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Chapter 29

# Hybrid Vigor in Drosophila

Experience has defined hybrid vigor as the evident superiority of the hybrid over the better parent in any measurable character as size, general vegetative vigor, or yield. For any one species it is left for us to show that, within the possible crosses of pure lines, hybrid vigor actually exists and what particular morphological and physiological characters express it best.

With this in mind, investigations with wild-type Drosophila of diverse geographical origin were begun in 1934 and continued to date. The group working on this problem has included Dr. Leslie E. Johnson, Dr. F. S. Straus, Miss Janice Stadler, Dr. S. Y. Loh, and myself. The material reported here is the result of our joint efforts. To specify the problem of hybrid vigor, five characteristics were chosen for investigation in eight inbred lines of Drosophila and a hybrid between two of the lines. The characteristics chosen were egg production throughout the full life of the fly, the days the females laid the eggs, the hatchability of the eggs, and the duration of life of the males and females in each line.

To determine egg production, a pair of flies of a particular line was placed in a quarter-pint milk bottle sealed with a paraffin paper cap on which was placed a disk of nutrient banana agar colored with charcoal. The female laid her whole day's egg output on this disk when it was properly seeded with yeast and a little acetic acid. The caps, a sample shown in Figure 29.1, were changed daily and the eggs were counted for each day.

The characteristic performances of the different pure lines and the hybrid are shown in Table 29.1.

The average egg production for the different inbred races varied from 263 to 1701 eggs. There is some correlation between the intensity of the inbreeding and the production of the particular race. Ames I and II are less inbred

races than Inbred 92 or Homozygous. Correlation exists between the egg production of the race and its fitness to survive as judged by its duration of life as measured by the survival of either males or females.

The hybrid race came from the cross Inbred 92 and Ames I. The mean productions of the parents were 389 and 1000 eggs respectively over the life-
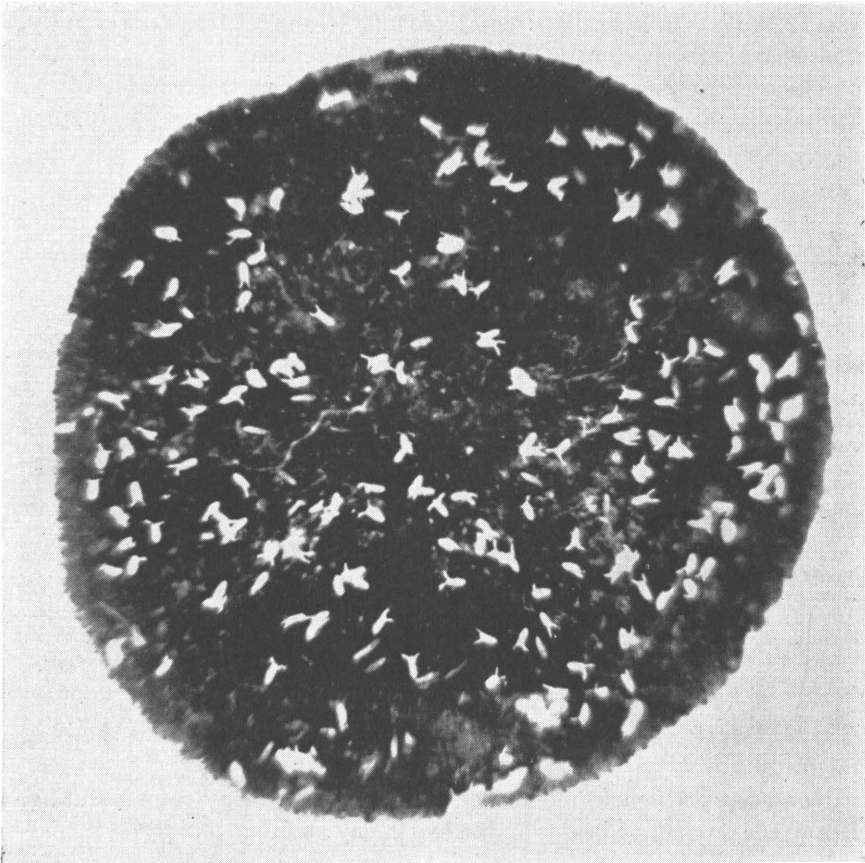


FIG. 29.1—Photograph of laying cap with eggs and some hatched larvae.

time period. The mean production of the hybrid was 2034 eggs, or 203 per cent greater than that of its high producing parent, Ames I, and 422 per cent greater than that of its other parent, Inbred 92. The hybrid showed more eggs than any of the pure races. The excess of the hybrid over the pure parents is greater in this particular experiment than in several other similar experiments.

The females of the different races showed average egg laying periods of 17.2 to 46.0 days. The hybrid lays eggs nearly as long as the higher producing

purebreds, 43.4 days, but does not exceed the range. The parents entering into the cross for this hybrid laid eggs for an average of 38.4 and 17.2 days respectively. Hybrid vigor is only 113 per cent for the length of the productive period.

The physiological fitness of the purebred races, as measured by their duration of life, ranged from 22 days to 58 days. The average life of the hybrid was 52.2 days. The hybrid's parents lived for 38.7 and 50.2 days respectively. This character showed little hybrid vigor—104 per cent.

These results show that egg production is the most favorable of the Drosophila characters analyzed for the study of hybrid vigor. The lifetime

TABLE 29.1

CHARACTERISTIC VARIATION OF DROSOPHILA RACES
IN EGG PRODUCTION AND DURATION OF LIFE

| RACE | EGG PRODUCTION | | SURVIVAL OF FEMALES IN DAYS OF LIFE | | DAYS FEMALES LAID EGGS | | SURVIVAL OF MALES IN DAYS OF LIFE | |
|---|---|---|---|---|---|---|---|---|
| | No. | Mean | No. | Mean | No. | Mean | No. | Mean |
| Ames II.......... | 56 | 1701 | 48 | 56.1 | 56 | 46.0 | 30 | 58.7 |
| Ames II.......... | 56 | 1511 | 41 | 51.5 | 56 | 40.0 | 39 | 53.6 |
| Princeton......... | 56 | 814 | 45 | 48.4 | 56 | 35.4 | 43 | 46.7 |
| Inbred 92......... | 56 | 389 | 43 | 33.4 | 56 | 17.2 | 42 | 44.0 |
| Florida-45 ........ | 54 | 610 | 49 | 28.5 | 54 | 22.4 | 52 | 32.6 |
| Oregon R-C-44.... | 54 | 413 | 49 | 36.4 | 54 | 28.7 | 48 | 35.5 |
| Swede-b-40....... | 53 | 398 | 50 | 26.7 | 53 | 16.5 | 50 | 35.7 |
| Homozygous 42... | 54 | 263 | 54 | 22.7 | 54 | 16.7 | 51 | 27.9 |
| Ames I.......... | 54 | 1000 | 51 | 50.9 | 54 | 38.4 | 50 | 49.6 |
| Hybrid.......... | 54 | 2034 | 52 | 50.0 | 54 | 43.4 | 51 | 55.4 |
| Pooled Variance... | d/f 537 | 236847 | ...... | ....... | d/f 537 | 179.5 | ....... | ...... |

distributions of egg productions for the inbred and hybrid races are shown in Figure 29.2.

Newly hatched females require a short period after emergence for maturing. Heavy egg production begins on the fourth day and rises rapidly to a maximum in early life. From the high point, production gradually declines. The rate of this decline varies with the different races. The average slope is shown by straight lines.

Drosophila egg production presents a single cycle as contrasted with the series of cycles or egg clutches observed in the egg production in certain other forms—the domestic fowl or the fungus fly, Sciara. This fact makes Drosophila egg production an easier character to study. The egg yield curve is determined by the initial high point in production and the rate of loss in productivity with age.

The form of the egg production curve in Drosophila fits in with Ashby's hypothesis of metabolic reserves being responsible for hybrid vigor. The hy-
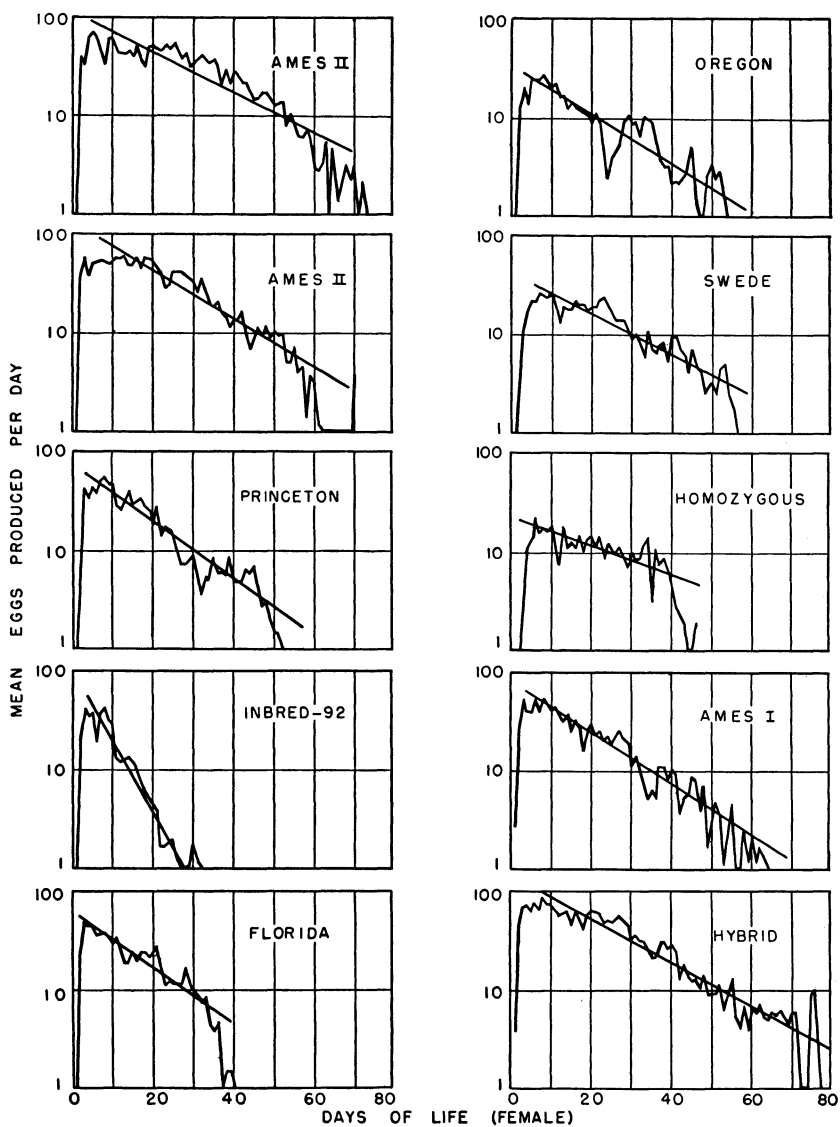
FIG. 29.2—Lifetime daily egg production of different races of *Drosophila melanogaster*, number of initial females tested, 53 to 56.

brid has a higher initial production than its parents, or, for that matter, any of the purebred races. The hybrid expends its metabolic reserves less rapidly than either of its parents. Taking all inbred races together, the hybrid utilizes its reserves at slightly less than the average rate. The hybrid is chiefly characterized by its large initial production. Examination of the pure lines indicates that there are slight differences in the rate of expenditure of the initial reserve, even when the obviously different Inbred 92 is not considered.

## WHAT IS HYBRID VIGOR?

These results show that the vigor of the hybrid is greatest for lifetime egg production, 203 per cent; is less if length of egg laying period is considered, 113 per cent; and is still less with life span as the character, 104 per cent. What is the explanation of egg production's high heterosis? Egg production is a character which is in turn dependent on other component characters. A simple breakdown would be, lifetime egg yield is determined by the length of egg laying period, the decline (slope) representing the loss of ability to produce eggs with age, and maximum egg production at the initial phase of the egg laying cycle. Length of egg laying period has already been shown to have 113 per cent heterosis. The slopes of the decreasing egg yields with advancing ages are Inbred 92—0.17, Ames I—0.06, and their $F_1$ hybrids—0.05. The hybrids show heterosis in that their egg productions decline less rapidly than their best parent, but the heterosis is only 121 per cent.

Maximum productions, as judged by the three highest days' average yields of the strains, are Inbred 92, 40.7; Ames I, 52.7; and $F_1$ hybrid, 81.4 eggs, or the heterosis is 154 per cent. The highest of the component heterosis values is only about half of that noted for lifetime egg yields. It seems not unlikely that if the division into components could be carried further, it would be found that the heterosis values would approach closer and closer to 100. The results consequently argue for heterosis, as the result of the combined action of two or more groups of distinct characters which, when jointly favorable, and as frequently truly multiplicative in action, lead to heterosis.

Analysis of the variation in egg production between races—the heritable fraction controlled through inbreeding—as contrasted with the variability within races—the fraction due largely to environment—shows that about 56 per cent of the lifetime egg production is fixed within the races and 44 per cent is due to gene segregation, environment, etc.

Consideration of the individual records further support this view. Contrasting the performances of the individual females within the hybrid groups with those in the different inbred races shows that the hybrid has no females with greater production than those of the inbred races. The hybrids are good because, on the average, all members of the cross are good producers. The hybrids include one female laying 3083 eggs and twenty-seven others laying between 2000 and 3000 eggs. The Ames I has one female laying 2016 eggs;

Ames II, in two similar experiments, has two females laying 3168 and 3108 eggs and thirty-two others laying between 2000 and 3000 eggs. The other pure races have no individuals laying more than 2000 eggs. Hybrid vigor contributes consistently high performance to all individuals rather than exceptional performances to a few. It is the consistency of high performance which calls for explanation.

## MEASURES OF HETEROSIS

As lifetime egg production is a difficult character with which to work, a less tedious measure of productivity was sought. The character chosen was daily egg yield 5, 6, 7, 8, and 9 days after the female emerged from the pupa. These records are at the general maximum of the female's productive life. The correlation with lifetime production is high.

### Chromosomal and Cytoplasmic Basis for Hybrid Vigor

The possibility of creating homozygous races of Drosophila through outcrossing offers a unique opportunity for analyzing the causative agents behind hybrid vigor. Hybrid vigor has been postulated as due to differences in allelic genes and to differences in the cytoplasms which combine at fertilization. The reduction in yield of inbred races is accompanied by increasing identity in both the combining alleles and the cytoplasms which combine to form succeeding generations. Both these factors have been invoked to explain the low yield of such inbred races. Production of homozygous types through outcrossing furnishes a contrast between these two possible causes of low productivity. The allelic genes are made homozygous so that any undesirable recessive gene would have full expression in the different races and thus lower the yield. The cytoplasms which combine are diverse and as such should give high yield to the individuals if hybrid vigor is an expression of differences in combining cytoplasm. This contrast is shown as follows:

| Genes tend toward | | Cytoplasms tend toward |
|---|---|---|
| | Inbreeding | |
| ♂ Gametes | | |
| | identity | identity |
| ♀ Gametes | | |
| | Homozygous by outcrossing | |
| ♂ Gametes | | |
| | identity | diversity |
| ♀ Gametes | | |

The effects of genes as contrasted with the effects of the cytoplasm may be measured by comparing inbred performance with that of a race made homozygous for the same genes. Table 29.2 shows this comparison.

For Princeton 1, the inbred progeny resulting from brother-sister mating for 28 generations had an egg production of 73.6 eggs per day over the test period. The homozygous Princeton 1 race, coming from the outcross breeding

system, had an average egg production of 62.7 eggs, or, the homozygous production was 10.9 eggs less than the inbred. The differences of the different inbred-homozygous comparisons range from − 22.0 to +10.6 eggs. In nineteen comparisons the inbred races produce more than the homozygous. In five instances the homozygous races yield more than the inbred. Of the nineteen trials in which the homozygous races had less production than the in-

TABLE 29.2

VARIANCE ANALYSIS FOR PROGENY OF IN-
BRED (BROTHER BY SISTER) AND OUT-
CROSS (LEADING TO HOMOZYGOSIS) MAT-
ING SYSTEMS

| Race | Mean Difference | Value F |
|---|---|---|
| Princeton  1 | −10.9 | 5.9* |
| Princeton  1 | − 2.8 | .3 |
| Princeton  1 | − 9.9 | 3.7 |
| Princeton  8 | + 6.6 | 2.1 |
| Princeton  8 | − 6.3 | 2.9 |
| Princeton  8 | − 1.3 | .1 |
| Princeton 10 | + 6.0 | 1.9 |
| Princeton 10 | + 0.1 | .0 |
| Princeton 10 | −15.9 | 9.6** |
| Princeton 10 | + 0.8 | .1 |
| Florida     2 | − 7.3 | .8 |
| Florida     2 | − 8.7 | 3.4 |
| Florida     2 | −20.5 | 20.1** |
| Florida     2 | −16.6 | 8.3** |
| Florida     5 | − 5.5 | .8 |
| Inbred      3 | +10.6 | 3.1 |
| Inbred      4 | −19.2 | 7.9** |
| Inbred      4 | − 9.6 | 2.6 |
| Inbred      4 | − 9.3 | 1.6 |
| Inbred      4 | − 6.6 | .7 |
| Inbred      9 | −13.7 | 4.4 |
| Inbred      9 | −21.1 | 7.6* |
| Inbred      9 | −16.2 | 3.9 |
| Inbred      9 | −22.0 | 7.9* |
| Average | − 8.4 | ............ |

breds, there are four differences which are highly significant and three differences that are in the significant range when account is taken of chance variations. In no instance was the homozygous egg yield significantly larger than that of the inbreds. The data were consistent in showing the homozygous poorer in egg production than the inbred, even though the particular homozygous is only a sample of the germ plasm of the highly inbred strain.

The average difference between the homozygous and inbred progeny is −8.38. Considering each observation as equivalent, the probability that the homozygous are on the whole poorer producers than the inbreds is well beyond the 1 per cent range by the test.

There are three major hypotheses to account for the vigor of race crosses. One hypothesis assumes an as yet unexplained physiological stimulation resulting from the union of gametes of unlike origin. The second hypothesis attributes hybrid vigor to the union of gametes carrying different favorable dominant genes for vigor, which cover up defects which may exist in each of the original parent races. The third hypothesis also depends on genic action. It assumes that the vigor of the hybrid comes from the association of unlike alleles brought in from the two parental races, these unlike alleles are postulated as contributing different, as well as like, chemical or physical stimulations favorable to the vigor of the hybrid. The results of these experiments presented in Table 29.2 are in favor of a genic basis rather than a physiologic stimulation as the cause of hybrid vigor, since throughout this work, diverse cytoplasm has shown less yield than like cytoplasm when put on a background of homozygous or inbred inheritance.

## INBREEDING EFFECTS ON HETEROSIS AS RELATED TO DEFECTIVE GENES

The creation of homozygous types tests the parent race for heterozygosity of particularly undesirable genes, lethals, and semi-lethals. Table 29.3 shows the results obtained in mating the homozygous races.

TABLE 29.3

GENOTYPES OF INBRED RACES FOR VIABLE, LETHAL, AND RECESSIVE VISIBLE ALLELES

| Race | Line | No. Lethal | Lethal | Recessive Visible | Total Isolation |
|------|------|-----------|--------|-------------------|-----------------|
| Princeton......... | 1 | 9 | 7 | 0 | 16 |
| Inbred............ | 9 | 0 | 17 | 0 | 17 |
| Florida........... | 5 | 98 | 20 | 2 | 118 |
| Florida........... | 2 | 0 | 12 | 2 | 14 |

The lethals observed were all in chromosomes 2 and 3. They range in frequency from 17 per cent for one race to 100 per cent for another. The visible recessives picked up were also semi-lethal. The mathematical model employed in inbreeding calculations postulates random recombination and fertilization. Conclusions are misleading when these postulates are not met. The above evidence for mechanisms to maintain heterozygosity in races even though the matings are of relatives as close as continued full brother × sister seems unmistakable. The defective genes are in the races. Residual defective genes can contribute both toward and away from greater heterotic effects in particular crosses. As these defective genes arise ultimately by mutation and as the number of the genes is large, the ultimate possible genetic changes are appreciable and may be an important force toward heterosis.

## EFFECT OF THE GENOME COMPONENTS ON HYBRID VIGOR

The combining capacity of a genome may be analyzed into its components —the individual chromosomes. To make this analysis, Dr. Straus in our genetics laboratory carried through duplicated experiments based on the cross of Inbred 92 and Ames I. This cross, as noted previously, showed high hybrid vigor. The following data were taken from his thesis (1942).

The results showed no cytoplasmic effects. Effects of reciprocal crosses also were found negligible.

The first step in these investigations required that 8 possible homozygous lines be created for the first, second, and third chromosomes. About 98.5 per cent of the genes would be homozygous in each of the eight types. Crosses of the eight different homozygous lines will give all the other types ranging from those heterozygous in one chromosome pair to those heterozygous for each chromosome. The productivities of these 27 different types together with their chromosomal constitution are as follows:

| | Type | Average Daily Egg Yield |
|---|---|---|
| Heterozygous for 3 chromosome pairs | 1 | 76.9 |
| Heterozygous for 2 chromosome pairs | | |
| 6 chromosome combinations | 2 | 64.7 |
| | 3 | 64.4 |
| | 4 | 51.5 |
| | 5 | 65.5 |
| | 6 | 66.5 |
| | 7 | 62.9 |
| Heterozygous for 1 chromosome pair | | |
| 12 chromosome types | 8 | 55.2 |
| | 9 | 52.7 |
| | 10 | 55.8 |
| | 11 | 60.6 |
| | 12 | 46.5 |
| | 13 | 53.6 |
| | 14 | 35.3 |
| | 15 | 56.1 |
| | 16 | 56.2 |
| | 17 | 52.4 |
| | 18 | 51.7 |
| | 19 | 41.7 |
| Homozygous | | |
| 8 types | 20 | 45.0 |
| | 21 | 51.3 |
| | 22 | 40.4 |
| | 23 | 36.3 |
| | 24 | 37.0 |
| | 25 | 35.9 |
| | 26 | 27.8 |
| | 27 | 31.9 |

The analysis of the variance of the 1440 daily egg productions in this completely balanced factorial experiment shows that the difference between chromosome effects makes the most important contribution to variation.

From type 20 to 27, all types are homozygous or are of zero heterozygosity. Types 8 to 19 have one chromosome heterozygous. Since each chromosome

enters in equal frequency, this means that one-third of the genes are on the average heterozygous. Types 2 to 7 have 2 chromosomes heterozygous, or the average of these types is two-thirds heterozygous. Type 1 is completely heterozygous or 100 per cent. Plotting the average egg production for the four groups shows the effects of different degrees of heterozygosity on the hybrid vigor.

The property of additivity of the heterotic gene effects would seem to be the logical explanation for this linear relation and also for the absence of interactions between the genes of the different chromosomes. It must be realized, however, that the chromosomes themselves represent interacting gene effects which give the block reactions. The trend so far considered is an average trend, each point, except that for the completely heterozygous, being based on several types. Interaction—combination effects which are larger or smaller than the sum of the chromosomal effects separately—may exist. Such effects, it is true, must be in opposite directions and equal. The factorial design of the experiment facilitates evaluation of these interactions. The data following gives the three levels of effect each of the chromosomes can assume, together with the two and three chromosome interactions.

VARIANCE ANALYSIS OF CHROMOSOMAL EFFECTS

| Source of Variation | d/f | Mean Square | Apportionment of Variation Per Cent |
|---|---|---|---|
| I chromosome..................... | 2 | 574 | 11 |
| II chromosome.................... | 2 | 1916 | 44 |
| III chromosome................... | 2 | 1010 | 22 |
| I and II chromosomes............ | 4 | 81 | 0 |
| I and III chromosomes........... | 4 | 62 | 0 |
| II and III chromosomes........... | 4 | 116 | 1 |
| I, II, and III chromosomes........ | 8 | 103 | 22 |

The effects of the direct order actions of the first, second, and third chromosomes are highly significant. None of the interactions show large variations. Nor are any of these interactions in excess of what would be expected from random differences. Apportioning the variance to its various chromosomes, 11 per cent is attributable to the first chromosome, 44 per cent to the second, and 22 per cent to the third.

Analysis of this material shows that the hybrid vigor of the egg yields receives a significant contribution from the heterozygosity of each chromosome pair, and that none of the chromosome interactions are significant. The homozygous chromosomes of the two parental inbreds do not differ in either their direct or interaction effects from zero.

The linearity of the effect on egg yield and the absence of interactions show that the chromosomes with their contained genes behave as integrated units

—much like major genes—with given degrees of dominance. Within each group the genes may have any known type of gene action so long as the quality of additivity of their effect between chromosomes is maintained.

## EFFECT OF CHROMOSOME LENGTH ON HYBRID VIGOR

The analysis of variance shows that the effects of the three chromosome pairs differed widely. These differences could be due to differential numbers of gene loci within the separate chromosomes, to varying magnitude of gene effects, or to both. The data do not allow us to positively distinguish between these hypotheses. Proportionality between the effects of the three chromosomes and their sizes would favor the first interpretation.

There are several different measures of chromosome size. These measurements of the different chromosomes may be compared with their heterotic effects in a least square test.

The proportionality between heterotic effects and chromosome lengths was as follows:

| | CHROMOSOMES | | | CLOSENESS OF AGREEMENT | |
|---|---|---|---|---|---|
| | I | II | III | 7-Day Data | All Data |
| Metaphase length............. | 1.56 | 2.21 | 2.80 | X | X |
| Salivary length.............. | 220 | 460 | 485 | X | X |
| Salivary bands............... | 1024 | 2134 | 2077 | XX | X |
| Per cent visible loci........... | .69 | 1.00 | .77 | XX | XX |
| Cross over length............ | .62 | 1.00 | .98 | XX | X |
| Observed heterotic {7 day.... | 248 | 386 | 325 | X significantly closer 1:20 or | |
| effect {All data.. | 192 | 305 | 174 | XX highly significant 1:100 | |

Excellent agreements are observed between per cent of visible loci or the crossing-over units with the heterotic effects of the chromosomes. Less agreement is noted between the number of bands in the salivary chromosomes and the heterotic effect. The metaphase lengths of chromosomes or the physical lengths of the salivary gland chromosomes are less closely related to heterotic effects. All comparisons of chromosome sizes with heterotic effect give excellent to fair correlations. In general, the heterotic effect is distributed according to random distribution of several genes to the various chromosomes. This favors the view that the heterotic effect is due to many gene pairs in each chromosome, rather than to one having a specific additive phenotypic effect. These genes would be randomly distributed to the different loci within the chromosome.

## GENOME CONTRIBUTIONS TO HYBRID VIGOR

Average combining ability of one inbred line when mated to several lines is called general. The genomes of an inbred line can be regarded as uniform

and good or bad according to the genes which they contain. These genes could be additive in effect making the genomes of uniform effect with other inbred lines. Specific combining ability represents unlike combining ability of the genomes from one race with those of a succession of other races. This variation in hybrids could be due to different allelic distributions as complementary or epistatic reactions of the different gene combinations with which the given genome was combined. The relative effects of general vs. specific combining ability for a particular group of crosses may be measured in data containing all possible combinations between a series of different inbreds. Table 29.4 presents the egg productions for the possible hybrids of five inbred races.

TABLE 29.4

EGG PRODUCTIONS OF 5 INBRED RACES AND THEIR CROSSES
AVERAGE YIELDS FOR 5, 6, 7, 8, AND 9TH DAYS
OVER 4 EXPERIMENTS

| FEMALE PARENT RACE | MALE PARENT RACE | | | | | TOTAL |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | |
| A.............. | .......... | 2509.0 | 2681.0 | 3479.4 | 2503.8 | 11173.2 |
| B.............. | 2908.6 | .......... | 2712.8 | 3427.4 | 1822.2 | 10871.0 |
| C.............. | 1804.8 | 2827.8 | .......... | 3298.8 | 3116.0 | 11047.4 |
| D.............. | 2321.4 | 3485.6 | 3215.2 | .......... | 3447.6 | 13467.8 |
| E.............. | 2109.8 | 1908.2 | 2498.2 | 3301.0 | .......... | 9817.2 |
| Total........ | 10144.6 | 10728.6 | 11107.2 | 13506.6 | 10889.6 | 56376.6 |
| Inbred Race Yields....... | 2595.2 | 2586.4 | 1996.6 | 2173.4 | 1859.4 | .......... |

Table 29.4 shows 14 of the race hybrids have higher average yields than their inbred parent races. The average hybrid produced 2818.8 eggs, the average inbred 2242.2, or the increase over the average inbred was 25.7 per cent. These data serve to re-emphasize the fact pointed out earlier, that characters built up of components of lesser characters generally show more heterosis than observed for each of the components taken separately.

The individual race crosses differed in their ability to unite into favorable hybrids. Race D is evidently high in its general combining ability. The other four races show about equal combining ability. Race D has this high general combining ability even though its own productivity is rather low—2173.4— eggs as against 2595.2 for another of the races.

For individual flies the range in egg production was from 0 to 146 eggs. The zero egg producing flies are an important class which give an insight into female sterility. We have dissected over 300 such flies. These observations have led to the opinion that this class is the result of a variety of causes and

definitely differs genetically and otherwise from that of the flies which produce even one egg in their lifetimes. For this reason and the fact that heterosis is a phenomenon of quantitative inheritance, we have excluded such flies from consideration in these studies.

The general analysis of the variations within these hybrid egg yields is presented in Table 29.5.

TABLE 29.5

DISTRIBUTION OF VARIANCE IN EGG PRODUCTION

| Source of Variation | Designation | d/f | Mean Square | Components of Variation | Per Cent Contributed |
|---|---|---|---|---|---|
| Total......... | ...... | 5624 | ............ | ................................ | ...... |
| Experiments.... | E | 3 | 116461 | W+11.2ERA+280EA+56ER +1400E | 7.3 |
| Races......... | R | 24 | 37405 | W+11.4ERA+57ER+45RA +225R | 15.4 |
| Inbreds...... | ...... | 4 | 12630 | | |
| Hybrids...... | ...... | 19 | 36811 | ............................ | ...... |
| Inbreds vs. Hybrids.... | ...... | 1 | 147779 | ............................ | .... |
| Ages.......... | A | 4 | 6665 | W+11.4ERA+285EA+45RA +1125A | 1.2 |
| Exp×Races.... | ER | 72 | 5800 | W+11.2ERA+56ER | 9.8 |
| Exp×Ages..... | EA | 12 | 18202 | W+11.2ERA+280EA | 6.8 |
| Races×Ages... | RA | 96 | 767 | W+11.4ERA+45RA | 0.2 |
| Exp×Races× Ages......... | ERA | 288 | 830 | W+11.2ERA | 3.1 |
| Residual....... | W | 5125 | 510 | W | 56.2 |

100.0

Table 29.5 presents data on the factors which may be of importance in the interpretation of heterotic effects. The percentage contribution of each factor is shown in the right hand column. The largest contribution, residual, is made by the variation within flies of a given age. It is half of the total observed. This variation shows what minor differences in seemingly constant conditions can be responsible for differences in egg yields.

Differences in races represent the next most significant contribution to yield variations—15 per cent. Major contributor to this effect is the difference between the productivities of the inbred parent races and their hybrids. These differences may be looked upon as the effects of additive genetic factors for yield, and the effects of specific gene combinations leading to the expression of dominance, overdominance, or epistacy in the phenotypes.

A point of currently even more importance brought out by these data is the dependence of yield on the close interrelation of environment and genotype. The interaction of experiment × race accounts for 9.8, and experiment, race, and age, 3.1 per cent of the variation. The total is 12.9 per cent. Even with great care to closely control conditions both within and between experi-

ments, the environment is sufficiently important to the yield of the particular race to account for nearly as much of the total yield variation as race alone. With widely ranging environments, given genotypes may show much more variation in phenotypic expression. The interaction terms show that genes in quantitative inheritance are not stable in their effects. In one condition the phenotypic reaction, in some degree, could be such as to suggest recessive action; in another dominant, in another additive or epistatic.

These interpretations may be brought out by another analytical approach. In analyzing data of this kind it has been customary to neglect the genotypic environmental interactions. This neglect finds expression in the models adopted to explain the yield. For the data above it is sometimes assumed that yield, $y_{ijk}$, may be accounted for by a basic value common to all crosses, the mean; deviations due to additive general combining ability of the different races $g_a$, $g_b$, etc.; deviations due to specific combining ability, $s_{ab}$, $s_{ac}$, etc., such as dominance variations, and epistatic effects common only to members of that particular cross; reciprocal effects, $r_{ab}$ vs $r_{ba}$, etc., of any differences between members of reciprocal crosses; and a term representing residual variations, $e_{abk}$, $e_{ack}$ etc., due to unknown causes. These variables are set up in the linear equation:

$$y_{ijk} = m + g_i + g_j + s_{ij} + r_{ij} + e_{ijk}$$

Analysis of the data for the contributions of these variables to the yield variance gives these results:

<div align="center">

EXPERIMENT 35

| | |
|---|---|
| General combining ability.... | 11.3% |
| Specific combining ability.... | 9.7 |
| Reciprocal effects........... | 2.3 |
| Residual effects............. | 76.6 |

</div>

Two sets of experiments are available. One is for five and the other for six inbred line hybrids. The test as presented above shows that 11.3 per cent of the variance is due to differences in general and 9.7 per cent to differences in specific combining ability. Differences in reciprocal crosses account for 2.3 per cent. Experiment 36 shows similar contributions attributable to general and to specific combining ability, but the effect of reciprocal crosses is insignificant. The two experiments are concordant in showing that general and specific combining ability account for most of the variation attributable to known causes. In both cases general combining ability is somewhat more important to productivity than specific combining ability.

These results from Drosophila are entirely without any previous selection for combining ability. They are comparable to the observations which were obtained in corn when combining ability was tested for the early crosses of inbred lines. It is significant that Sprague's analyses of such crosses show general combining ability twice as important as specific. This difference is like that of Drosophila but gives even more emphasis to general combining ability.

In later corn hybrids, the products of more stringently selected inbreds, the emphasis was reversed. The specific combining ability was zero to five times as important as the general. Improved utilization of hybrid vigor has seemingly selected and fixed general combining ability in the approved inbreds. Further progress is dependent on specific combining ability. One comparison weakens this evidence. On exactly the same ten inbred combinations one set grown at Ames, the other at Davenport, the specific combining ability was five times that of the general at Ames, while at Davenport the general and specific were identical. A place × genotype interaction in the general-specific combining ability similar to that observed above for Drosophila egg yield is also important even in these highly selected lines.

## SIGNIFICANCE OF ENVIRONMENT-GENOTYPE INTERACTION IN HETEROSIS

An experiment by Dr. Loh evaluating the significance of early or late testing furnishes data on the part played by environment in the stability of the hybrid phenotypes (1949). Fifty full brother × sister lines were formed from each of three wild stocks having different geographical or chronological origin. Each line was then doubled and mated full brother × sister for as long as possible, or until 37 generations were reached. The average productivity of the initial lines crossed to the same synthetic strain at the start of the inbreeding was Ames 1947, $179 \pm 2$; Ames 1943, $176 \pm 3$; and Amherst 1947, $166 \pm 3$ eggs for the 5, 6, and 7th days after the hybrid females hatched. All surviving inbred lines were crossed to the same synthetic stock, and the hybrid females tested at the 8, 9, 16, 23, and 30th generations. The results were consistent for the three stocks. The egg productions of the hybrids declined 2.4 eggs per generation on the average. This result was surprising, but may possibly be accounted for by the fact that the inbred lines surviving in the three stocks were becoming more and more like the synthetic tester due to the fact that they were cultured on the same media and in the same way. The favorable gene differences between the crossed lines became less each generation and resulted in a progressive lowering of hybrid yield. As the generation times were confounded with time of year, it was also possible that the egg yields showed some effects of the progressive changes in season.

The surviving inbred lines were tested for egg yield on the 21, 26, and 31st generations. They showed an average decrease in egg yield of 4.3 eggs per generation. This decrease was greater than that observed for the inbreds × synthetic cited above. This was not entirely unexpected, although it did indicate continued and persistent heterozygosity in the inbred lines to a much greater extent than was sometimes realized. The inbred lines produced 20 to 40 per cent less than the hybrids. The differences became greater as the inbreeding advanced.

The inbreds of the 15, 24, and 34th generations were crossed in all possible

ways. The line crosses were 17, 30, and 62 per cent better than their inbred parents. They were also 2 to 4 per cent better than the inbred × synthetic crosses. Figure 29.3 shows these trends for the three types of progenies.

Coefficients were calculated for the like cross performances at different generations. The synthetic × inbred lines had correlations for the 1, 8, 9, 16, 23, and 30th generations. Like numbers of comparisons were available in the reciprocal crosses, inbred lines × synthetic. The correlations were similar for generations and their reciprocals. The average for the thirty comparisons was −0.01. In terms of the data, the synthetic × inbred line cross of one generation gave no information on the relative performance of the same cross in a succeeding test. The hybrids showed random variation within themselves, but at the same time averaged out to be distinctly better than the inbreds.

The inbred lines of the 21, 26, and 34th generations were crossed in all possible ways. Again the correlations between the productivities of the like crosses in different generations showed variation. The average correlation was 0.25. The performance of the cross uniting two of these inbred lines did have some predictive value for the performance of like crosses made subsequently. Again these hybrids showed most of the variation within the crosses to be random, but that the yield level of the hybrids was significantly better than the inbred parent lines. The over-all value for larger yield came as a consequence of the cross rather than as an effect of specific cross differences.

This fact is brought out in another way. The inbred crosses were analyzed for general and specific combining ability, as described earlier, for the three different generations of inbreeding, 15, 24, and 34. The average results were:

$$\begin{array}{ll} \text{General combining ability} \ldots \ldots & 12\% \\ \text{Specific combining ability} \ldots \ldots & 5 \\ \text{Residual variation} \ldots \ldots \ldots \ldots & 83 \end{array}$$

These results are comparable with those presented earlier. General and specific combining ability can be estimated for each line in the particular crosses and experiments. The values can be compared as between the different generations, to determine how consistent in combining ability is the behavior of each line. The correlations for these comparisons were as follows:

|  | 15 and 24 | 15 and 34 | 24 and 34 |
|---|---|---|---|
| General combining abilities...... | −0.02 | −0.27 | −0.17 |
| Specific combining abilities....... | 0.13 | 0.02 | 0.26 |

These correlations are so small as to indicate that combining abilities are not consistent from one generation (in this case also season) to the next. The hybrids are uniformly better than the inbreds in yield, but again the genotypic system does not appear to have a fixed reaction. The explanation of this fact appears in Table 29.4 where a high experiment genotype interaction was observed. It means that each genotype may react differently to different environment. As these environments change from place to place, season to sea-

son, and even between simultaneous carefully controlled experiments, it is not surprising that the general and specific inheritance effects show variations. A particular fitting of strains to place and season, etc., appears essential for highest yields. In view of this conclusion, it is important to remember that this effect is within hybrids, and that hybrids, in general, are distinctly better than the inbreds (see Fig. 29.3).

Through the kindness of Dr. G. F. Sprague, making available certain of his extensive data on $F_1$ crosses of some 62 inbred lines of corn, we have been able to extend this analysis and compare the stability of general and specific
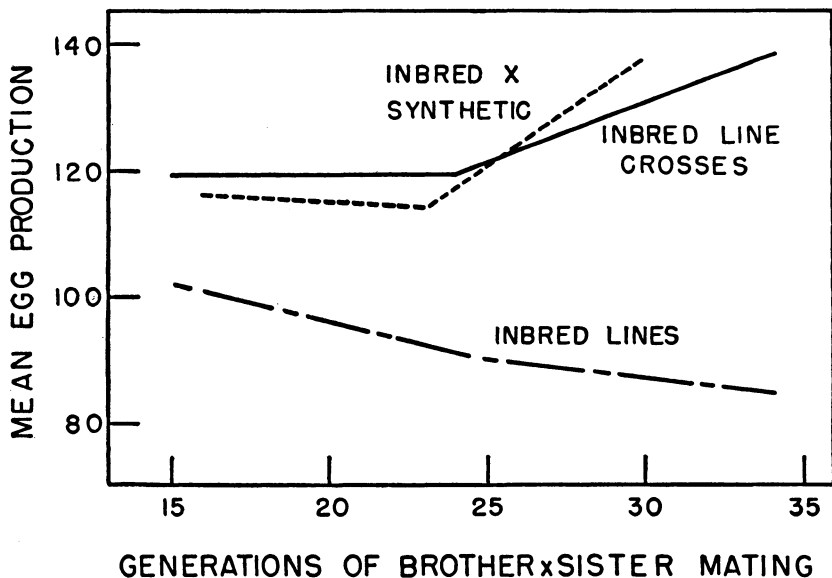


FIG. 29.3—Changes in productivity with advancing generations of brother × sister mating of inbred lines, inbred line crosses, and inbred × synthetic.

combining ability in the two species. The trials were conducted yearly from 1940 to 1948. The $F_1$ hybrids were planted in ten different areas chosen as representative of the different climatic conditions of Iowa. Any one trial may contain all possible single crosses of 4 to 14 inbred lines. The trials contain many individuals, and are replicated several times so the record for the $F_1$ is an average of numerous $F_1$'s instead of an individual as in the data on Drosophila. As would be expected, a particular cross was occasionally lost from a test. When this happened, the missing plot value was calculated from the mean and the general combining abilities of the lines entering the test. The specific value was considered zero. The data for the general and specific combining abilities of the lines in the remaining plots were used for further study. Our study considered the first order values, as these are the only values which have operational significance in breeding for heterosis.

General and specific combining abilities are strictly applicable to the particular experiment from which they are calculated. The values for the different lines vary with the group of lines from which they are calculated. This is a serious defect, for the results have no significance unless they may be used for the prediction of the future performance of the particular line or line combination. As the interest is in the operational use of these parameters in guiding breeding work, the theoretical objections to comparing successive values for general or specific combining abilities are outranked by the practical consideration. This study measures the repeatability of the estimates of general and specific combining ability for particular inbred lines when the crosses are grown in different locations, different years, and in different combinations.

Sixty-two inbred lines were the parents of the $F_1$ crosses. The data include 451 determinations of general and 2033 estimates of specific combining ability. As pointed out above, these determinations are not of equal weight because of differences in numbers and lines in the different $F_1$ hybrid tests. However, for the purposes of this comparison they are regarded as of equal weight, since it is on this basis that the data will be used for guiding future breeding operations.

The intraclass correlation between the repeated tests of the general combining abilities was 0.29; that for the specific combining abilities of the repeated crosses of the pairs of lines was 0.27. These correlations are definitely higher than those observed for the Drosophila data. They are high enough to be of reasonable importance in practice. The data for the general combining abilities become of somewhat greater value when the determinations are restricted to particular regions of the state, the over-all correlation becoming 0.31. When arranged within years but allowing free range over the 10 different geographical regions of the state, the over-all correlation becomes 0.53. The specific combining abilities do not show an equal improvement in predictive values when subdivided by these categories. Specific combining abilities drop when the data are subdivided by geographical regions of the state, the over-all correlation becoming 0.18. When the subdivision is made by years, the over-all correlation becomes 0.34.

These results reemphasize the effects of the environmental-genotypic interactions on performance as discussed earlier. The corn hybrids are fitted to the geographical regions of the state by selection of the place of planting for season of maturity. Little or no selection is possible for fitting the plantings to the vagaries of the different years. The effects are noted in the intraclass correlations. Double selection for genotypic environmental correlation when the data are subdivided by years leads to definitely increased correlations for the general combining abilities of the particular lines and to slightly increased correlations for the specific combining abilities of these same lines. Where the years $\times$ genotypic effects are allowed to express themselves, the correlations are no greater than those of the whole or are reduced.

## SUMMARY

Consideration of egg production and other component characters in *Drosophila melanogaster* shows that hybrids are uniformly better producers than inbreds even though the inbreds be the parents of the hybrids. The hybrids themselves are not exceptional in production when contrasted to the best random bred individuals. Rather, hybrid vigor contributes consistently high performance to all individuals rather than very superior performance to a few.

Lifetime egg productions show greater heterosis than any of the component factors which ultimately determine it. Length of egg laying period has 113 per cent heterosis, maximum egg production 154 per cent, and resistance to decline in vigor, as measured by egg production with advancing age, 120 per cent, while the over-all character lifetime egg yield has 203 per cent heterosis. Heterosis appears to be a consequence of the combined action of two or more groups of distinct and more elementary characters which when jointly favorable lead to generally high yields.                           .

Tests show that hybrid vigor is attributable to nuclear contributions of the two parents rather than to possible cytoplasmic differences in the uniting gametes. Inbred races frequently contain or soon attain mechanisms to slow down or prevent reaching complete homozygosis through continued close inbreeding. Lethal genes, deficiencies, or defective genes residual in all stocks or acquired through mutation, balance to prevent free interchange of genes within chromosome groups, and thus retard or stop the formation of the homozygous types. In the light of these results, mutations as a heterosis mechanism assume much greater importance than ordinarily supposed.

When the egg yields were analyzed by the degree of heterozygosity it was found that flies homozygous for all loci in chromosomes I, II, and III or 0 heterozygous, produced 38.2 eggs on the average. Those heterozygous for one-third of the unlike parental genes in the cross produced 51.5 eggs on the average. Those heterozygous for two-thirds of the unlike parental genes laid 62.6 eggs, and those heterozygous for all unlike parental genes, three-thirds heterozygous had a mean yield of 76.9 eggs. The differences are additive, about 12.9 eggs being added with each increase of one-third of the genes heterozygous. The additivity of the mass gene effects would suggest additivity of the individual gene actions on egg yield. This is an important point but does not necessarily follow, because the dominance or recessiveness or interallelic interactions could be balanced by the mass of gene pairs comprising one-third of the heterozygous loci.

Study of the contributions to the heterosis made by the different chromosomes shows that they are all first order contributions, there being no interaction between chromosome pairs. Comparison of the heterosis attributable to the different chromosomes with different measures of the numbers of gene loci which they contain, shows that as the method of chromosome measure-

ment approaches what appears to be the likely loci number, the better this method agrees with the heterosis which is observed when the chromosome is made heterozygous. The evidence favors several to many gene pairs per chromosome as necessary for the heterotic effects.

Heterotic effects of parental genomes as shown by a series of $F_1$ hybrids were analyzed. For the individual the most significant contribution to variation was that due to a large number of unanalyzed causes. This component contributed over half of the total variation. Differences due to races contributed 15 per cent, while those due to race-experiment and age interactions, 13 per cent. The interaction term shows that genes in quantitative inheritance are not stable in their effects. In one condition the genes could react as recessives; in another as dominants; in a third, show epistacy.

The dependence of yield on the interrelation of environment and genotype is of even greater importance. The model customarily chosen to represent genetic and environmental effects ordinarily considers the interactions of these terms zero when in truth they may be quite large. The data on both Drosophila and corn general and specific combining abilities of inbred lines show these interactions to be of major importance. Further progress in the utilization of heterosis appears to lie in the adjustment of the hybrid genotype to the environment.