GENETIC ANALYSIS OF A "PLATEAUED" POPULATION OF DROSOPHILA MELANOGASTER[1]

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SELECTION has long been recognized as a primary force in the evolution of species under either the natural or domesticated state. Yet perplexing cases occasionally arise where no response to artificial selection is observed in a population even though genetic variation is apparently present. This situation has been observed in *Drosophila melanogaster* populations under selection for such quantitative traits as bristle number (MATHER 1941; SCOSSIROLI 1954; RASMUSON 1955); wing length (ROBERTSON and REEVE 1952); and fecundity (BELL, MOORE and WARREN 1955). Similar results have been reported for poultry populations under selection for high egg production (LERNER and DEMPSTER 1951; DICKERSON 1955; and YAMADA, BOHREN and CRITTENDEN 1958).

Attempts to penetrate the haze surrounding the genetics of plateaued populations have led to such concepts as genetic inertia (DARLINGTON and MATHER 1949), coadapted gene complex (WALLACE 1953), genetic homeostasis (LERNER 1954) and genetic slippage (DICKERSON 1955). While differing in detail, these concepts present a common theme of genetic variation being conserved in a population at the expense of selection response. In some manner, genetic deviations beyond certain limits around the mean of a population place these individuals at a disadvantage from a fitness standpoint.

During a comparison of different methods of selection with *D. melanogaster*, a closed population under selection for high fecundity was observed to plateau early in the experiment. Marked inversions for all major chromosomes in this species provide a unique analytical tool to evaluate the importance of lethal, sterility and subvital factors in relation to the plateau for fecundity.

MATERIALS AND METHODS

The closed population of *D. melanogaster* analysed was the one developed in the second selection experiment reported by BELL, MOORE and WARREN (1955) after the population had been selected for high fecundity over a period of 47 generations. The population was reproduced each generation by selecting, on individual and family merit, 40 females along with their nonsib mates out of a total population of 400 pairs. One generation previous to the genetic analysis, the closed population was reduced to 20 families by combining the selected

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families two at a time. A rapid response to selection had been observed in this closed population during the early generations of selection; however, after reaching a peak of performance at the seventh generation no further progress was evidenced and the data suggested a decline. This decline occurred regardless of continued selection with the selection differential showing little change in magnitude.

The genetic analysis, which afforded an objective determination of the frequencies of deleterious factors in the closed population, was made possible by a marked-inversion technique (a modification of MULLER's method for constructing homozygous stocks) utilizing an analyser stock obtained from DR. A. B. BURDICK, Department of Biological Sciences, Purdue University and having the following genetic construction:

sc^{81} B In-S w^{a}sc^{8}; In-SMI, al Cy sp^{2}/Pm ds^{33k}; Ubx^{130} e^{s}/C Sb; pol.

The *B*, *Cy* and *Ubx* chromosomes contain the important recombination suppressors. Lines extracted by this technique are referred to as "isogenic" or "homozygous" even though the degree of homozygosity is not expected to be complete. The increased recombination between one pair of chromosomes due to the presence of a heterozygous inversion in a non-homologous pair was first pointed out by STURTEVANT (1919). However, our experience has led to confidence in the above marked inversions. Breakup of the marked chromosomes is rare and inbred lines formed by this method show a greater degree of inbreeding depression, an indirect measure of homozygosity, than that found in inbred lines formed by many generations of full sib-matings.

The type of genetic analysis employed in this study is unique with *D. melanogaster* and provided for the determination of sex-linked lethals plus autosomal sterility, subviable and lethal factors. The mating system used is shown in Figure 1. Virgin females from each of the 20 families, which constituted the closed population, were mated individually to males of the analyser stock as shown in Cross I. Approximately the same number of females was used from each family. The male progeny from Cross I would represent in their sex chromosome the gametic array of sex-linked genes from their dams, while the female progeny would have their sex chromosome from the closed population balanced with the marked sex chromosome from the analyser stock. Evidence of subviable or lethal sex-linked genes would be revealed by the sex ratio of progenies from the individual females in Cross I.

A single *Cy Ubx* F_1 male from the progeny of each Cross I mating was backcrossed to an analyser female in Cross II so that duplicate samples of chromosomes from the closed population would not be obtained. This male carried one wild type chromosome from each of the chromosome pairs in the original female. To duplicate in the Cross III progeny the same wild type chromosomes which were originally sampled, *B Cy Ubx* daughters of each Cross II were backcrossed to their respective sires. One hundred and thirty-two matings were made in Cross III and 126 reproduced.

If a recessive autosomal lethal were present in a chromosome sampled from the closed population, the homozygote in the Cross III progeny representing this

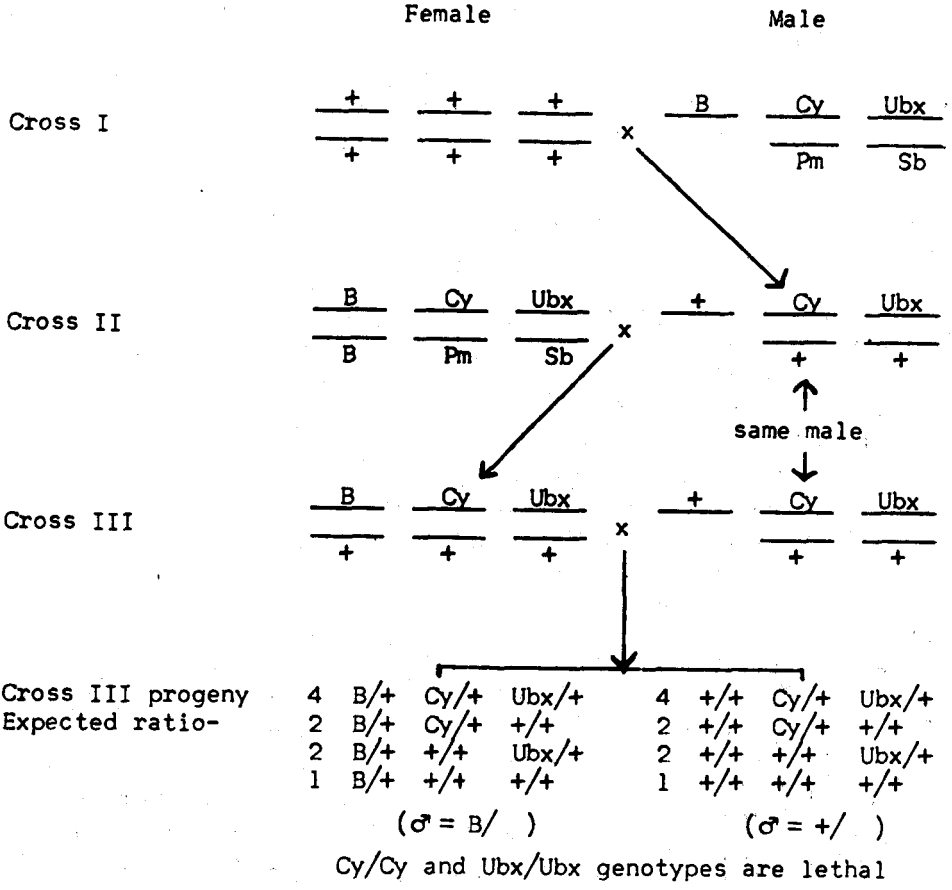

FIGURE 1.—System of mating utilized for detecting subviable, lethal and sterility factors in the plateaued population (explanation in text).

chromosome would be missing. Thus, the frequency of lethals in a population and the chromosomes with which they are associated can be determined directly.

The ratio of fertile isogenic lines to the total directly measures the frequency of sterility factors in the closed population. Rather than a particular type failing to appear in the Cross III progeny, as with a lethal, it would instead be sterile.

The study was extended to differential viability among the progeny of Cross III when classified according to sex and chromosome type. By comparing through a factorial analysis the frequencies of the various types, the effects of heterozygosity, sex, and each of the major chromosomes on viability were observed. Also, differences in viability among the extracted wild type genomes were investigated.

In the plateaued population, genetic variation for the selected trait, fecundity, was estimated from data obtained on six of the "isogenic" lines and their hybrids. These estimates are in addition to those reported earlier on correlations among relatives (BELL, MOORE and WARREN 1957).

Experimental conditions including media, population densities, temperature, humidity and light were made as similar as possible to those provided for the closed population during the selection experiment and were thought to be optimum for fecundity.

RESULTS

Sex-linked lethals: The frequency of sex-linked lethals in the closed population was estimated from an analysis of the sex ratio of Cross I progenies resulting from the mating of virgin females from the closed population to analyser males. The progeny from 57 of the Cross I matings were counted and classified by sex. One count was made of the progeny two days after they began emerging. At this time a male was randomly chosen from each progeny group to be used in Cross II matings. A second count of these 57 Cross I progenies was made one week later and prior to the emergence of any F_2 flies.

Eight of the 57 samples analysed (or 14.04 percent) gave a significant deviation from a normal 1:1 sex ratio, with a shortage of males in every case. These results are summarized in Table 1. Of the eight which deviated from the normal, three of the samples (or 5.26 percent of the total) gave a good fit to a 2 female:1 male sex ratio which indicated the presence of a sex-linked lethal in the original female. When those samples which gave a significant deviation from a normal sex ratio were excluded from the analysis, the total sex ratio of the remaining 49 samples gave a significant deviation from a normal 1:1 sex ratio with an excess of females. This is evidence of subviability factors and suggested the relative viability analysis in a subsequent section. The observed frequency of possible sex-linked lethals was too low to be a major deterrent to selection response. In fact, the estimated frequency of sex-linked lethals is very little higher than would be expected from the reported natural mutation frequencies. DEMEREC (1937),

TABLE 1

Sex ratio analysis of eight Cross I progenies which gave a significant deviation from a 1:1 sex ratio and the goodness of fit of these to a 2 female:1 male sex ratio

	Number of progeny			Chi-square values	
Cross I mating	Male	Female	Total	1:1	2:1
1	105	166	271	13.73**	3.59
2	158	209	367	7.09**	15.45**
5	87	143	230	13.63**	3.37
6	77	107	184	4.89*	6.03*
95	66	91	157	3.98*	4.99*
157	86	130	216	8.96**	4.08*
172	58	88	146	6.16*	2.67
29	112	154	266	6.63**	9.18**
Remaining 49 matings	4060	4308	8368	7.34**	

* Significant at the .05 level of probability.
** Significant at the .01 level of probability.

in studies on mutability in *D. melanogaster*, reported the frequency of spontaneous lethals in chromosome I of various wild type stocks to range from 0.066 ± 0.03 percent to 1.09 ± 0.15 percent.

Autosomal lethals: Autosomal lethals could cause a population to respond no longer to selection if some mechanism were present which maintained the lethals at relatively high frequencies. In the event of lethal heterozygote superiority for the selected trait or for fitness, selection would tend to maintain the lethal and closely linked genes in equilibrium. Another mechanism might be a lethal located within an inversion with the inversion heterozygote superior to the homozygous wild type. Selection would favor the inversion, with a resulting increase in the frequency of the lethal.

Homozygotes for each chromosome of the genome originally sampled would be expected among the progeny resulting from Cross III of Figure 1 if no lethal factors were present. The failure to observe individuals homozygous for any particular chromosome pair would lead one to suspect a lethal factor in that particular chromosome.

Of the originally sampled genomes, 126 were successfully carried through the prescribed mating plan of Figure 1 and resulted in Cross III progenies which were recorded by sex and type. Individuals homozygous for all three major chromosomes were found in 113 (or 90 percent) of the progeny groups. If one considers that sex-linked lethal factors were eliminated at Cross I, then a liberal estimate for the frequency of autosomal lethal genomes is ten percent. One could accept this estimate and conclude that autosomal recessive lethal factors were not causing the plateau in selection response by either a balanced lethal complex or by superiority of the lethal heterozygote. However, there is evidence that the actual lethal frequency was considerably lower than 0.10.

Some of the Cross III progenies had relatively few individuals which made the presence of a complete homozygote unlikely since the probability of obtaining such an individual here was only one out of 18. This can be appreciated by observing in Table 2 the total number of individuals in each of the 13 Cross III progenies not yielding any homozygous individuals. With ten of the 13 cases having less than ten observations each, it is obvious that the estimate of lethals at ten percent is too high. To obtain a more realistic estimate, the remainder of Table 2 provides the probabilities of obtaining one or more of the complete homozygotes in each mating. If we assume no chromosomal interaction for lethality and a conservative 0.50 probability level, the frequency of genomes containing an autosomal lethal is estimated to be 7.1 percent. The frequency of second chromosome lethals was found to be 3.2 percent and that of third chromosome lethals was estimated to be 3.9 percent.

BERG (1941), in studies with the Nikita Botanical Garden populations of *D. melanogaster*, reported a lethal frequency of 14.49 percent among 1,905 chromosomes studied. WALLACE (1950) observed a lethal frequency of 4.6 percent after seven generations from an original lethal-free population. REEVE and ROBERTSON (1953) reported that lethal factors occurred in a population of *D. melanogaster* under selection for increased wing and thorax length which were associated with

TABLE 2

A summary of the 13 Cross III matings which could possibly possess autosomal lethal factors

Cross III mating	Homozygous chromosomes absent	Number of progeny	Probability of observing the absent genotype if nonlethal
59	II	9	.97*
152	II	58	.99*
204	II	48	.99*
215	II	17	.99*
285	III	2	.56*
294	III	1	.33
4	III	4	.80*
82	III	1	.33
371	III	3	.70*
375	III	3	.70*
53	III	6	.91*
58	II, III	2	.21
112	II, III	1	.11
Total number of autosomal lethals			9
Estimated frequency in the population based on the total sample of 126 genomes		9/126	.071

* Lethal assumed present for probabilities greater than .50.

the third chromosome. They concluded, however, that the lethal factors could hardly explain the refractory response to selection which they observed.

The observed frequency of autosomal lethals in the closed population under consideration in this study was as low or lower than that reported for nonselected populations. Obviously, lethal factors at a frequency of less than four percent did not contribute significantly to the lack of response to selection for high fecundity.

Sterility factors: Evidence suggesting that sterility genes were infrequent in the closed population is provided by its reproductive history. Each generation the population was reproduced from 40 single pair matings. Seldom did the frequency of unsuccessful matings exceed five percent.

The frequency of sex-linked sterility genes would be a direct function of the proportion of males in Cross II matings of Figure 1 which failed to produce progeny. These males would possess a random sex chromosome from the plateaued population while the autosomes would be heterozygous for the marked inversions. A total of 160 individual male matings were made in Cross II and 146 reproduced. If one assumed all 14 failures to be of genetic origin rather than environmental, the frequency of sex-linked sterility factors would be only nine percent. Autosomal recessive sterility factors would be made homozygous in the isogenic progeny by the outcross technique, and their effects would be observed by the inability of isogenic lines to reproduce. Less than five percent of the extracted lines were sterile or failed to reproduce in the first generation after extraction. Therefore, neither sex-linked nor autosomal sterility factors were

likely causes for the plateau in response to selection for fecundity in the closed population. In fact the frequency of sterility factors in this population is less than that reported by DOBZHANSKY (1951) for natural populations.

Differential viability

After eliminating the possibility of lethal and sterility factors as causes of the plateau in response to selection for fecundity, the possibility of recessive subviable genes was considered. These are usually associated with inbreeding depression. While deleterious when homozygous, they may display heterozygote superiority, as suggested by WALLACE (1950), due to overdominance or pseudo-overdominance. Such superiority of the heterozygote for the selected trait, fecundity, would cause a plateau in response even though genetic variation was present within the population. Likewise, heterozygote superiority for fitness would operate within the population to cause a plateau in response for the selected trait. By observing the relative viability of the various genotypic classes in the progeny of Cross III, it is possible to compare levels of homozygosity with inversion heterozygotes. In addition, these data made possible the identification of the genetic unit or factor with which the effect was associated.

Genetic factor analysis: The genetic factors of sex and the three major chromosomes, I, II, and III, were analysed in a 2^4 factorial manner. Sex was at two levels i.e., male or female, and each of the three chromosomes was at two levels i.e., homozygous wild type or heterozygous inversion type. The frequency of flies for each of the 16 classifications (2^4) in the Cross III progeny were grouped by the family from which the original females were taken and standardized to an equal expectation of 2.78 percent for the least frequent class (+/+ +/+ +/+) by the genotypic frequencies from Figure 1. The average values over all families are given in Table 3. For the factorial analysis, the data were transformed to arcsin $\sqrt{p}$, where p is the percentage of flies for each standardized classification

TABLE 3

Mean percentage of heterozygous inversion and wild type flies in Cross III progenies summarized by sex and genotype and standardized for expected genotypic ratio. Larger values indicate superior viability

Genotype by chromosome*			Relative viability**	
I	II	III	Males	Females
B/+	*Cy*/+	*Ubx*/+	2.28	2.80
B/+	*Cy*/+	+/+	2.71	2.67
B/+	+/+	*Ubx*/+	2.74	3.21
B/+	+/+	+/+	2.29	2.38
+/+	*Cy*/+	*Ubx*/+	3.21	3.03
+/+	*Cy*/+	+/+	3.18	2.40
+/+	+/+	*Ubx*/+	3.16	3.04
+/+	+/+	+/+	2.58	2.19

* Males are *B*/ or +/ for chromosome I.
** Expected value = 2.78.

within each family. In three cases missing values were obtained by the method given by WALKER and LEV (1953). The transformed values were summed over all families, and the totals were compared in linear contrasts which were so made that differences, d, which were negative represent a superiority of females over males in comparisons involving sex, and homozygous wild types over the inversion heterozygotes in comparisons involving chromosomes. The variance of the transformed variable becomes that of the binomial distribution and takes the value of $\sum_{i=1}^{18} 821/N_i$, where N_i equals the total number of individuals produced in the ith family, and the fraction $821/N_i$ is summed over the 18 families which yielded data suitable for this analysis. With one degree of freedom the test gives an approximation to d/σ which is compared with the normal deviate.

When the totals for each sex over all Cross III progeny were compared in Table 4, no significant difference between the frequency of males and females was observed. This seems to contradict the significantly higher frequency of females among the progenies of Cross I (Table 1). Apparently the sex-linked low viability factors present in Cross I progenies were eliminated during the series of matings shown in Figure 1. Each wild type chromosome I was carried in the hemizygous male in Cross II matings, and through the outcross technique additional selection was exerted against sex-linked subvital genes by requiring these males to survive long enough to sire Cross III matings.

Further comparisons in Table 4 involve the homozygous wild type with the heterozygous inversion type for each chromosome pair. The homozygous wild type was superior at chromosome I, possibly of equal viability at chromosome II

TABLE 4

Genetic factor analyses involving the effects of sex, each major chromosome and their interactions on the relative viability of heterozygous inversion types versus *wild types in the Cross III progenies. The values approximate* d/σ *as described in the text*

	Genetic factors	Comparison values†
1.	Sex	0.02
2.	Chromosome I	−2.05*
3.	Chromosome II	1.30
4.	Chromosome III	3.90**
5.	Sex × I	−2.85**
6.	Sex × II	0.32
7.	Sex × III	−1.24
8.	I × II	−0.69
9.	I × III	−0.46
10.	II × III	−2.69**
11.	Sex × I × II	0.07
12.	Sex × I × III	0.62
13.	Sex × II × III	−0.36
14.	I × II × III	−1.01

* Significant at the .05 level of probability.
** Significant at the .01 level of probability.
† Males minus females where sex is involved; inversion type minus wild type where chromosomes are involved.

and inferior to the inversion heterozygote at chromosome III. Recessive subviable factors were rare in the closed population, if present. Although the marked-inversion chromosomes of the analyser stock are not normal, the vigor of the inversion heterozygotes in the Cross III progenies more nearly approaches that of wild populations than that of inbred populations.

The comparisons involving the main effects practically eliminated subvitals as a cause for the plateau in response to selection; however, the complete factorial was made and some of the interactions between the main factors are of interest from an evolutionary standpoint. The first three interactions, which involve sex with each of the major chromosomes, indicate whether or not the sexes differ in viability for any of the three major chromosomes over and above that due to the average effect of sex or the three major chromosomes alone. The sex of the individual influenced the relative superiority of chromosome I isolated from the closed population (Table 4). The values in Table 3 reveal two causes for this "Sex × I" interaction. The first is the superiority in chromosome I of the inversion heterozygous ($B/+$) female class over the hemizygous ($B/\ $) male class. The second cause is independent of the marked chromosome I and is interesting from an evolutionary viewpoint. The males exceed the females in the last four clases in Table 3. The two sexes in each class are summed over identical genotypes; however, the males are hemizygous for the wild type chromosome I while the females are homozygous for the same chromosome. These results provide evidence that sex-linked genes respond differently in the sexes when exposed to evolutionary forces. On the one hand, in females there would be selection for heterotic loci or coadapted gene complexes, as in most cross-fertilized diploid organisms. The reduced viability in the chromosome I homozygous females suggests this to be the case. On the other hand, the transmission each generation of a portion of the sex-linked genes through hemizygous males suggests strong selection for genes with favorable additive effects for viability. Superior viability cannot in this case be dependent upon heterotic loci. This dilemma is circumvented through a form of genetic dimorphism. Natural selection would favor those genes which produced superior viability when present either as a single dose in the hemizygous male or as diploid in females with superiority due to allelic interaction. Thus, one would expect inbreeding depression in homozygous females but not in males, with the consideration restricted to sex-linked genes. The results agree with this expectation.

Extending the analysis to the possibility of interaction between sex and chromosomes II and III, one finds in Table 4 that neither of these comparisons is significant. The common behavior of chromosomes II and III in both sexes would be expected from an evolutionary standpoint since the sexes presumably do not differ for these chromosomes.

Interactions between chromosomes, due to differential effects upon viability for the three major chromosomes, were tested by comparisons between heterozygous and homozygous types. These interactions, if significant, are indicative of epistasis between chromosomes for viability. The values for chromosomes "I × II" and chromosomes "I ×III" were not significant although the interaction of

chromosomes "II × III" was highly significant (Table 4). This reflects an epistatic effect between chromosomes II and III in favor of the homozygous wild type, and it is in addition to the average effect of each of the chromosomes alone. The absence of interaction between chromosome I and either of the other major chromosomes may be a function of the dual nature of selection on chromosome I. None of the three factor interactions are large enough to merit discussion.

Genome analysis: We have established that the wild type chromosomes extracted from the plateaued population possessed few lethal, subviable, or sterility factors. The viability of wild type homozygotes was about equal to that of the standard heterozygous inversion types. However, the factorial analysis does not measure genetic variation in viability among the wild type genomes.

The homozygous wild type for any particular chromosome sampled from the closed population cannot be compared directly with that from another genome since each set of Cross III progenies (see Figure 1) was reared in separate cultures. However, the viability of the homozygous wild type relative to the standard inversion heterozygote can be determined within each culture for each of the three major chromosomes and these relative viability values are comparable for all genomes sampled from the closed population.

The measured variable for viability becomes the ratio of flies which were homozygous wild type for each of the three major chromosome pairs and for sex to the total number of flies for the Cross III progeny of each genome sample, adjusted to an equal expectation according to the genotypic frequencies in Figure 1. The ratios were transformed to arcsin values for the analysis. Thus, each set of Cross III progeny, which represents a single wild type genome sampled from the closed population in Cross I, had six measurements reflecting the viability of each wild type chromosome when homozygous relative to the standard inversion heterozygotes present in the same culture. The six values by chromosome and sex represent I♂, I♀, II♂, II♀, III♂ and III♀. Cross III progenies with less than five individuals in any one classification were excluded. Such a restriction automatically excludes those few genomes previously identified as carrying lethal factors. Also, it might exclude some semilethals or subvitals; however, percentage values based on less than five individuals are unreliable. A total of 34 wild type genomes had Cross III progenies suitable for analysis. The variation in viability was statistically analysed for the three main effects of sex, chromosome, and genome, plus the interaction of these effects. The general model for the design is:

$$Y_{ijk} = \mu + S_i + C_j + SC_{ij} + G_k + SG_{ik} + CG_{jk} + SCG_{ijk},$$

where

Y_{ijk} is the arcsin $\sqrt{\text{percentage}}$ of wild type individuals of the total emerging in the kth genome for the jth chromosome in the ith sex.

μ is the average of all the Y_{ijk}.

S_i is the fixed effect of the ith sex; $i = 1,2$.

C_j is the fixed effect of the jth chromosome; $j = 1,2,3$.

SC_{ij} is the interaction of the ith sex with the jth chromosome.

G_k is the effect of the kth randomly chosen genome; $k = 1, \ldots, 34$.

SG_{ik} is the interaction of the ith sex with the kth genome.

CG_{jk} is the interaction of the jth chromosome with the kth genome.

SCG_{ijk} is the interaction of the ith sex with the jth chromosome with the kth genome and is used as the error estimate.

The analysis of variance for these viability data is presented in Table 5. The highly significant effect due to "Sexes" appears to contradict the comparison of "Sex" in the factorial analysis (Table 4). Actually, both analyses are reflecting the sex differences apparent in the summary of mean values given for genotypes in Table 3. In every case the hemizygous wild type chromosome I males (+/) are superior to the homozygous I females (+/+). This effect contributes to the significant mean square due to "Sexes" in Table 5, since this analysis was made on viability values for wild type chromosomes. The comparison in the factorial analysis of Table 4 included marked chromosome types as well as wild type. The superiority of $B/+$ females over $B/$ males has been pointed out earlier. Therefore, no over-all sex difference is observed over both marked and wild type chromosome I; however, these viability differences are revealed in Table 4 by the "Sex × Chromosome I" interaction. The highly significant effect shown in Table 5 among "Chromosomes" confirms other studies showing non-homologous chromosomes to vary in the magnitude of their effects on quantitative traits.

The most significant aspect of Table 5 is the small and insignificant variance due to differences among "Genomes" in contrast to the large and statistically significant "Sex × Genome" interaction. While not differing in their average effect on viability, the genomes exhibited differential behavior in the sexes. Apparently, those genomes contributing to superior viability in one sex lead to below average viability in the other sex. Presumably a type of genetic dimorphism had developed which would maintain genetic variation in a population. Those genes involved would reach an equilibrium frequency whereby positive natural selection within one sex would be counterbalanced by a negative selection in the opposite sex. In essence, this amounts to a negative genetic correlation between viability in the two sexes.

TABLE 5

Analysis of variation in relative viability among wild type genomes sampled from the closed population

Source of variation	Degrees of freedom	Mean square
Sexes (S)	1	192.64**
Chromosomes (C)	2	61.72**
Genomes (G)	33	6.48
S × C	2	11.24
S × G	33	24.46**
C × G	66	6.40
S × C × G	66	5.49

** Significant at the .01 level of probability.

Heterozygous versus *homozygous wild type:* The conclusion that the wild type genomes were not different in their average viability values rests on the comparison of homozygous wild types. While genetic variation in viability was present as revealed by the "Sex × Genome" interaction, another possible source in the original population and not revealed above would be a superior viability for heterozygous wild type individuals. Such a situation would not necessarily inhibit response to selection for high fecundity if genetic variation for the selected trait were present and not completely correlated with the viability factors. However, a comparison involving heterozygous wild types in addition to homozygous wild types was desirable as a further check on the type of genetic variation in the closed population plus a validation of genetic interactions found in the earlier analyses.

The homozygous lines extracted from the closed population had been reproduced by sib-matings for approximately 25 generations before the above analyses revealed the need for a comparison involving the heterozygous wild type. Because these lines may have changed during this period, new homozygous lines were extracted from each of three randomly chosen lines. This re-extraction of homozygous lines followed the same mating outline given in Figure 1. This provided the three "old" homozygous lines (184–1, 262–2, and 276–2), designated 1, 2, and 3, and their corresponding re-extracted "new" sublines 1a, 2a, and 3a. Virgin females from each of the six lines were mated separately with analyser stock males in the same manner as Cross I of Figure 1. The resulting *B*/+ *Cy*/+ *Ubx*/+ female progeny were then intercrossed with the +/ *Cy*/+*Ubx*/+ male progeny within lines and all possible combinations between lines as illustrated in Figure 2 to produce progenies which were classified by sex and genotype. They were expected to occur in frequencies identical to those for the Cross III progenies in Figure 1. The matings in Figure 2 are analogous to the combination of six inbred lines in all possible combinations; however, the hybrid progeny from reciprocal crosses were pooled to simplify the statistical analysis.

In order to contrast the viability of heterozygous wild types *versus* homozygous wild types, it is necessary to summarize these data in the same manner as described in the *Genome analysis* section. The measured variable was the ratio of flies which were wild type (homozygous or heterozygous depending upon the mating involved) for each of the three major chromosome pairs and sex to the total number of flies for each mating. The values were adjusted to an equal expectation according to the genotypic frequencies for Cross III progeny in Figure 1. The statistical model is the same as in the previous section except the main effect of "Genomes (G)" is more appropriately labeled "Matings (M)" since it represents the wild type genotype in either the heterozygous or homozygous state as prescribed by Figure 2. Each mating has six measurements, as before, which reflect the viability of either homozygous or heterozygous wild type chromosome pairs relative to the standard inversion heterozygotes in the same culture. In this manner a comparison is possible among wild type flies with different degrees of heterozygosity without the confounding influence of different culturing conditions. Obviously, such use of the standard inversion heterozygotes as environ-

mental controls makes the questionable assumption that genotype by environment interactions are negligible.

The analysis of variance for these viability data is presented in Table 6. The main effects "Sexes" and "Chromosomes" are highly significant as in Table 5.

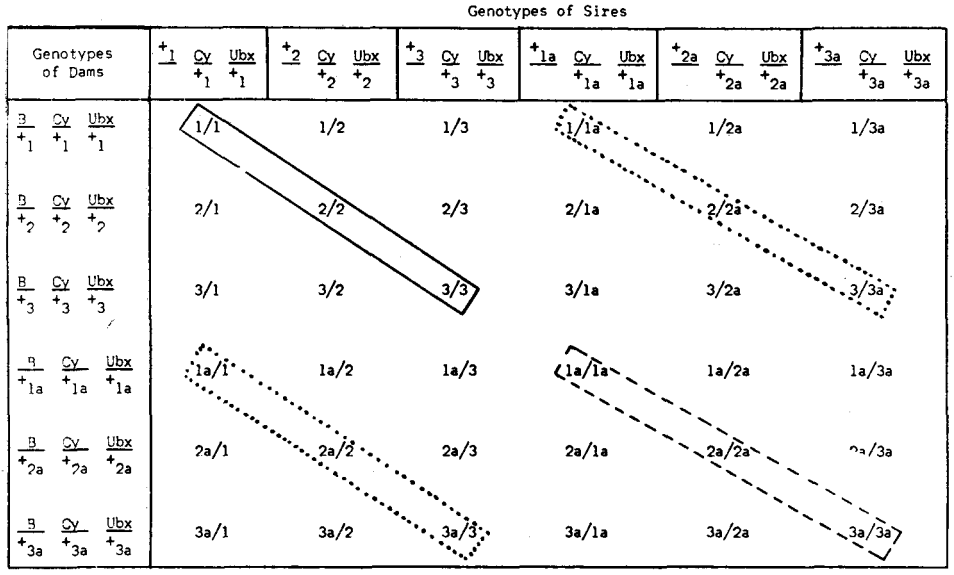

FIGURE 2.—Mating combinations involving marked inversion heterozygotes carrying wild type chromosomes from six homozygous lines. Listed in each cell are the sources of wild type chromosomes in the resulting progeny. See text for identification of those combinations which yield the different levels of heterozygosity.

TABLE 6

Analysis of variation in relative viability among the progenies resulting from the crossing of six "homozygous" lines

Source of variation	Degrees of freedom	Mean square
Sexes (S)	1	173.42**
Chromosomes (C)	2	343.49**
Matings (M)	20	10.22*
Between levels of heterozygosity†	3	4.41
Between homozygous "old" lines	2	1.69
Between homozygous "new" lines	2	46.50**
Between heterozygotes within lines	2	20.38*
Between heterozygotes between lines	11	4.91
S × C	2	8.90
S × M	20	16.31**
C × M	40	4.92
S × C × M	40	4.80

* Significant at the .05 level of probability.
** Significant at the .01 level of probability.
† Homozygous old lines *vs.* homozygous new lines *vs.* heterozygotes within lines *vs.* heterozygous between lines.

However, the most interesting aspect of Table 6 is the significant differences observed between matings. This is in contrast to the nonsignificant differences among the 34 genomes found earlier. These differences are due to (1) varying levels of heterozygosity, or (2) original genome differences not detected earlier, or (3) mutations within the homozygous lines since their extraction during the period of sib-matings. The design of the experiment diagrammed in Figure 2 makes possible a breakdown of the variation among the 21 matings to test these hypotheses. The data were partitioned according to homozygosity and heterozygosity of the wild-type chromosomes into four probable levels of heterozygosity. (1) Matings with all "+" chromosomes from the same "old" homozygous lines are enclosed by solid lines in Figure 2. (2) Matings with all "+" chromosomes from the same "new" lines are referred to as "homozygous new sublines" in Figure 2. (3) Matings with "+" chromosomes from an "old" line combined with those from its "new" subline are referred to as heterozygous within lines and these matings are enclosed by dotted lines in Figure 2. (4) Matings with "+" chromosomes from different lines, which include the remaining matings in Figure 2, are referred to as heterozygous between lines. This partitioning associates significant genetic effects with particular lines or crosses. Table 6 shows that the significant differences among "Matings" were not due to differences among levels of heterozygosity. Likewise the homozygous "old" lines, representatives of the original genomes, were not significantly different. Only the third hypothesis remains and the results are compatible with the occurrence of new genetic variation arising within the homozygous lines since their original extraction from the closed population. All significant differences among matings were limited to those involving the "new" homozygous lines.

The highly significant "Sex × Mating" interaction in Table 6 confirms the same type of interaction, "Sex × Genome", found earlier in Table 5 and supports the hypothesis of genetic dimorphism for viability. This sex by genotype interaction, also describable as a negative genetic correlation for viability in males and females, remains the sole identifiable source of genetic variation in the original plateaued population.

The selected trait: Not too many years ago one would have been consistent with population genetic theory to suspect that prolonged selection had fixed the "favorable" alleles and, thereby, eliminated genetic variation for the selected trait. Without harboring such an idea for the case at hand, the similarity in relative viability of the genomes from the plateaued population suggests that additional information is needed regarding genetic variation for the selected trait, fecundity.

Parent-offspring regression analyses of the fecundity data for each generation of selection in this closed population revealed that estimates of additive genetic variation were essentially zero for the generations showing no response to selection; yet, indications of nonadditive genetic variation remained (BELL *et al.* 1957). The marked-inversion technique makes possible an experimental test for genetic variability in the plateaued population by contrasting extracted genomes in both the homozygous and heterozygous state. Following the mating procedure

outlined in Figure 1, six new homozygous lines were extracted. These homozygous lines were crossed in all possible combinations including reciprocals. Daily egg numbers were obtained for two days (fifth and sixth days of age) on each of five daughters in each of three replications for each of the 36 crosses. An analysis of variation based on the cross means for each replication is summarized in Table 7. Highly significant differences observed among crosses indicate that genetic variation was present in the closed population even though it did not respond to selection. A breakdown of the 35 degrees of freedom for "Crosses" is especially enlightening. If the genetic variation were solely additive in nature, significance should be confined to the "Between inbreds" and "Between hybrids" effects, with the "Between levels" effect showing nonsignificance. But if the genetic variation were largely, or entirely, nonadditive, "Between levels" and "Between hybrids" should be significant, with "Between inbreds" being nonsignificant. The results support the hypothesis that the genetic variation in the closed population was nonadditive in regard to fecundity.

If the six inbred matings are excluded from these data, the remaining 30 F_1 and reciprocal crosses fit GRIFFING's (1958) Modified Diallel Design, Method 3. Provided the underlying assumptions are met, he has shown that this design yields unbiased estimates for the additive and nonadditive genetic variances in the parent population. The analysis of variance based on this model and presented in Table 8 reveals the general combining ability effects (GCA) to be nonsignificant with the corresponding estimated component of variance slightly negative. Since this component estimates one half the additive genetic variance in the parent population, we have another piece of evidence that the additive genetic variation for fecundity had been exhausted in this plateaued population.

An additional point of interest in Table 8 is the highly significant mean square associated with the specific combining ability effects (SCA). The corresponding variance component in the absence of epistatic effects provides an unbiased estimate of the nonadditive genetic variance in the parental population. This evidence confirms the earlier report (BELL *et al.* 1957) that genetic variation was present in the plateaued population under study, but it was nonadditive in nature.

TABLE 7

Analysis of variation in fecundity among the inbred and hybrid combinations of six genomes extracted from the closed population

Source of variation	Degrees of freedom	Mean square
Replications (R)	2	349.58
Crosses (C)	35	388.01**
Between levels of heterozygosity†	1	4962.54**
Between inbreds	5	163.90
Between hybrids	29	268.91*
R × C	70	142.17

* Significant at the .05 level of probability.
** Significant at the .01 level of probability.
† Inbreds *vs.* hybrids.

TABLE 8

Analyses of variance for the modified diallel crossing system involving F_1 and reciprocal crosses among six homozygous lines extracted from the closed population

Source of variation	Degrees of freedom	Mean square	Component of variance
Replications (R)	2	252.0	....
Crosses (C)	29	268.9	....
a. GCA	5	112.1	— 47.6
b. SCA	9	492.9**	164.8
c. Reciprocal	15	186.8	....
R × C	58	163.4	163.4

** Significant at the .01 level of probability.

DISCUSSION

The present genetic analysis of a "plateaued" population of *Drosophila melanogaster* has been more effective in revealing factors which have not contributed to the plateau rather than those responsible for it. This would constitute a serious criticism were not such factors as sterility, lethals, and subvitals widely reported to limit the response to selection. Selection experiments involving bristle number in Drosophila which illustrate this point are those of MATHER (1942), RASMUSON (1955), CLAYTON and ROBERTSON (1957), and SCOSSIROLI (1954). Other studies reporting such factors are those of ROBERTSON and REEVE (1952) with wing length in Drosophila and that by LERNER and DEMPSTER (1951) involving shank length in fowl. The distinguishing feature between the experiments discussed here and those reported in the literature is the fact that the selected trait, fecundity, is a major component of fitness. The significance of this point must remain largely a matter of conjecture until the selection response for fitness traits has been more thoroughly investigated.

The causes of a plateau must be scrutinized in light of their effects on the gain expected from selection. The expected gain ($\triangle \overline{G}_g$) in a trait per generation of mass selection may be expressed by the equation

$$\triangle \overline{G}_g = h^2 i$$

where h^2 is the heritability of the selected trait and i is the selection differential. Through either a reduction in effective heritability or by lowered selection intensity, several features may prevail which cause a decrease or plateau in response to selection (LERNER 1958). Obviously, a gradual worsening of the environment during the course of a selection experiment could lead to an apparent plateau when actually the average genetic merit would be improving. The performance of control populations and those under other methods of selection indicated that an environmental trend of this kind had not occurred in this experiment (BELL *et al.* 1955). Genotype-environment interactions in the presence of a fluctuating environment over generations could cause a plateau in response even though h^2 and i were positive within generations. Such a situation would be most difficult

to detect and would amount to an over-all reduction in the effective heritability. Likewise, heritability in a population could be reduced as selection exhausts the additive genetic variation. Increasing frequencies of lethal, subviable and sterility factors which are part of a superior heterozygote system will lead to an increase in the nonadditive genetic variance and eventually reduce the additive portion. An example of single gene heterosis associated with a lethal has been reported by MUKAI and BURDICK (1959).

The selection differential may decline due to a reduction in the phenotypic variation or due to a negative genetic correlation between the selected trait and reproductive fitness. In the extreme case where genes approach fixation from selection or inbreeding, the phenotypic variation would become reduced by the amount of the genetic variation. The selection intensity in turn becomes a function of the environmental variance and heritability approaches zero. A negative genetic correlation between the selected trait and fitness would reduce the effective selection intensity. The possible role of natural selection for some component of fitness to impede the effectiveness of artificial selection has not been eliminated in the present case. A more complete investigation should include other fitness traits, such as fertility and developmental rate and the relationship of these to fecundity.

Any thought that the closed population had reached a physiological or an environmental ceiling in fecundity can be discarded since other populations under different methods of selection consistently performed at higher levels even though they were initiated from the same base stocks and existed under quite similar environmental conditions.

After considering all available evidence, the major factor contributing to the lack of response in this particular plateaued population appears to be an exhaustion of the additive genetic variation due to prolonged selection. A significant amount of nonadditive genetic variation was observed and selection differentials remained large due to this nonadditive genetic variation plus environmental effects. The influence of lethals, semilethals, subvitals and sterility factors were found to be negligible. These findings agree with those reported by FALCONER and KING (1953) in their investigation of selection limits for body size in the mouse. A decomposition of the nonadditive genetic effects into those due to dominance, overdominance, epistasis, and genotype-environment interaction is limited until more refined statistical tools are developed.

SUMMARY

In an attempt to identify the genetic factors causing a plateau in response to selection for high fecundity in a closed population of *D. melanogaster*, 126 genomes were extracted from the population by the marked-inversion technique and the frequencies of lethal, sterility, and subvital factors were determined The observed frequencies of these factors were sufficiently low to eliminate them as major causes for the lack of response.

Genetic variation for viability in the plateaued population as measured by

adult emergence was found to be associated with sex, the three major chromosomes, and a sex by genome interaction. While the extracted genomes did not differ in their average effect on viability, they exhibited differential action in males *versus* females. Such an interaction would be an effective force for retaining genetic variation in a population in spite of continued selection.

Evidence was presented which identified the exhaustion of the additive genetic variation in fecundity, the selected trait, as the primary cause for the plateau in selection response in this closed population. Nonadditive genetic variation was present to eliminate the possibility that selection, plus the inevitable inbreeding which occurs in a closed population, had rendered the population homozygous.

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