INHERITANCE OF EGG SIZE IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

It has been demonstrated in a few cases that differences in size and height are inherited in the same way as are other Mendelian characters, but in most cases it has been found that size differences cannot be explained upon such a simple basis. Such cases are generally referred to multiple factor differences, but it has rarely been possible to demonstrate the presence of these postulated factors. At most the inference is made probable by reference to the spread of the F_2 as compared with the F_1 generation. Drosophila is the only form in which all of the linkage groups are so well known that it is possible to locate definitely any factors that determine or modify the size of particular parts or organs. The egg of Drosophila is especially favorable material for such genetic study, not only because here any postulated factors can be traced, but also because the size of the egg has been found to be independent of the effects of the environment and even of the size and age of the female producing the egg.

The eggs of various mutant stocks of Drosophila exhibit characteristic differences in size. Whether this variation in size is one of the effects of the gene that also conditions the mutant character, or whether it is the result of other genes present in the stocks could only be learned by crossing.

The size of the egg might be determined either by its own genetic composition or (through the egg membranes) by the germinal constitution of the female that produces it. Since the egg before maturation and the tissue cells of the female have identical genetic factors, it is not possible to discover by genetic methods whether the egg or the parent tissues determine the size of the egg. However the size of the egg is determined, the inheritance is not strictly maternal (although the term has sometimes been used in this sense) because egg size is as characteristic of the female as is any somatic character.

The size of the egg is fixed before the entrance of the sperm and can not possibly be changed by the genes from the male. The fertilized egg, that develops into the F_1 embryo, should have the size characteristic of the P_1 maternal race and the egg hatching into the F_2 embryo will be expected to show first the effects of the genes brought in by the P_1 sperm. The size of F_1 eggs (hatching into F_1 embryos) is therefore characteristic of the P_1 female, and the size of the F_2 eggs is characteristic of the F_1 female.

METHODS

The females, whose eggs were to be measured, were isolated in one-fourth pint milk-bottles. The eggs were laid on ripe banana, that had fermented in a yeast solution for at least twenty-four hours. To facilitate the removal of the eggs from the bottles, the food was placed between layers of paper toweling on an ordinary microscopic slide. Four thicknesses of the toweling were used and a small piece of the fermented banana, the size of a grain of corn, was placed between the two uppermost layers. The strips of toweling were then thoroughly saturated with the fermented banana juice. If too small an amount of the juice is used the food becomes dry, if too great the juice is likely to run off into the bottle, and drown the flies. Food prepared in this way should keep moist for twenty-four hours. Food that has fermented for more than four or five days becomes too acid. The object of placing the banana between the layers of toweling is to prevent the flies from burying their eggs in the banana. Eggs thus buried are likely to have portions of the banana clinging to them and are difficult to measure accurately. Eggs laid on the moist paper may be removed in a clean condition. Covering the banana with the single layer of toweling in no way reduces egg laying.

In order to avoid loss from hatching, it was necessary to measure the eggs at intervals of about twenty hours. All stocks and isolated females were kept in an incubator where the temperature was approximately 25°C. For measurement the eggs were removed from the food with a dissecting needle, the point of which had been flattened into a spatula-like form and then ground to an oblique, blade-like edge. Working under a binocular, the eggs could be rapidly scooped up with the needle and removed to a slide where they were placed in rows in a film of water for measurement under the compound microscope. For measurement, the low power objective and a No. 2 Leitz micrometer eye-piece were used.

DESCRIPTION OF THE EGG

The egg of *Drosophila melanogaster* averages about .5 millimeter in length. It is white in color and its chorion shows hexagonal sculpturing due to the flattened follicle cells surrounding it. Near one end of the egg, which corresponds to the anterior end of the embryo, is found a pair of slender filaments. The function of these appendages is unknown, but it has been suggested that they may aid in keeping the egg afloat. The micropyle cone is found at the anterior end of the egg. Figure 1 shows the egg in position for measuring.

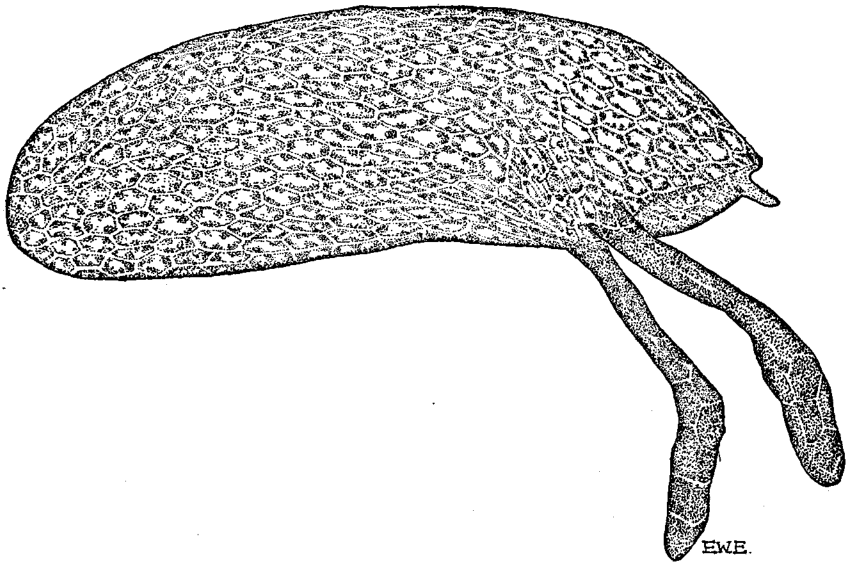

FIGURE 1.—The egg of *Drosophila melanogaster*. Drawn by Miss E. W. EMMART.

VARIATION IN DIFFERENT MUTANT STOCKS

Most of the stocks examined for egg size were being kept for some one or more mutant characters. Some of these stocks had been inbred for a long time. Wild stocks which had recently been brought into the laboratory and which carried no observed mutation were also measured. Over a hundred stocks were examined. In determining the range of egg size, a minimum of one-hundred eggs were measured from each stock, and, in cases where the stocks were extensively used in crosses, as many as a thousand eggs were measured. Several pairs of flies were isolated so that eggs from a number of females would be included in the measurements.

Table 1 shows the range of 26 stocks taken at random. The numbers given at the head of the columns, ranging from 22 to 31 divisions or units, represent the length of the egg in units of the eye-piece micrometer. A unit equals .018 mm. The name of the stock usually indicates the single

TABLE 1

Size range of various stocks.

STOCKS	UNITS AND FREQUENCIES									
	22	23	24	25	26	27	28	29	30	31
Apricot			2	5	8	20	34	23	7	1
Florida wild				2	9	33	43	11	1	1
X ple		1	2	8	25	32	25	5	2	
Abrupt						2	29	43	23	3
Purple			1	22	37	33	6	1		
w_a v f					2	10	38	36	13	1
Bar	3	6	23	51	15	2				
Dichaete				11	27	40	19	3		
Lobe				11	48	29	8	4		
Aristaless				4	11	29	37	18	1	
Morula					1	12	30	42	15	
Facet				1	3	17	47	26	6	
Oblique					3	29	40	24	4	
Cream					3	23	35	28	11	
Eyeless			1	5	10	34	31	14	5	
Wild Ku		2	8	45	19	25	1			
s_c ab cv c_{t6} t v				12	36	38	12	2		
7 ple		1	3	5	12	29	28	19	3	
Curly				6	44	40	9	1		
Shaven				2	15	30	23	20	9	1
Cardinal			4	23	43	25	4	1		
Ruby	1	3	14	33	34	14	1			
Ivory			4	34	32	24	6			
Vestigial	2	8	24	22	20	18	4	2		
Sooty				2	9	23	32	16	17	1
White					1	11	38	40	10	

It is seen that there is considerable individual variation in the egg lengths in these twenty-six stocks. The ranges determined are fairly constant for the different stocks.

mutant character of that stock. The different stocks show much individual variation. Records made at different periods indicate that the range for a stock is fairly constant. The most extreme stocks have quite different means. The means of others range between these extremes. No one condition can be designated as the normal or wild type. The stock listed as Florida wild had been in the laboratory only one generation when the record was made.

In studying the genetic behavior of the size variations, the two most divergent stocks were most extensively used. These are abrupt, which has a mean of 29.0 units (from the measurement of one-thousand eggs), and bar, which has a mean of 24.7 units. There is very little overlapping of the extremes of these two stocks. Apricot-vermilion-forked and apricot were also used extensively. Ruby was used in several crosses.

CONSTANCY OF THE CHARACTER

One of the first steps was to determine whether egg size is dependent upon environmental conditions. It is well known that the adult vinegar fly varies greatly in size, depending upon food conditions. In order to determine the relation of the size of the eggs to that of the female which lays them, a virgin female and a single male were placed in a bottle containing scarcely enough food to carry through an average brood. The first females to emerge were large, while the last ones were much smaller. The eggs of the large females were compared with those of the small ones. Since wing length has been found to be a fairly reliable index of the size of flies, this was used as a measure. The wing length given is the distance between the anterior cross-vein and the tip of longitudinal vein III. Abrupt females and hybrid females from the cross of abrupt by bar were used. Since the heterozygous condition of a character has sometimes been shown to be more susceptible to environmental influences than the homozygous condition, flies of both constitutions were tested (see table 2). The

TABLE 2

Effect of size of female upon egg size.

	ABRUPT 1*		ABRUPT 4*		F_1 (ABRUPT × BAR) 1*		F_1 (ABRUPT × BAR) 4*	
Mean wing length....	66.0	86.4	64.8	85.4	69.6	91.2	68.4	91.2
Mean egg length.....	28.9	28.7	29.1	29.5	26.9	27.0	27.5	27.0

* These numbers are culture numbers.

The mean length of eggs of sister flies which differ in size. Eggs laid by small females average as long as those of large females.

means for egg length were computed from the measurements of two-hundred eggs in each case. The results show that neither in the homozygous nor in the heterozygous condition is there any correlation between the size of the female and the size of the egg she lays. Small females lay very few eggs, but the eggs laid have an average as large as that of larger females.

As a culture ages the bottle becomes dryer. Consequently the first flies to emerge develop under conditions of much greater moisture than do those which are last to emerge. Since the size of the egg did not change, we may conclude that moisture has no effect upon egg size.

The relation of the size of eggs to the age of the female producing them was also examined. Eggs were measured at different periods in the life of the same female. The means were computed for the eggs in lots of fifty each. Records were made for two females from the abrupt stock and from one wild-stock female (Wild Ku). Table 3 gives the data. The age of the female at the beginning of each lot is given. In the case of the two abrupt females there is a slight decrease in size with age. This decrease, however, is very small. In the case of the wild female there is no indication of any decrease in the size of the egg of older flies.

TABLE 3

Effect of age of female upon egg size.

WILD KU

Age (days)........	2	5	7	34	38	41	43	46	51	59
Mean egg length...	26.3	24.9	25.7	26.1	26.5	26.9	26.6	26.0	26.4	26.7

ABRUPT 2

Age (days)........	2	7	10	12	14	18
Mean egg length...	28.9	29.0	28.9	28.6	28.7	28.5

ABRUPT 4

Age (days)........	2	7	9	11	13	16
Mean egg length...	29.1	28.9	28.9	28.4	28.3	28.4

This table shows that there is little, if any, change in the length of the egg, which is correlated with the age of the female laying it.

Another environmental condition, temperature, was also examined. Three temperature conditions were utilized,—one ranging from 28° to 30°C, another from 23° to 26°C, and a third from 10° to 20°C. In order to have all conditions (excepting temperature) as nearly identical as possible, the daily egg production of a single female (previously mated to a single male) was divided among the three temperature conditions. Females reared from the eggs of a single female but subjected during development to different temperature conditions, were compared as to egg size. Two abrupt females were used in this experiment. Table 4 gives the mean lengths of two-hundred eggs. The egg size under all these conditions was found to be constant.

TABLE 4

Effect of temperature upon egg size.

	INCUBATOR 23° to 26°C	INCUBATOR 28° to 31°C	COLD 10° to 20°C
Abrupt 4........	28.8	29.3	28.8
Abrupt 8........	28.8	28.6	28.5

The mean egg-lengths for flies reared under different temperatures indicate that temperature does not influence egg-size.

In spite of what has just been said with reference to egg size, there was evidence that some conditions do, to a slight extent, influence egg size; for individual females showed some variation from day to day. What the factors are that cause such fluctuations was not determined. Nevertheless, the constancy of egg size in general makes it an excellent character for a study of genetic behavior.

STOCKS USED IN CROSSES

The two stocks most used in crosses were abrupt and bar. The mean of abrupt is 29.0 and that of bar 24.7. Figure 2 shows the range in egg size in these two stocks. There is practically no overlapping of the extremes. Figure 3 shows the range of individual means for a number of females from each stock. Each point of the graph represents the mean of fifty eggs from a single female. While there is some individual variation, the difference between the nearest extremes of the two stocks is distinct. Another stock, apricot-vermilion-forked, was used to some extent. It had been kept for about a year as a stock combining these three sex-linked characters. The mean of apricot-vermilion-forked, computed from five-hundred egg measurements, was 28.5. Table 1 shows that this stock also has a long egg but not as long as the abrupt stock.

Ruby has a short egg with a mean of 25.4 (computed from five-hundred eggs). It is, however, not as short as bar.

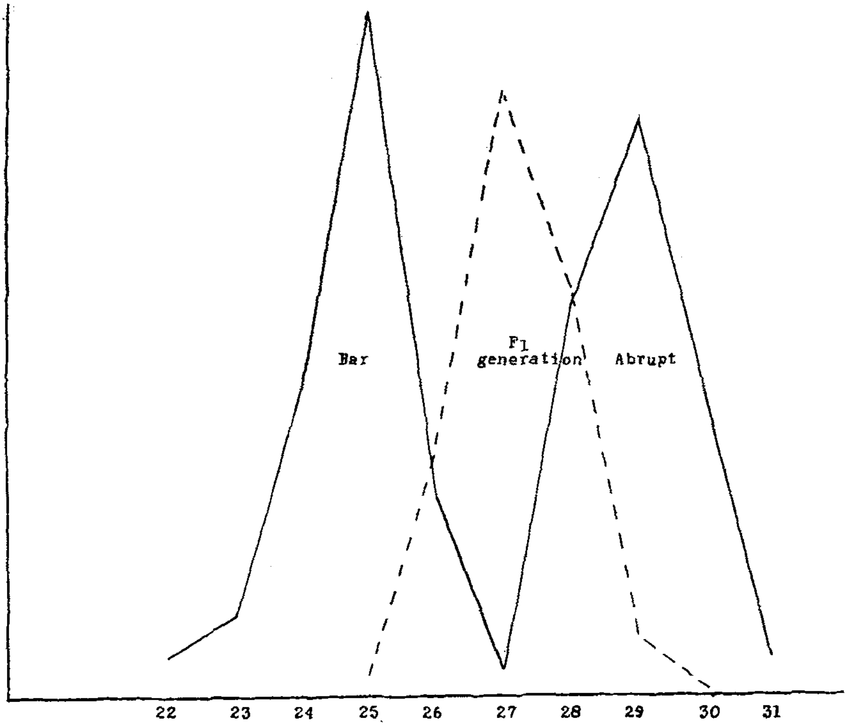

FIGURE 2.—Curves for egg length of bar and abrupt, together with that of the F_1 generation from crossing the two stocks.

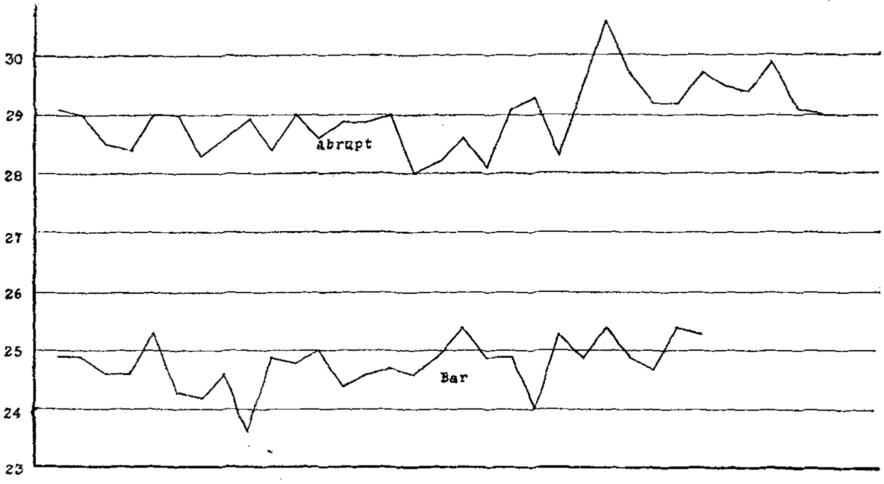

FIGURE 3.—Showing individual variation of females from the bar and abrupt stocks. Each point on the graph is an individual mean from fifty eggs. There is some individual variation but the two stocks behave quite differently.

EGG SIZE AND MUTANT CHARACTER

The following experiments were made to determine whether the genes that condition egg size are identical with those which produce the mutant characters of the two stocks abrupt and bar. The curves for the range of abrupt (long) and for bar (short) are shown in figure 2. The curve for egg measurements from F_1 flies is also included. It is seen that the curve for eggs from F_1 flies falls almost exactly intermediate between the two stocks. The crosses were made in both directions, but no significant difference was found between the reciprocal crosses (table 5).

TABLE 5

Comparison of range of reciprocal crosses of bar by abrupt.

UNITS	25	26	27	28	29	30	31	MEAN
Bar female by abrupt male	6	80	208	100	6			27.1
Abrupt female by bar male	6	65	152	142	29	6		27.4

There is no significant difference in egg-length between reciprocal crosses of bar and abrupt.

From figure 4 it is seen that the egg size is independent of the mutant character of the stock. This figure shows the results of the F_2 generation from the cross between bar female and abrupt male. Due to the amount of labor involved in determining the genetic constitution of the eggs of any one female (the measurement of at least fifty eggs) the number of individuals considered is small. Fifty eggs were measured from each of nine females. All of these females were F_2 segregated bars. Although a very few individuals were considered, both parental extremes were realized. That the egg size is independent of the mutant character is evident from the fact that some of the bar females show an egg size identical with that of the abrupt stock. There was segregation of the long egg that is characteristic of the abrupt stock, but these long eggs were produced by bar females. Thus the F_2 generation shows that egg size segregates independently of the mutant characters in the stocks.

GENETIC BEHAVIOR OF LONG EGG CONDITION FOUND IN ABRUPT STOCK

An abrupt female was crossed to a bar male and the F_1 male was back-crossed to an abrupt female. This cross was made to test whether any of the factors involved in producing the long egg of the abrupt stock are located in the X chromosome. The eggs measured were produced by F_2 females from this back-cross. Figure 5 shows the origin of the X and of the second chromosomes carried by these females. The letters inside the chromosomes indicate whether the particular chromosome came from the long or short stock. Figure 5 shows that every female derives both of her sex chromosomes from the long-egg stock (abrupt). From among the F_2 females forty were tested for egg size. Fifty eggs from each female were

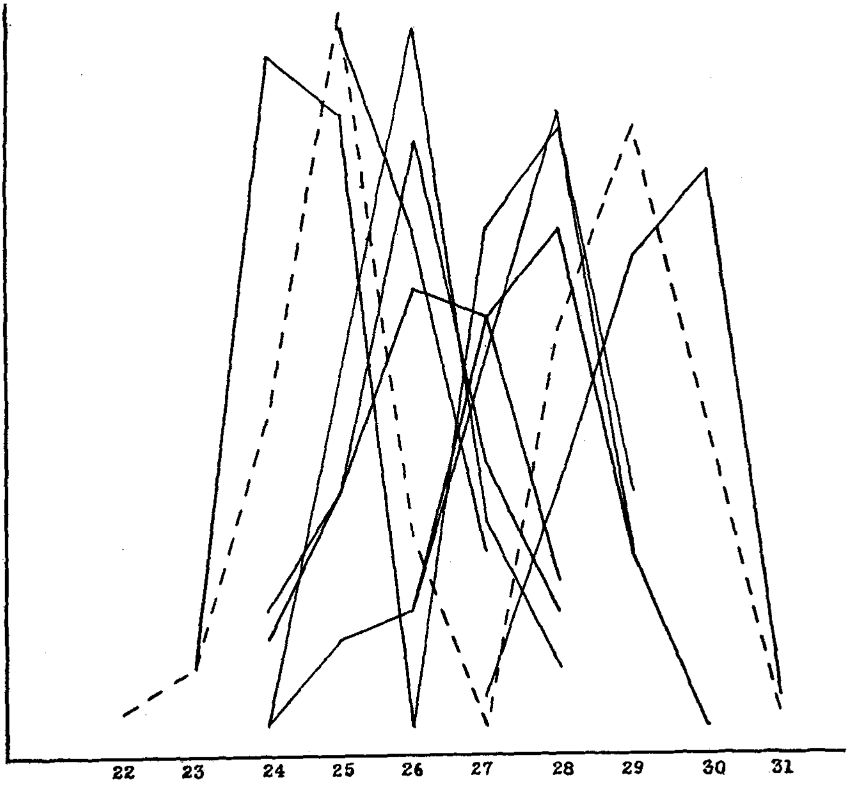

FIGURE 4.—In this graph are shown the F_2 results of the cross, bar female by abrupt male. The females tested were all segregated bars and they are indicated by the solid lines in the graph. The dash curve to the left shows the range of the bar stock and the same type of curve to the right, the range of the abrupt stock. Segregation of both parental types was obtained here.

measured and the range of the means for these females is given in figure 6. Every one, with a single exception, fell within the range of the abrupt (long) stock. The range shown by the graph in figure 6 may be compared with the range for abrupt shown in figure 3. If the long egg condition is due to a single autosomal gene we would expect only half of these F_2 females to be pure for the long condition, but all of the females show the long-egg tendency. The only explanation that will satisfy this condition is that the factor for long egg (or at least the main one) is located in the sex chromosome.

In the graph of figure 6 two lines are shown. The dash line represents the means of females which show the abrupt character and the solid line represents the means of females which do not show the abrupt condition. Abrupt is a recessive mutation in wing venation and tests have shown that the gene for abrupt is located in the second chromosome. Females which show the segregated abrupt character are known to have received both of their second chromosomes from the abrupt (long) stock. Likewise females from this cross which do not show the abrupt character are known to have received one of their second chromosomes from the bar (short) stock. The average of the normal (non-abrupt) females is lower than that of the abrupt females. The same results are shown in figure 7. Here the dotted curve represents the range of five-hundred eggs from the pure abrupt stock. The dash curve shows the range of five-hundred eggs from ten segregated abrupt females taken at random from those shown in figure 6. The solid curve represents the range of five-hundred eggs from ten of the normal females shown in figure 6. The curve for the segregated abrupt females practically coincides with that of the abrupt stock. The mean of the curve of the normal females is somewhat less than that of the abrupt

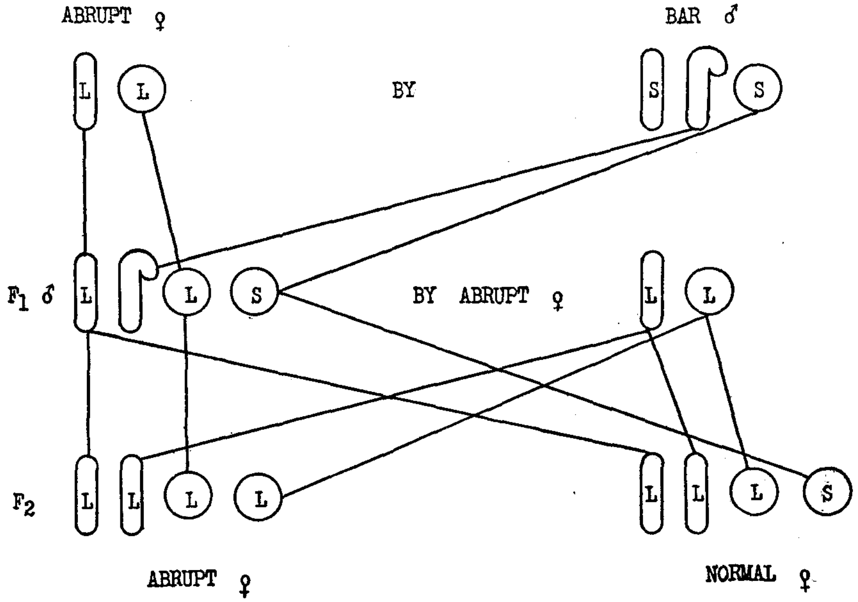

FIGURE 5.—Showing the origin of the sex chromosomes and third chromosomes in F_2 females from the cross as indicated above.

stock. Two interpretations may be placed upon the significance of this difference between wild and abrupt females. It is possible that some minor factor for the long (abrupt) condition is located in the second chromosome, since the abrupt females which here are known to have not only both X chromosomes but also both of their second chromosomes from the long stock, produce longer eggs than normal females, which are known to have but one second chromosome from the long stock. Another explanation, which further data show to be correct, is that a factor which tends to produce the short length found in the bar stock is located in the second chromosome; for, the normal females which show an average range less than pure abrupts have derived one of their second chromosomes from the bar stock. Linkage data (discussed later) indicate that both the above-

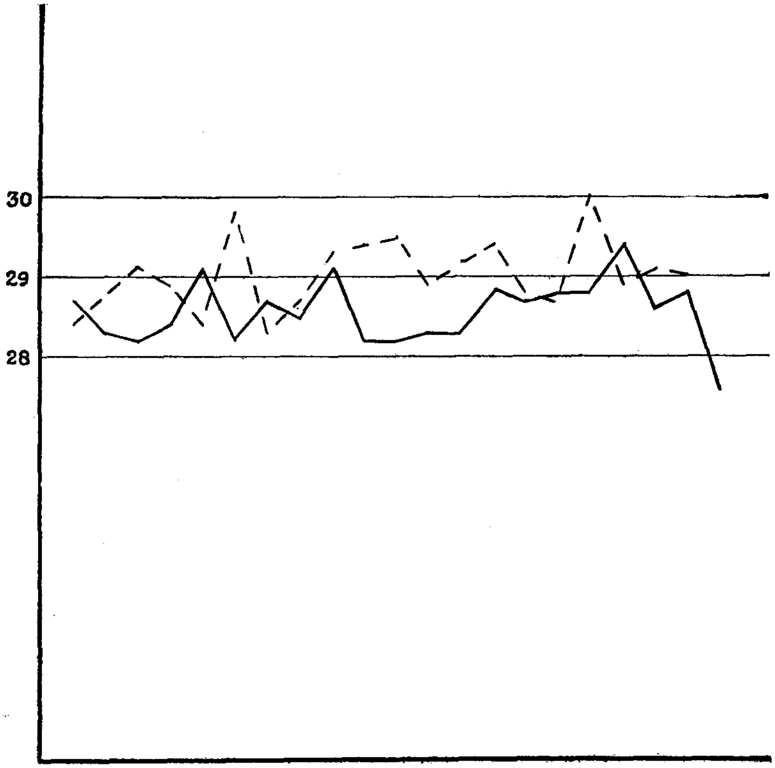

FIGURE 6.—Results of back-crossing an F_1 male from the cross, abrupt female by bar male, to an abrupt female. Each point in the graph represents an egg mean (from 50 eggs) for an F_2 female. The dash line indicates the range of segregated abrupt females, and the solid line, wild females from the same cross. The abrupt females lay eggs which average slightly larger than those of wild females, but practically all of the females examined from this cross fell within the range of pure abrupts. (See figure 3.)

mentioned conditions may be true. That there are in the second chromosome of the bar stock one or more genes which decrease the size of the egg and one or more genes in the same chromosome of the abrupt stock which increase the length of the egg. Figure 5 shows the origin of the second chromosomes in these two types of females. From the data just considered it follows that at least one major factor for the long egg condition belongs to the sex-linked group.

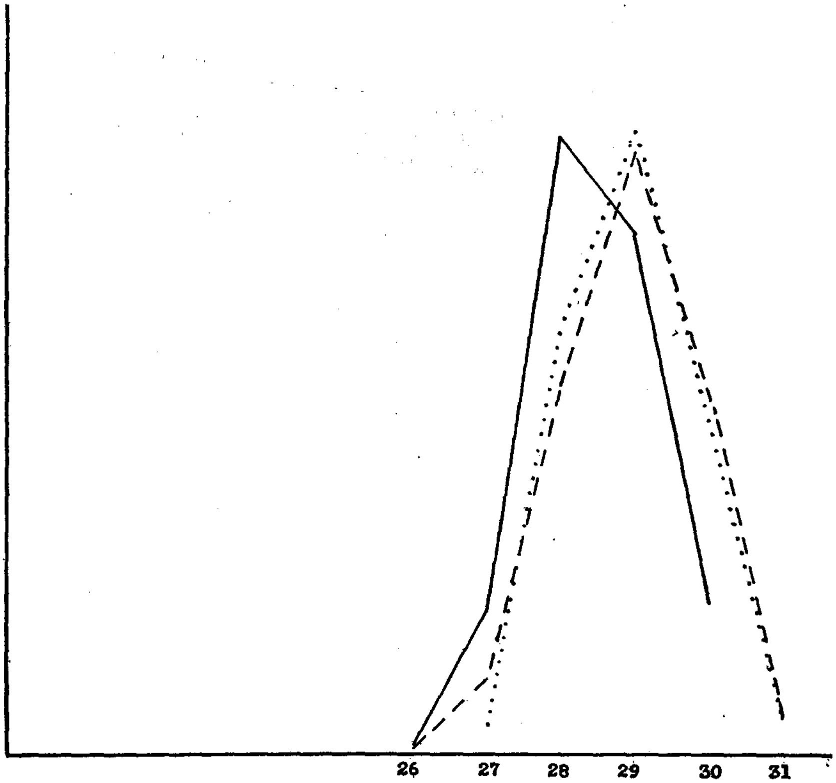

FIGURE 7.—The mass results of the data given in figure 6. The dash curve shows the range of segregated abrupt; solid curve, segregated wilds; and dotted curve, pure abrupt stock.

To further check the data just given, a back-cross was made from the reciprocal of the previous cross. An F_1 male from the cross, bar female by abrupt male, was back-crossed to an abrupt female. Eggs were measured from F_2 females from this cross. Figure 8 shows the origin of the sex chromosomes and the second chromosomes in these females.

Figure 8 shows that each female receives one member of her pair of X chromosomes from the abrupt stock and the other member from the bar stock. Figure 9 shows the range of the means of twenty-eight females from the F_2 of this back-cross. This graph shows that the F_2 behavior here is quite different from that shown by the reciprocal cross (figure 6). The difference between these females and those of the reciprocal cross is that in the former cross they receive both their X chromosomes from the abrupt (long) stock and here one of the two X chromosomes comes from the bar (short) stock. Only about half of the females have a mean which falls within the range of the pure abrupt (long), the rest falling below. Other data have indicated that one of the main factors for long egg is in the sex chromosome. If the long condition is due entirely to a sex-linked gene we would not expect any of these females to have a mean within the range of pure longs; for, as shown in figure 8, none of these females are homozygous for the X chromosome from the long stock. It has already been shown that the heterozygous condition has a range midway between those of the abrupt and bar stocks. Since some of these females (in fact half) show a mean within the range of the abrupt stock it seems probable that there is also an autosomal gene involved in making the egg long. The dash line shown in figure 9 represents the range of ten females that show the abrupt character. These females, as shown in figure 8, receive both their second chromosomes from the abrupt (long) stock. Since there is evidence that an autosomal gene is involved in making the egg long and since we know

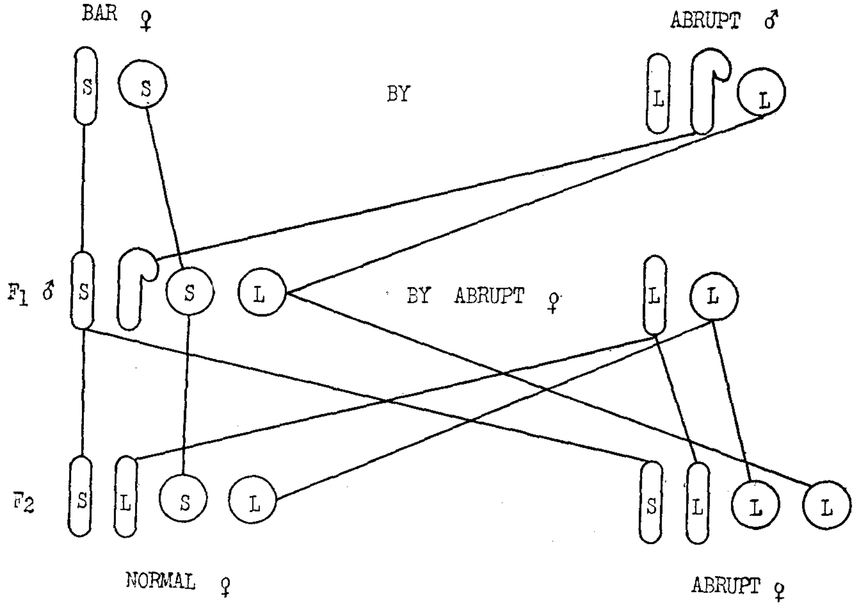

FIGURE 8.—Showing origin of sex chromosomes and third chromosomes in F_2 females from cross as indicated in figure 6.

that the females showing the abrupt character receive both their second chromosomes from the abrupt (long) stock, their behavior should indicate whether the autosomal gene is in the second chromosome. If the autosomal gene involved is in the second chromosome, all of the females showing the abrupt character should have means which fall within the range of the abrupt stock. We find that some of these females have means which fall outside the known range of the abrupt stock, showing that the most important autosomal gene increasing the length of the egg is not in the second chromosome.

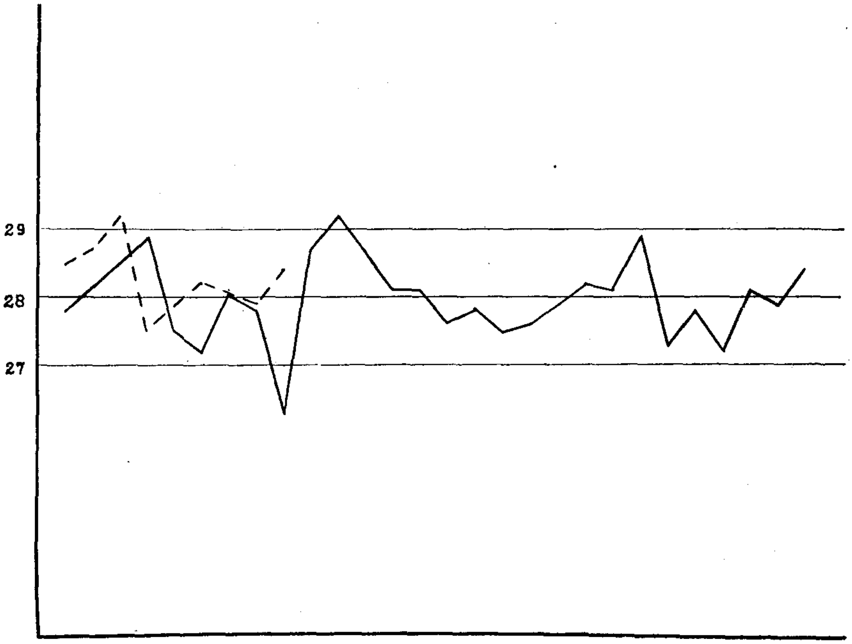

FIGURE 9.—Range of means of individual females from back-cross of F_1 male (from cross of bar female by abrupt male) to an abrupt female. The solid line indicates range of all F_2 females, and the dash line, the segregated abrupt females.

Thus from this back-cross we have three lines of evidence as to the genes involved in producing the long egg condition found in abrupt stock:

1. When the F_2 females carry only one X chromosome from the abrupt (long) stock, half of them show a mean which falls below the range of the abrupt stock. This substantiates the previous indication, that one of the main factors producing the long egg is in the sex chromosome.

2. Since about half of these females have means which fall within the range of abrupt stock, it seems probable that there is also an important autosomal gene involved which makes the egg long.

3. The most important autosomal gene involved in making the egg long is not carried by the second chromosome.

LINKAGE DATA FOR THE ABRUPT STOCK (LONG)

Since evidence was found that at least one gene involved in making the egg long, is carried by an autosome, the following linkage experiments were planned to locate more accurately this gene. In testing the linkage group to which such autosomal genes might belong, abrupt females were out-crossed to three stocks each of which was known to carry a factor for one of the three autosomal linkage groups. Purple was used in the second chromosome, cardinal in the third, and eyeless in the fourth. The abrupt stock was out-crossed to each of these stocks, and the F_1 male was back-crossed to these respective stocks. Purple has a mean of 26.2 units, cardinal a mean of 26.0, and eyeless a mean of 27.8. These means were computed from the measurement of two-hundred eggs. It will be observed that all of the mutations to which abrupt is back-crossed show an egg size less than that of abrupt. In the F_2 of such back-crosses, eggs were measured from females showing the character of the stock to which abrupt was out-crossed and also from females lacking this mutation. Thus a female known to be homogametic for the origin of a given chromosome pair is compared with a female known to be heterogametic for the origin of the same chromosome pair. The former receives both members of a given pair of chromosomes from the stock to which abrupt is out-crossed and the latter carries one member of this pair from the abrupt stock. Thus, if the back-cross is made to the chromosome group to which a "long" autosomal gene belongs, the females which show the mutation of this group should have a shorter egg length than the normal females; for, the normal females are known to possess one chromosome of this pair from the abrupt (long) stock. Figure 10 shows the origin of the pair of autosomes in which the long gene is located, for these two types of females.

In crossing any two stocks which differ in egg size the usual range of the F_1 females is mid-way between the two. However, there has been observed some variation in the range of F_1 flies. This fluctuation is probably due to the effect of some environmental factor not determined. This variation is a possible source of error in interpreting the results, so that slight differences have not been considered as significant. Table 6 gives these data. It will be seen that in the out-cross to the fourth linkage group (eyeless) the normal and eyeless from the F_2 generation show ranges that are practically identical. The cross to the second linkage group (purple) shows some difference in range between the two types of females. In the cross to the

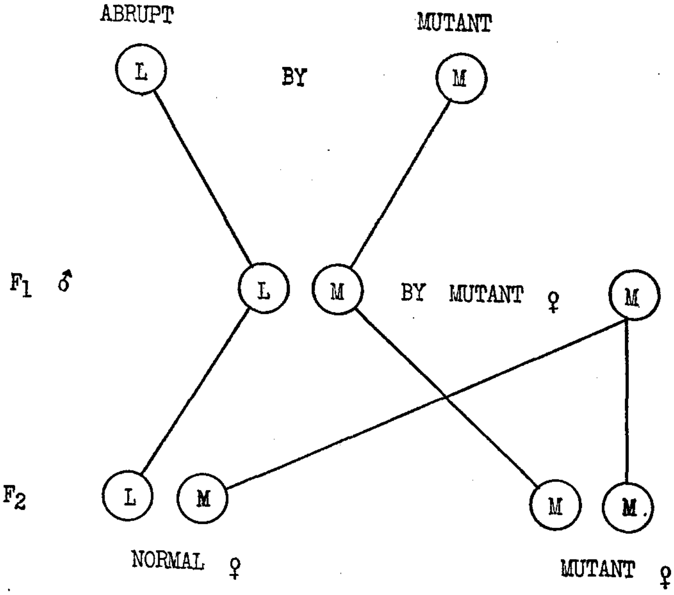

FIGURE 10.—Showing the origin of any pair of autosomes which may differ as to genes influencing egg length in the two stocks crossed. This type of cross was made, utilizing recessive mutants in each linkage group, in determining the linkage relations of the abrupt stock.

TABLE 6

Linkage relations of long egg condition in abrupt stock.

Cross to second group

Units	24	25	26	27	28	29	30	31	Mean	χ^2	P
Purple female		19	65	77	35	4			26.7	31.04	.000005
Normal female		6	42	65	79	8			27.2		
Cross to third group											
Cardinal female	9	95	81	14	1				25.5	294.76	.000000
Normal female		2	14	62	95	27			27.7		
Cross to fourth group											
Eyeless female				14	60	90	36		28.7	3.76	.3
Normal female			1	14	77	80	28		28.6		

The application of the χ^2 test indicates that the difference found in the tests with the second and third linkage groups is significant. Thus, there are genes in the second and third chromosomes which affect egg size.

third linkage group there is a rather striking difference in range between the normal and cardinal females. There is here no evidence that there are any genes in the fourth chromosome which influence egg size. There is some evidence that the second chromosome bears one or more genes which increase the length of the egg and very strong evidence that the most important gene or genes for increasing the length of the egg are in the third chromosome. The application of the χ^2 test indicates that the differences found in the case of the second and third linkage groups are significant. The principal autosomal factor for which we had previously found evidence (figure 9) is probably in the third linkage group and there is also a minor modifier which belongs to the second linkage group.

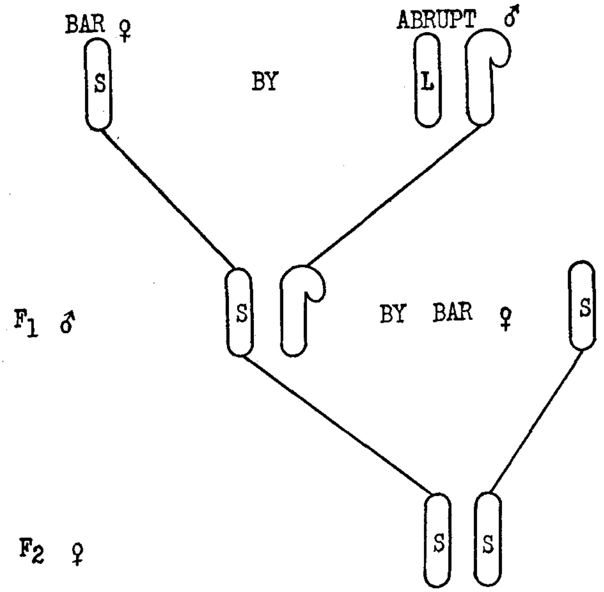

FIGURE 11.—Showing the origin of the sex chromosomes in the females from the cross as indicated in figure 9.

From the foregoing experiments with the abrupt stock we have evidence that there are two principal and one minor factor involved in making the egg long. One of the major factors is sex-linked and the other belongs to the third linkage group. A minor factor showing linkage to the second group slightly affects the length of the egg. The effects found may be due to a single gene or to the combined action of several genes which have accumulated in particular chromosomes.

GENETIC BEHAVIOR OF THE SHORT EGG OF BAR STOCK

A bar female was crossed to an abrupt male and the resulting F_1 male was back-crossed to a bar female. This cross was made to test whether any genes involved in producing the short egg which is characteristic of bar, is sex-linked. Figure 11 shows the origin of the sex chromosomes in these F_2 females. In this cross, the counteracting influence of the "long-producing" factors carried by the abrupt stock, must be considered. However, since here both of the sex chromosomes of the F_2 female come from the bar stock, we have eliminated the sex-linked "long" gene of abrupt. Half of these females will carry in one of their third chromosomes a "long-producing" gene from the abrupt stock. The other half of these F_2 females should be free from the effects of either of the two "long-producing" genes and since both of their sex chromosomes come from the bar (short) stock, they should be homozygous for any "short-producing" sex-linked genes. By comparison of figure 12 with figure 3 it will be seen that considerably less than half of these females have a mean which falls within the range of pure bar. It seems probable therefore that there are no sex-linked factors which produce the short egg condition of bar.

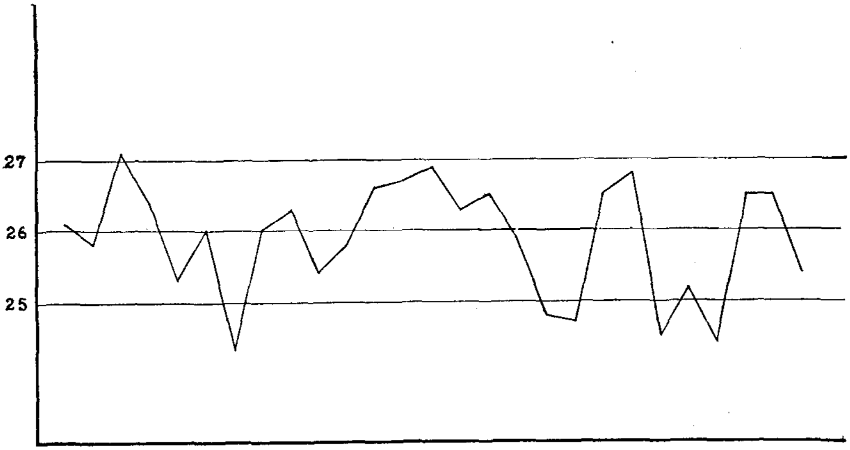

FIGURE 12.—The range of means of individual females from the back-cross of an F_1 male from the cross bar female by abrupt male, to a bar female. Since many of these means fall outside the range of pure bars, the most important genes for the short egg are not in the sex chromosome.

If there were a single autosomal gene which makes the bar egg short, then half of these F_2 females should be homozygous for this gene and should have the range of pure bars. However, the counteracting effect of the "long" autosomal gene must be kept in view. Half of these females also carry this gene and with the free assortment of these two genes we would expect one-fourth of these F_2 females to be homozygous for the "short" gene and free from the effects of the "long" gene. This in fact, is about the proportion of pure "shorts" that are realized.

It seems therefore that there is a single major autosomal factor that produces the short condition of bar.

LINKAGE DATA FOR THE BAR STOCK (SHORT)

The linkage behavior of the short condition was tested in the same way as in the case of abrupt. Here it was possible to use dominant mutations in each group. The F_1 males showing the dominant mutation were backcrossed to the bar stock, and in the F_2 generation, females showing the dominant mutation were compared (as to egg size) with females lacking this character. The chromosomal complexes of both types of females are shown in figure 13. Here the F_2 females which show the dominant mutation will receive from the bar stock but one member of the pair of chromosomes under consideration, while in the case of the females lacking the dominant character, both chromosomes of this pair must come from the bar stock. Since all of the stocks used averaged considerably larger than the bar stock, the females showing the dominant character in the group to which the "short" autosomal factor belongs, should average larger than

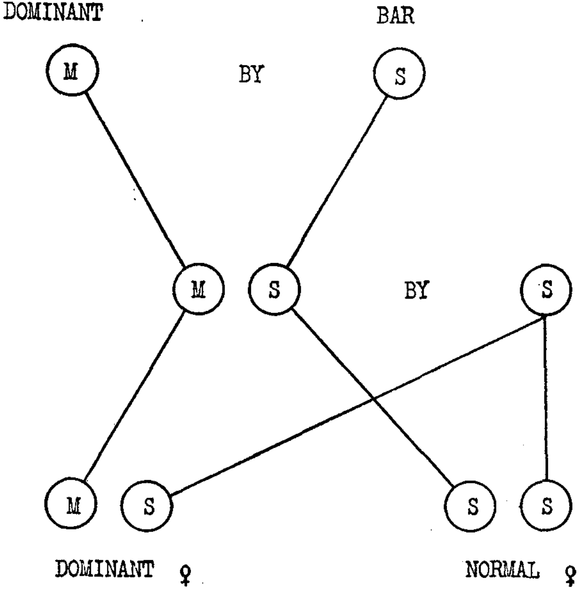

FIGURE 13.—Showing the origin of autosomes in crosses made to determine the linkage relations of the bar stock.

the normal females. In crosses to linkage groups in which the "short" autosomal factor does not belong, there should be no difference between the two types of females. Curly and lobe$_2$ were used for the second chromosome and dichaete for the third. Since both the characters, bar and eyeless, affected the shape of the eye, it was not possible to obtain a test for the fourth linkage group. These linkage data are shown in table 7. There is evidence for one or more genes in the second chromosome which decrease the length of the egg. This is shown by the two crosses, to curly and to lobe$_2$, for, in both cases the females known to be heterogametic for the origin of their second chromosomes average considerably larger than those females known to be homogametic for the same pair of chromosomes, both members of which have originated in the bar stock. There is a similar difference in the cross to dichaete, a third-chromosome dominant, but here the difference is rather slight and, if significant at all, is indicative of a minor gene affecting egg size. The evidence for a "short" gene in the second chromosome is substantiated by data given earlier in figure 7. It was shown that segregated abrupt (second-chromosome) females produce eggs that average longer than those lacking this character and one possible explanation of this would be that the second chromosome of the bar stock carried one or more genes which decreased the length of the egg.

TABLE 7

Linkage data for short egg condition in bar stock.

Cross to second group

Units	22	23	24	25	26	27	28	29	Mean	χ^2	P
Lobe$_2$ female		1	11	85	85	18			25.5	114.84	.000000
Normal female		9	53	126	12				24.7		
Curly female			4	37	76	73	9	1	26.5	108.52	.000000
Normal female			14	87	75	21	3		25.6		

Cross to third group

Dichaete female			10	80	83	27			25.6	20.48	.001250
Normal female		2	29	94	57	17	1		25.3		

The differences found in the tests with the second linkage group indicate that there are genes in the second chromosome which influence egg length. It is questionable whether the difference found in the case of the third group is significant.

Thus, it appears that we have in the second chromosome one or more genes which largely determine the shortness of the bar egg and there may be in addition a minor modifier in the third chromosome.

THE GENETIC BEHAVIOR OF APRICOT-VERMILION-FORKED STOCK

The stock apricot-vermilion-forked has an egg mean of 28.5 units. This is seen to be almost as extreme as abrupt. If the two stocks are crossed the F_1 females show a range more or less intermediate between the two. These data are shown in table 8. Since the F_1 mean is so near that of the two parent stocks, it is evident that the two stocks must have in common some of the principal factors which determine the long egg condition.

TABLE 8

Results of crossing two long-egg stocks, abrupt and w_a v f.

STOCKS	NUMBER OF EGGS MEASURED	UNITS AND FREQUENCIES						MEAN
		26	27	28	29	30	31	
w_a v f....................	500	3	54	190	181	68	4	28.5
F_1 of w_a v f by abrupt.....	200		11	84	73	31	1	28.6
Abrupt..................	500		8	147	214	117	14	29.0

Since the range of egg length in the F_1 generation of the cross abrupt by w_a v f is so near that of the two stocks crossed, it is assumed that some of the principal genes influencing egg length in the two stocks are identical.

In the cross of apricot-vermilion-forked to dichaete (table 9) the F_1 male was back-crossed to an w_a v f (apricot-vermilion-forked) female. Dichaete has an egg range much shorter than w_a v f but the F_2 females, receiving both X chromosomes from the w_a v f stock, showed a range practically identical with that of the pure w_a v f stock. This would indicate that here,

TABLE 9

Comparing range of w_a v f stock with F_2 females from back-cross of F_1 male in cross w_a v f female by dichaete male, to w_a v f female.

STOCKS	NUMBER OF EGGS MEASURED	UNITS AND FREQUENCIES						MEAN
		26	27	28	29	30	31	
w_a v f....................	500	3	54	190	181	68	4	28.5
F_2 females from back-cross.	400	8	73	184	112	23		28.2

The similarity in range of the two types of females tested indicates that some of the principal genes determining egg length are sex-linked.

as in abrupt, a sex-linked gene plays an important part in making the egg long.

Having evidence that both $w_a\ v\ f$ and abrupt carry in the sex chromosome an important gene for their long egg condition, linkage experiments were carried out with $w_a\ v\ f$ to determine its autosomal condition with respect to egg size. Table 10 gives the results of these linkage crosses. $w_a\ v\ f$ was crossed to purple, cardinal and eyeless, recessive mutants from the second, third and fourth chromosome groups, respectively. These crosses were made in the same manner as were the crosses in the case of abrupt. There was found no evidence for any genes in the second chromosome which modify the length of the egg. In the test with the fourth linkage group the difference between the two types of females is significant and indicates that there are one or more genes in the fourth chromosome which modify the length of the egg. The evidence for any modifiers carried by the third chromosome is questionable. Thus it seems that in the $w_a\ v\ f$ stock we have the same sex-linked modifiers that exist in the abrupt stock, but the autosomal genes involved are not the same as those found in the abrupt stock. It seems that the second-chromosome genes found effective in the case of abrupt are here lacking. This is quite

TABLE 10

Linkage data for long egg condition in $w_a\ v\ f$ stock.

Cross to second group

Units	23	24	25	26	27	28	29	30	Mean	χ^2	P
Purple female	2	11	92	74	20	1			25.5	6.92	.135888
Normal female	1	6	73	94	26				25.7		

Cross to third group

Units	23	24	25	26	27	28	29	30	Mean	χ^2	P
Cardinal female			51	64	58	25	2		26.3	16.54	.002
Normal female		1	22	78	62	30	7		26.6		

Cross to fourth group

Units	23	24	25	26	27	28	29	30	Mean	χ^2	P
Eyeless female			8	40	73	59	18	2	27.2	57.12	.000000
Normal female			3	9	42	83	53	10	28.0		

There is a significant difference between the two types of females in the test with the fourth linkage group, and in this group only is there unquestionable evidence for factors influencing egg length.

possible for it will be shown that the sex-linked gene or genes in abrupt have alone the same effect that they have in conjunction with the autosomal genes.

SHORT EGG SIZE OF RUBY STOCK

The mean for ruby, computed from the measurement of five-hundred eggs, was found to be 25.4 units. It shows a range much shorter than the average but the mean is somewhat higher than that of bar (mean 24.7).

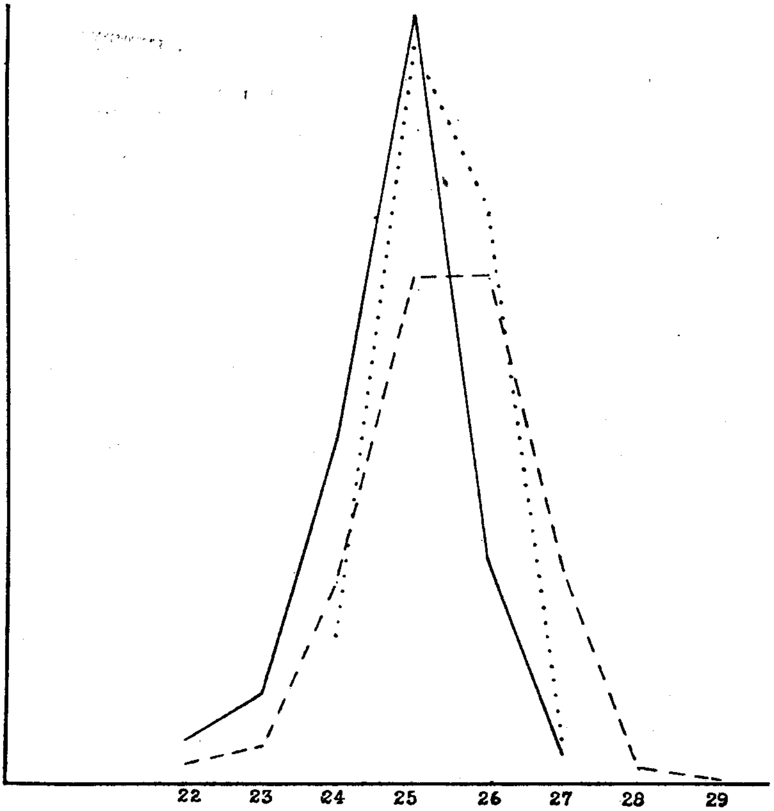

FIGURE 14.—Results of crossing the two short-egg stocks, bar and ruby. The solid line indicates the range for bar; the dash line, for ruby; and the dotted line, for the F_1 generation from crossing the two.

To determine whether the same factors were involved in the two "short" stocks, they were crossed (see figure 14). It is seen that the F_1-generation size range is also short as in the two parental stocks. It follows that the principal factors which make bar short must also be carried by the ruby stock. However, since the ruby stock consistently ranges slightly higher than bar, it must have a somewhat different genetic constitution. The ruby stock was selected for ten generations, breeding only from the shortest eggs and the results are shown in table 11. It is seen that selection reduced the range of the ruby stock to practically that of bar. Thus by selection it has been possible to eliminate the factor or factors which tended to slightly increase the egg size of ruby.

TABLE 11

Effect of selection in ruby stock.

STOCKS	NUMBER OF EGGS MEASURED	UNITS AND FREQUENCIES								
		22	23	24	25	26	27	28	29	MEAN
Ruby stock.....	500	7	13	68	167	168	71	5	1	25.4
Ruby (selected).	200		1	23	134	42				25.1
Bar stock......	500	14	30	113	257	77	9			24.7

It is seen that selection was effective in reducing the length of the egg of the ruby stock.

F_1 BEHAVIOR IN CROSSES OF VARIOUS STOCKS

In the crosses between various stocks, the range of egg size of the F_1 flies is of interest in that there is a general tendency for the mean of egg size to be intermediate between the two parental stocks. The nearness with which the F_1 mean approaches the median between the two stocks is rather striking. Table 12 gives the results of these crosses. A series of crosses are shown for bar, w_a v f and abrupt. The column to the left shows the different stocks to which they were crossed. These stocks are arranged in order of their mean sizes beginning with the short-egg

TABLE 12

The F_1 behavior in crosses of various stocks.

STOCKS	BAR MEAN, 24.7		w_a v f MEAN, 28.5		ABRUPT MEAN, 29.0	
	Stock mean	F_1 mean	Stock mean	F_1 mean	Stock mean	F_1 mean
Bar.........					24.7	27.1
Ruby.......	25.4	25.3				
$Lobe_2$.......	26.5	25.5	26.5	27.4	26.5	27.2
Dichaete....	26.8	25.9	26.8	28.1	26.8	27.4
w_a v f.......					28.5	28.6
Abrupt......	29.0	27.1	29.0	28.6		

This table shows that the F_1-generation range is always intermediate between any two stocks crossed.

stock, bar, and ending with abrupt (long). In adjacent columns are given the means of the stock to which these three stocks were crossed and the mean of the F_1 generation of each cross. Examination of the table will show that the mean of the F_1 generation is always intermediate between any two stocks crossed. It will also be seen that when any stock is crossed to a series of stocks which successively increases in size, the F_1 means increase in the same manner, although not exactly in the same ratio. This regular intermediate nature of the F_1 generation is of interest. Such a behavior would be expected if we had here a number of rather small modifiers for egg size. The stocks varying in range would each have a different combination of them, and the F_1 generation from crossing such stocks would be intermediate. From the foregoing experiments we have found some evidence for small modifying factors.

GENERAL DISCUSSION

Measurements of various stocks of flies showed that each seemed to have an egg size of its own. No one condition was found which might be called "wild-type." The sizes found to be characteristic of the different races were very constant and seemed to be in no way affected by any environmental conditions tested. They were also not correlated with any size or age differences in the female.

Conditions similar to those found here with respect to independence of egg size and size of female, have been described in other forms. The smaller breeds of the domestic fowl frequently produce larger eggs than do some of the larger breeds. Here also the size difference of the egg is due to genetic constitution rather than environmental conditions. EAST (1913) found in Nicotiana that the corolla spread of the flower practically showed independence of the size of the plant.

If the nature of the size character could be traced to its last analysis, it would probably be found that we are dealing with some anatomical relation of the female reproductive organs which govern the formation of the egg. If so, in the study of egg size we are dealing with a condition which is the result of this character. However, the character and its result are both expressions of the same genetic constitution, and the results are in no way altered.

In two stocks selection was carried out for ten generations. These stocks were abrupt and ruby. In the case of the former the strain was carried by selecting only the very longest eggs, and in the latter only the shortest eggs were used. The selection in the abrupt stock showed no increase in egg size. This is more or less to be expected since the stock was, to begin with, the most extreme stock examined. In the case of ruby it was possible by selection to reduce the egg size to a range practically identical with that of bar. Table 11 gives these results. It was possible to eliminate whatever genetic differences existed between bar and ruby.

It was possible to locate in all four chromosomes, genes that affect egg size. The factors found were quite variable in their effect upon egg length. It is not known whether the effects correlated with particular chromosomes are due to a single gene or to the action of several genes in that chromosome. From the F_1 behavior (discussed in the last section) of various stocks it seems probable that there may be many minor factors, each having only a very slight effect, which contribute to the variations found in egg size. The results of selection of the ruby stock indicate that there must have been one or more minor factors which it was possible to eliminate. By linkage experiments it was possible to locate some of these minor factors.

In discussing the foregoing linkage experiments it has been stated that the stocks, abrupt, bar and $w_a\ v\ f$, each carries specific genes which increase or decrease the length of the egg. This is perhaps an overstatement of what has actually been determined, for in this study no wild type is known and very little is really known as to the factors for egg size carried by the stocks to which the above-mentioned races were crossed in determining the linkage relations. The linkage experiments indicate only that there are, with respect to egg size, certain factor differences between the stocks crossed, and just how the factors in any linkage group react upon the length of the egg, is unknown. The fact of chief interest, however, is that there exist these genes which are responsible for the variations in the length of the egg.

In determining the long egg length found in the abrupt stock two major factors were found, one sex-linked and the other belonging to the second group of linked characters. In the F_2 generation of the cross, bar female by abrupt male (figure 4), we obtained an individual with an egg mean typical of pure abrupt. This shows that the autosomal gene in abrupt is capable of giving the extreme abrupt condition without the aid of the sex-linked gene, for here both sex chromosomes came from the bar stock. This segregated "long" must have received both of its second chromosomes from the abrupt stock, for the F_1 behavior of this cross shows that a fly heterozygous for both the sex-linked and autosomal factors never shows the extreme range of pure abrupt. In figure 6 we have evidence that the sex-linked factor in abrupt stock is capable, in the presence of the autosomal factor in a heterozygous condition, of producing the pure abrupt size. Here all the females are homozygous for the sex-linked factor, but about half of them must be heterozygous for the autosomal factor. However, all of them have the range of pure longs.

Due to the presence of the two major factors in abrupt, each of which in the absence of the other is probably capable of producing the long egg condition, the effects of the "short" gene in bar was frequently submerged in the crosses shown. However, in figure 6 we see that females heterozygous for the "short" factor from bar, show a lower range than those known not to carry this gene in either chromosome. Thus the "short" factor in a heterozygous condition slightly counteracts the effect of the sex-linked factor in a homozygous condition.

In a back-cross of an F_1 male from the cross, abrupt female by bar male, to a bar female, a few F_2 females were examined for egg size (data not given). Only six were measured, but two of these showed the range of pure shorts. Here all females received from the abrupt stock one sex chromosome which carried the "long" sex-linked gene. It follows that the "short" factor of bar in a homozygous condition produces its full effect even in the presence of the counteracting influence of the "long" factor in a heterozygous condition.

We have dealt with variations that are exceedingly minute. The most extreme variants considered have shown a difference of less than .2 millimeters. Nevertheless it has been possible to show that a number of genes are involved in bringing about these variations. To what an extreme degree of minuteness a genic change may be manifested is one of the interesting results of this study.

In conclusion, I wish to express my gratitude to Prof. T. H. MORGAN for valuable advice and criticism during the course of this study. I am also indebted to Dr. A. H. STURTEVANT for many helpful suggestions.

SUMMARY

1. Of the various stocks examined, many show characteristic and constant ranges, for egg size. No one condition found could be classed as "wild-type."
2. The factors for egg size proved to be entirely independent of those responsible for mutations in the races where the unusual sizes were found.
3. Egg size is, as far as tested, independent of external conditions. It is also independent of the size and age of the female which lays the egg.
4. Factors for egg size were found in all four linkage groups.
5. From the study of the abrupt stock, genes which influence the size of the egg, were located in the first, second and third chromosomes.

6. Linkage experiments with the bar stock indicate that there are one or more genes in the second chromosome which affect the size of the egg.

7. Genes affecting egg size were located in the first and fourth chromosomes from tests with the w_a *v f* stock.

8. In crossing any two stocks, the egg size of the F_1 females was almost invariably intermediate between those of the two parent stocks.

9. There is evidence that in addition to the factors already mentioned, there are several minor ones which have a slight influence upon the size of the egg.

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