was the product of, a better ability of the flies to exploit the available food and to convert it into "Drosophila material." In view of this fact, one is led to conclude that the increase in weight, too, had a positive selective value. We shall discuss its significance later.

It would appear, therefore, that new polygenic systems have arisen from the genotypes of the two original strains which have conferred on their carriers a remarkably higher viability and have thus increased substantially the fitness of the populations to the conditions in which they existed.

That new and better adapted genotypes were produced seems beyond any possible doubt, but they need not necessarily have been the product of a long continued selection. If this had been operating on the products of genetic recombination resulting from the mixture of the two sets of genes present in the Oregon-R and the *white-Bar* strains, in each of the four populations a new gene constellation would have been synthesised through a progressive process of casting off the less adapted and preserving the most favourable polygenic combinations. But the observed higher adaptive level could have been the result of a different process, namely that complementary-acting genes were brought into combination at the F_1 and stayed in the populations without selection at the F_1 gametic frequency, plus or minus sampling fluctuations. In this latter case the higher fitness

TABLE 12
Heterosis in the crosses between the two original strains

Fecundity of females					
Cross	No. ♀♀ tested	Mean 10 d	No. eggs/day 15 d	Total 10 d	No. eggs 15 d
♀ <i>w-B</i> × ♂ +	12	49.67	59.93	5961	8942
♀ + × ♂ <i>w-B</i>	12	39.05	40.54	4686	7229
Fertility					
Cross	No. eggs		No. adults	Percentage	
♀ <i>w-B</i> × ♂ +	1000		906	90.6	
♀ + × ♂ <i>w-B</i>	1000		900	90.0	

of the populations would have been produced by a sort of heterotic response due to the complementary action of the genes, and it would have been achieved in one step, at the first generation, rather than through a steady progress extending over several generations. Viability factors such as those measured in this investigation do in fact often show heterosis when two unrelated strains are crossed.

Two tests on viability factors and one on body weight were accordingly performed on the two reciprocal F_1 hybrids of the original strains to see whether this alternative interpretation was plausible. The pertinent data were collected following the previously described techniques. As can be seen in tables 11 and 12, the hybrid values show a clear-cut heterosis reaching values similar and sometimes higher than those of the samples derived from the populations after many generations. This being the case, the previously discussed

interpretation attributing the higher adaptive level of the populations to the action of a long continued selection should be rejected.

7. COMPETITIVE ABILITY OF THE NEW GENOTYPE

If indeed no selection for quantitative traits had occurred and the populations had just been maintained in hybrid condition over the whole experiment, other questions arise. Thus, in view of the fact that, except for the May 1948 test for fecundity, all the measurements of viability factors and of morphological traits were performed on the progenies of samples taken from the populations, and were cultured under optimal conditions allowing both heterotic and non-heterotic individuals to survive, how could the reported higher fitness obtain? Similarly, how could the indication of progressive change in fecundity be explained? No wing measurements were performed on the heterotic hybrids, but it was hard to believe that they could have had wings smaller than the parental strains, as it is known that wing length is positively correlated with body size (Zarapkin, 1934; Buzzati-Traverso, 1947*b*). This being true, how could the data presented in table 9 be accounted for? And, finally, how could the increase in population size to be discussed in the following section (8) be expounded?

Because of these questions an experiment was required which might discriminate between the two alternative interpretations and explain the causes of the observed changes. If the observed change in viability had been due to the effects of natural selection, which had produced a new genotype better adapted to the culture conditions, it follows as a necessary consequence that this new combination of genes would have a better survival value in population bottles than strains which had not been subjected to similarly stringent selection. This supposition could be tested if the wild-type flies derived from the experimental populations were put in competition with the original *white-Bar* strain, using the same technique of breeding that had been followed previously. Should the greater viability be the result of a new combination of genes selected over many generations then one would expect that the rate of elimination of the *Bar* and *white* alleles would be faster than in the four original populations. If, on the contrary, the greater viability was due to some sort of complementary action of genes maintained at their F_1 frequency, then no such difference between the results of the old and the new selection experiments could be observed. Two new populations, referred to as Selected 1 and 2 (PS1, PS2), were therefore started with 10 virgin females and 10 males taken from Population 1, and 10 virgin females and 10 males taken from the *white-Bar* strain.

Table 13 and fig. 7 give the results obtained in the course of the first fifteen generations. The comparison with similar data of the four original populations shows beyond doubt that in the Selected populations the rate of elimination of the *white* and *Bar* alleles was

much greater than in the first experiment. After 15 generations the mutant alleles were still present with a frequency of about 15 per cent. in the four old populations, but had already practically disappeared in the new ones. These data seem therefore sufficient to warrant the interpretation that the change in physiological and morphological characters was due to the action of natural selection which in the

TABLE 13
*Selection rate in two of the original four populations and
in the two new (Selected) populations*

Generation	P ₃		P ₄		PS ₁		PS ₂	
	q_B	q_w	q_B	q_w	q_B	q_w	q_B	q_w
1	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
3	0.625	0.625	0.330	0.340	0.132	0.132	0.031	0.031
5	0.399	0.366	0.383	0.390	0.043	0.043	0.030	0.022
7	0.276	0.352	0.163	0.179	0.014	0.029	0.014	0.032
9	0.242	0.283	0.181	0.179	0.023	0.025	0.049	0.041
12	0.201	0.229	0.130	0.160	0.015	0.027	0.029	0.018
15	0.148	0.156	0.080	0.117	0.027	0.034	0.035	...

course of several generations has produced from the combination of the two original genotypes new polygenic constellations which ensured a better viability to their carriers and thereby made the populations progressively better adapted to their environments.

FIG. 7.—Elimination of the mutants *white* and *Bar* in Populations 3 and 4 and in the two Selected Populations. On the abscissa the number of generations, on the ordinate the gene frequencies.

8. NATURAL SELECTION MECHANISMS IN EXPERIMENTAL POPULATIONS

The growth of a population, whether natural or experimental, is known to be the result of the interaction of two groups of factors; the "biotic potential" of the species and the "environmental resistance" (Chapman, 1928).

"The biotic potential represents the potential rate of increase of the species under given conditions. It is realised if there are no restrictions of food, no toxic waste products, etc. Environmental resistance can be measured by the difference between the potential number of organisms which can appear during a fixed time in consequence of the potential rate of increase, and the actual number of organisms observed in a given microcosm at a determined time. Environmental resistance is thus expressed in terms of the reduction of some potential rate of increase, characteristic for the given organism under given conditions" (Gause, 1934).

The increase in number of organisms per unit time depends in the first place on the potential number of offspring (b) which the organism can produce per unit time; the total potential number of organisms present at a given time in a given environment will be given by the product of such potential increase by the number (N) of organisms present at the time considered. If there were unlimited food supply and no other limiting factors the population would grow according to an equation of geometric increase, where the constant b is the rate of potential increase of the organism. Under experimental conditions, as well as in nature, food is limited and other factors may further interfere with the growth of the population; therefore the potential geometrical rate of population growth is not realised, and its limitation is due to a complex of factors which are described under the general term "environmental resistance." In a limited environment with a constant supply of food, such as our population bottles, the potential geometric increase at every moment will be only partially realised, depending on how near the already existing size of the population reaches the maximum number (K) of individuals that can live within the limits of the chosen biotope or its numerical equilibrium. The difference between the maximal population possible and that already realised, expressed in terms relative to the maximal population, shows the number of "still vacant spaces" at a given time in a given microcosm ($K-N/K$). In accordance with this formulation, in populations like the ones of *Drosophila melanogaster* here considered, which were started with a number of adults much smaller than that of the population at numerical equilibrium:

(1) The rate of growth will increase rapidly at first, will reach a maximum and then decline until attainment of the numerical equilibrium.

(2) The environmental resistance will be very small at the beginning and then will increase to a constant maximum value when the population has reached its maximal number, and correspondingly:

(3) The intensity of the struggle for existence, measurable by the ratio of the unrealised part of the potential increase over the realised part of the potential increase, is very little at the beginning of the experiment, but later increases considerably and very rapidly.

The quantitative treatment of the interaction between the biotic potential and the environmental resistance has been expressed by

Verhulst and by Pearl and Reed by means of the logistic curve : $\frac{dN}{dt} = bN \frac{K-N}{K}$. A general discussion of this conception can be found in Gause (1934).

Pearl and his co-workers (see Pearl, 1927) demonstrated that the growth of genetically homogeneous populations of *Drosophila melanogaster* follows the logistic curve. This interpretation is borne out by the data of L'Héritier and Teissier (1933), Bodenheimer (1938) and of the writer so far as homogeneous populations are concerned. The experiments reported here, however, are concerned with populations

FIG. 8.—Comparison between the observed population growth (circles and broken line) and the calculated logistic curve (solid line).

which are changing genetically rather than with purely demographic phenomena. The experimental populations were started with equal numbers of two genotypes. While numerical equilibrium was being reached and later, a struggle for existence took place between the various genetic combinations present. The end result was a change in the biotic potential itself, which led to a further increase in the number of flies present in the bottles, beyond a first equilibrium value, as can be seen in fig. 8. This shows that the classical logistic curve is not realised in genetically heterogeneous populations. It accordingly seems worthwhile to analyse how this may occur.

The two groups of flies originally present in the first bottle differed in the shape and colour of the eyes and, we can now say, in fitness or biotic potential. This original population was accordingly genetically

heterogeneous. At the beginning there were two genotypes which had to face only a small environmental resistance. From the second generation on, however, the struggle for existence became much more complicated because of genetic recombination, which led to an increase in the number of genotypes present. How large this increase was is unknown. It must have produced phenotypic variability in the considered components of fitness beyond the range of variation of the original strains ; but more important for the present discussion, it must also have produced a range of biotic potentials sufficient to permit the operation of natural selection within the conditions prevailing in the bottles. This range must also have changed continuously during four years.

The ways in which different genotypes became represented by different average numbers in the next generation are certainly numerous and we can only attempt to list a few, with particular reference to the factors which have been measured in this investigation.

In the first place, adult flies having different genetic constitutions may show different percentages of sterility, as was the case for our Oregon-R and *white-Bar* stocks. In the second place, flies of one sex can be differentially attracted by flies of the opposite sex having other genotypes ; this seems to be at least in part the cause for the elimination of *white* gene reported by Reed and Reed (1950) and by Merrell (1953), who showed that both red- and white-eyed females discriminate against white males in mating. In the third place, flies genetically different can have different average fecundities, as was shown above. In the fourth place, selective fertilisation might occur. Certain types of sperm might fertilise certain types of eggs more easily than others ; there is no satisfactory experimental evidence on this point, but the possibility cannot be excluded. In the fifth place, different genotypes may differ in fertility as was shown above, and this in turn may depend on a series of factors such as presence of lethals or semilethals, the ability to grab food, capacity to assimilate it, etc. In the sixth place, developmental periods may be different, as shown above. In the seventh place, hardiness in the adult stage can be different, as was found in our experiments in terms of longevity. In the eighth place, resistance against diseases at various life stages can be different. Different values of each of these and similar factors will contribute to the differential success of any genotype in competition, provided that they are genetically determined and extend beyond the range of environmental variation.

While discussing the new adaptation level reached during the experiment (Section 6) it was pointed out that the total potential viability of the populations had increased by a factor of 8. The intensity of the struggle for existence under the experimental conditions employed can be evaluated by the ratio of the unrealised part of the potential increase to the realised part. The four populations reached their first numerical equilibrium after 33 days at an average value

of 704 flies per bottle. The integrated differential equation of the logistic curve best fitting the experimental data is :

$$\frac{704}{1 + e^{4.03 - 0.2344t}}$$

From this expression the values shown in table 14 can be obtained. The lowermost figures of this table give an index of the struggle for existence. It will be noted that this has increased over 1000 times from the outset of the growth of the population to the 33rd day. Is it possible to evaluate the magnitude of competition in the population bottles in a more direct way? Using Pearl's data (1932) on the influence of density of population upon egg production in *Drosophila melanogaster*

TABLE 14

Growth of populations of *Drosophila melanogaster*. The observed values are the average of the four parallel experiments. b (biotic potential) = 0.2344; K (maximum population) = 704

Day	N observed	N calculated	$bN - \frac{dN}{dt}$
3	16	24.40	0.036
6	51	47.63	0.073
9	94	90.02	0.141
12	179	160.73	0.297
15	281	263.67	0.600
18	392	385.46	1.212
21	465	499.29	2.448
24	583	586.66	5.024
27	657	639.44	9.989
30	669	670.77	20.278
33	704	687.16	42.481

one can estimate the number of eggs actually laid in conditions like the ones prevailing in our experiment. Using this estimate and the average viability data presented above, one can compute the number of zygotes that did not reach the adult stage for every one that did. The figure thus obtained, 554, shows again how intense selective processes were under the experimental conditions.

It should be mentioned, furthermore, that with the progress of the experiment it was noticed that the four experimental populations did not remain at the original equilibrium of 700 flies but slowly approached and later exceeded the average number of 900 flies per bottle (fig. 8). At the same time it was noted that the number of adults emerging from each bottle was positively correlated with the number of individuals present in the population when eggs were being laid in that particular bottle. The correlation was curvilinear, as one would expect, in view of the fact that the maximum number of adults which can be recovered from a certain amount of food is practically constant beyond a certain number of fertilised eggs present in the original culture bottle. Buzzati-Traverso (1947b) has shown

that it is not possible to obtain more than an average of about 140 adult flies from between 300 and 1000 eggs in experiments in which the same type of vial and an inbred strain of *Drosophila melanogaster* were used and the eggs were placed on the medium all at the same time. It seems safe to conclude that the increased viability and ability to exploit the available food made the populations better adapted to the environmental condition in the sense that they became more numerous. If it were possible to calculate from our data the value of the biotic potential at the end of the experiment it is certain that we would find a figure larger than the original 0.2344.

The consequence of these results for problems of demography will not be discussed here (Buzzati-Traverso, 1954); but a conclusion seems ineluctable: that the intensity of natural selection increased during the experiment, even though the environmental conditions were constant. This conclusion pertains only to the action of natural selection on traits determined by polygenes, for no such increase in the intensity of the struggle for existence is considered by the current mathematical theories of evolution in which the effects of selection on the frequencies of genes primarily affecting a single trait have been analysed. A new theoretical treatment of natural selection in polygenic systems therefore becomes necessary.

One more comment seems pertinent. Most discussions of evolution from the standpoint of genetics consider the selective value of a certain gene, or chromosome type alone, and, so to say, *in vacuo*. The fact is that the change in frequency of a single gene (or chromosome) during a number of generations means that the individuals carrying its allele will produce more or fewer adult offspring in the next generation than the individuals not carrying it. The factor which is decisive for changing frequency is a *productivity differential* involving of necessity the whole genotype and not one gene alone, for the latter will have different survival values in different genetic milieus. Some extreme mutants, like those mostly used in laboratory experiments, may affect specifically the productivity of its carriers to such an extent as to make the effects of the rest of the genotype and of the interactions of the mutant with it insignificant. But under natural conditions the commonest case is very likely that of "small" mutants where the natural selection mechanism probably involves many genes at one time. Thus, a continuous increase in the intensity of natural selection fostering both productivity and change in polygenic traits may be of cardinal significance in evolutionary events.

Finally, I have shown that a given physiological or morphological trait may differ in degree in parallel experiments, and that, as a consequence, the improvement in fitness realised in the four populations described above was not identical after a certain number of generations. In view of the fact that we are dealing with characters which are controlled by many genes this is not surprising. The very exacting sieve of natural selection acts throughout the whole life cycle

of an organism and it is well possible that the same results, namely an increased total productivity or an adaptive change in morphological characters, can be reached in various ways through the action of various constellations of genes. For example, one of these may guarantee success during larval development, another may insure a hardier adult life. Each of two individuals carrying either of these constellations may show an identical total productivity value and yet the constituent factors, taken separately, can be very different. The complex genetic conditions which underlie the observed changes in fitness need not be the same in the four populations, nor indeed in all of their members. The only fact of significance is that one end result was achieved : higher productivity.

9. EVOLUTIONARY SIGNIFICANCE OF CHANGE IN POLYGENIC TRAITS

J. B. S. Haldane has pointed out (1949), that in view of the arbitrariness of distinctions between systematic categories, the most reliable measure of evolutionary rate is one which relates changes in the dimensions of the body of an organism or its parts, to time, whether expressed in units of geological time or in generations. It is generally accepted, however (see, *e.g.*, Fisher, 1930 ; Timoféeff-Ressovsky, 1939 ; and Simpson, 1944 and 1954) that mutations important for evolution are small, as measured in terms of structural change, and therefore that evolution can be explained in terms of the accumulation of small mutants affecting quantitative traits. This opinion seems substantiated by two considerations, that (*a*) differences between subspecies, species and higher systematic categories are ordinarily of a quantitative nature and accordingly fall under the control of polygenic systems, and that (*b*) a great majority of characters used by the taxonomist are in terms of measurements or ratios between measurements.

The data presented above bring evidence to show that evolutionary changes of the same degree as those observed by the paleontologist and the morphologist which may have taken millions of years to produce, can be observed within the limited span of a few dozens of generations. The variation in the size, and therefore shape, of the wings, as well as that concerning the size of the body are of this type. Furthermore, the correlation of reduction in wing size and increase in body size demonstrates that a change must also have occurred in the values of the coefficients of relative growth. These changes are significant for an understanding of evolutionary events not only from the viewpoint of qualitative differences but of quantitative ones also. If, in the case of the shortening of the wings, we take the values derived from Population No. 2 at its 55th generation (157.7 ± 0.726) and compare it with that of the smaller of the two parental strains namely *white-Bar* (159.8 ± 0.787), we can calculate

that the average rate of change per generation is 0.00024 per cent. This change corresponds to a little less than one-twenty-thousandth of the standard deviation of the value of Population 3. This means that the observed rate was of the same order of magnitude of the fastest evolutionary change, heretofore recorded, namely the length/height index of the human skull. This fact brings further evidence to indicate that known genetic processes are quite adequate to account for the evolutionary changes observed in the fossil record.

The analysis presented above brings still other evidence which may be of significance for an understanding of historical evolution. Paleontologists have found many examples of progressive increases in size within phyla. This seems to be a general trend among terrestrial vertebrates, and similar evidence is accumulating for other animal groups (see e.g. Stenzel, 1949). Castle (1932) argued that phylogenetic increases in size of this sort may be caused by competition among individuals in a given population of the same species and that the larger individuals or those attaining a large size earlier in life than others would be at an advantage over the smaller ones. Competitions *in utero* between mammalian embryos would be favoured by genetic conditions which would result in a more rapid rate of development, and thus a larger size at birth and maturity. It is quite possible that a similar phenomenon has actually occurred in our populations, where, as it has been shown, genotypes producing a faster development have been favoured.

My material also illustrates another evolutionary process. When the wild-type flies from the first natural selection experiment were put in competition with the original *white-Bar* strain, the two mutant alleles were eliminated at a much faster rate than in the four original populations, as a result of the greater fitness which had been attained by the "selected" wild type. Let us now consider a condition in nature in which there is a steady migration from Population A to Population B. As a result of this accretion of new variability by the latter, natural selection may produce genotypes better adapted to the environmental conditions of Population B by incorporating the favourable migrating genes and discarding the less favourable ones. In the course of time the pressure of selection against the latter would increase and they would accordingly be eliminated more and more rapidly, as was the case in our "new experiment" (see section 7). In this way a very elementary sort of isolating mechanism may arise.

New experimental data always raise new questions and broaden the scope of our investigations, and this attempt to analyse the action of natural selection on polygenic characters has accordingly proved to be quite rewarding. Its main significance lies in the fact that it may encourage the use of similar techniques in order to reach a deeper insight into the factors responsible for the "advancement of all organic beings, namely, multiply, vary, let the strongest live and the weakest die."

10. SUMMARY

1. Four parallel populations of *Drosophila melanogaster* were maintained in approximate numerical equilibrium for a period of about 4 years (100 generations) using Pearl's technique.

2. The foundation stock of each of the four populations consisted of equal numbers of males and females of a wild-type Oregon-R strain and of another strain homozygous for *white* and *Bar*. About 2,000,000 flies were classified for sex and phenotype. After about 30 generations the mutants were completely replaced by their normal alleles.

3. Samples of the two original strains and of the four populations were tested at different times for a series of physiological and morphological traits.

4. The following changes occurred in the experimental populations: (i) Sterility of females of the two original strains disappeared. (ii) A very marked and progressive increase in the average fecundity of females took place. (iii) The rate of development of the flies increased. (iv) The adult longevity of the flies increased. (v) Length and width of the wings become smaller. (vi) The average body weight of the flies increased.

5. The observed improvement in fitness by a factor of 8 over a period of about 80 generations as well as the morphological changes are interpreted as being due to the action of natural selection which, making use of the genotypes of the two original strains, has produced new polygenic systems better adapted to the conditions of the experiment.

6. To test the validity of this interpretation two new populations were started in which the wild-type flies derived from the previous experiment were put in competition with the original *white-Bar* strain. The rate of elimination of the mutant alleles was in this case much faster.

7. The mechanisms of natural selection in experimental populations are discussed and it is shown how the genetic heterogeneity of a population may affect its growth. This will follow a curve different from the logistic derived from the assumption of a constant value for the biotic potential of an organism which, on the contrary, falls under the control of natural selection. In fact the average number of adults in the four populations departed from the logistic curve calculated from a constant biotic potential. It is further concluded that the intensity of natural selection is bound to increase under constant environmental conditions if one takes into consideration polygenic systems.

8. The evolutionary significance of changes in polygenic traits is discussed. The observed rate of change in wing size is shown to be of the same order of magnitude as the fastest known rate of evolution as revealed by the fossil record. The increase in body size, too, is in agreement with progressive increase in the dimensions of animals observed by paleontologists for many phyla.

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