evidence obtained from meiotic prophase configurations and from the types and frequencies of the various quartets at anaphase II indicates that these new types of chromosomes arise from crossing-over in a heterozygous pericentric inversion rather than from aberrant centromere behavior as previously suggested. The presence of metacentric chromosomes indicates that the inversion is of approximately equal length in the two arms of the altered chromosome and the frequencies of novel quartet types show that crossing-over occurs within the inversion with a high frequency. The ratio of comparable quartet types resulting from three- and four-strand double crossing-over agrees with that expected if crossing-over involves the four chromatids at random.

[1] Giles, Norman H., Jr., *Genetics*, **28**, 512–524 (1943).
[2] Hillary, B. B., *Bot. Gaz.*, **102**, 225–235 (1940).
[3] Beadle, G. W., and Emerson, S., *Genetics*, **20**, 192–206 (1935).
[4] Weinstein, A., *Ibid.*, **21**, 155–199 (1936).
[5] Darlington, C. D., *Jour. Genet.*, **39**, 351–361 (1940).

STUDIES ON THE DEVELOPMENT OF THE EYE: EVIDENCE THAT THE LOBE², LOBE⁴, LOBE⁵ AND EYELESS² MUTANTS OF DROSOPHILA MELANOGASTER DEVELOP IN A MANNER SIMILAR TO BAR*

BY ARTHUR G. STEINBERG

DEPARTMENT OF GENETICS, McGILL UNIVERSITY

Introduction.—Studies in the field of developmental genetics, although primarily directed toward an understanding of how the gene acts, have in many instances led toward a better understanding of the developmental mechanics of the organisms studied. This is most certainly true of *Drosophila melanogaster*, in which our knowledge of its embryology, both descriptive and experimental, has been derived from studies concerning the development of various mutant forms.

Studies on the development of the Bar mutation of *D. melanogaster* have led to a hypothesis concerning its (the mutation's) mode of development which, if capable of generalization, would add further information to our growing knowledge of the embryology of Drosophila. These studies showed that the Bar eye disc was smaller than that of wild type at all

larval stages from 36 hours after hatching, the earliest stage studied, to pupation. They demonstrated that the rate of increase in size of the Bar eye disc was the same as that of wild type and that histological differentiation proceeded at the same rate as in wild type (Steinberg 1941*a*, 1941*b* and 1943). They have also established that both a second chromosome inhibitor of Bar, and temperature bring about their effect on facet number without affecting the size of the eye disc, although it had been shown by Medvedev (1935) for Lobe,[c] glass[2], eyeless[2] and wild type, and by Steinberg and Abramowitz (1938) for double-Bar, double infra-Bar, Bar, infra-Bar and Bar[4] that the size of the eye discs of the mature larva is directly proportional to the number of facets in the imaginal eye.

The hypothesis based on these studies postulates that the reduced size of the Bar eye disc is due to the participation of fewer cells in the initial formation of the eye disc, and that this smaller size of the eye disc determines the range over which the facet number of the adult eye may vary; the variation in the facet number, once the eye disc is formed, is postulated to be due to the presence of cells labilely determined to form either head chitin or facets. The ultimate fate of these cells is dependent upon various extrinsic and intrinsic factors—such as temperature, nutrition, modifiers, etc. (Steinberg 1941*a*, 1941*b* and 1943). Reduced to its bare essentials the hypothesis postulates two main steps in the determination of the facet number of Bar, the first in the embryonic stage when the size of the eye disc and hence the possible range of variation of facet number are determined, the second in the larval stage when the fate of the labilely determined cells is fixed.

Some evidence exists which indicates that at least the first step postulated in the determination of the facet number is present in other mutant forms as well as in Bar. The evidence is derived from the data of Medvedev and of Steinberg and Abramowitz cited above which showed that a reduction in the facet number of the imaginal eye is associated with a proportional reduction in the size of the eye discs of the mature larvae. Hence all of the eight mutants studied in this respect show indications of the presence of the postulated first step in the determination of the adult facet number. There is no evidence available concerning the presence or absence of labilely determined cells in any mutant type other than Bar. This paper reports the results of experiments designed to obtain such evidence for four mutants of *D. melanogaster*.

Materials and Methods.—The mutants used were the alleles Lobe[2], Lobe[4] and Lobe[5] (L^2, L^4 and L^5, respectively) located at 72.0 in the second chromosome and eyeless[2] (ey^2) located in the fourth chromosome. The stocks bearing the mutants were maintained in mass cultures.

These mutants were chosen for study because previous experience had shown that the facet number of flies of each of these genotypes is extremely

variable even in isogenic stocks raised under uniform conditions, and that
the facet numbers of the left and right eyes of flies of each of these mutant
types are largely independent of each other. It was reasoned that this
variability is due, at least in part, to an extreme sensitivity of the labilely
determined cells to minor fluctuations in the environment in which the
larvae develop.

If the variation in facet number from fly to fly within a given genotype
is due simply to changes in the size of the eye discs, it follows that the coef-
ficient of variability of the facet number should not be greater than that of
the size of the eye discs of mature larvae of the same strain. On the other
hand, if the variation in facet number from fly to fly is associated with the
presence of labilely determined cells, the coefficient of variation of the facet
number should exceed that for the size of the eye discs. Likewise, if the
variation in facet number between the left and right eyes of a given fly is
due solely to variation in the size of the left and right eye discs it follows
that the left-right correlation values for the eye discs should be the same
as that for the corresponding facet numbers, while, on the other hand, if
the variation is due to the presence of labilely determined tissue, the left-
right correlation of the eye discs should exceed that of the facets.

The experiment consisted, therefore, in counting the facet number of
the left and right eyes of flies of each of the four mutant strains and of de-
termining the areas of the left and right eye discs of mature larvae of each
of the four. The facet counts were made by dissecting off the cornea of the
eye, mounting it on a slide in a drop of water and then projecting the image.
This method permits of great accuracy in the determination of the facet
number, hence the statistics determined from these data are also accurate.
The areas of the optic discs were determined by means of planimeter trac-
ings of camera lucida drawings of eye discs newly dissected from mature
larvae in a Ringer's solution (NaCl, 7.5 g.; KCl, 0.35 gms; $CaCl_2$, 0.2
g.). Because the discs are curved they must be flattened against the slide
in order that the camera lucida drawings be accurate. The process of
flattening, involving tapping with a needle, distorts the shape of the disc
and doubtlessly introduces variations in the area of the eye discs, which
may be either increases or decreases. In addition there are random errors
introduced in the course of the planimeter tracings. These errors tend to
increase the variability of the eye discs within a group, and precisely be-
cause they are random to decrease the left-right correlation calculated
from the measurements. Consequently, the values of the statistics cal-
culated from the eye disc measurements are shifted in such a way as
to reduce their sensitivity. A further source of error is the relatively poor
control of the ages of the larvae (discussed in a following paragraph).
Since the eye discs increase in size as the larvae age, any variation in ages

would increase the variability of the disc size from fly to fly and again re-duce the sensitivity of the statistics.

The imagos for the facet counts were raised on the standard corn meal-agar-molasses food medium, reënforced with dried brewers' yeast. The larvae used for eye disc measurements were obtained as follows: 20–30 females were permitted to lay eggs on "wafers" of food (approximately 2% Carragar plus 12.5% molasses in water), over a 24-hour-period. At the end of this period the wafers were transferred to refrigerator dishes which were half filled with the standard food medium. Larvae which had crawled up the sides of the dish preparatory to puparium formation were considered mature and were the source of the eye discs measured in the various experiments.

With one exception, all experiments were done at 25 ± 0.2°C. The exception involved the Lobe[4] flies raised for facet counts when, due to a heat wave, the temperature fluctuated between 24°C. (when ice was added to the incubator) and 28°C. It was usually closer to the latter than the former.

TABLE 1

MEAN NUMBER OF FACETS IN THE LEFT AND RIGHT EYES, AND THE MEAN AREAS OF THE CAMERA LUCIDA DRAWINGS OF THE EYE DISCS OF MATURE LARVAE OF EACH OF THE FOUR MUTANT TYPES STUDIED. THE AREAS OF THE CAMERA LUCIDA DRAWINGS ARE IN SQUARE INCHES × 100. THE LINEAR MAGNIFICATION = 114.06 ×

| | | FACETS | | | EYE DISCS | | |
| | | LEFT EYE | RIGHT EYE | | LEFT EYE | RIGHT EYE | |
GENO-TYPE	SEX	$\overline{X} \pm \sigma_{\overline{x}}$	$\overline{X} \pm \sigma_{\overline{x}}$	N	$\overline{X} \pm \sigma_{\overline{x}}$	$\overline{X} \pm \sigma_{\overline{x}}$	N
L^2	♂	106.0 ± 9.5	92.9 ± 9.1	35	48.9 ± 2.1	49.7 ± 2.0	25
	♀	134.4 ± 12.7	142.9 ± 10.5	31	51.5 ± 2.5	53.4 ± 2.2	24
L^4	♂	117.6 ± 10.7	104.0 ± 8.9	26	48.9 ± 1.7	49.0 ± 1.6	25
	♀	126.9 ± 9.3	147.2 ± 8.6	37	56.1 ± 1.9	60.5 ± 2.5	23
L^5	♂	135.8 ± 11.1	151.5 ± 9.8	26	50.2 ± 1.8	51.0 ± 2.6	25
	♀	153.8 ± 10.5	159.2 ± 11.3	22	52.6 ± 2.2	54.8 ± 2.0	25
ey^2	♂	279.8 ± 22.4	339.4 ± 22.6	19	79.0 ± 3.6	77.2 ± 3.3	25
	♀	289.1 ± 21.7	290.8 ± 22.2	16	76.6 ± 5.2	74.2 ± 4.2	25

Data.—The mean facet number of the imaginal eye and the mean areas of the camera lucida drawings of the eye discs will be found in table 1. They are presented for the purpose of orientation and as a summary of the raw data from which tables 2 and 3 have been derived.

Table 2, which presents the correlation coefficients of the facet numbers in the left and right imaginal eyes and of the larval eye disc sizes, shows that in every case *r* (correlation coefficient) is greater for the eye discs than it is for the facet numbers. Furthermore, of the eight comparisons only two, namely L^2 females and L^4 males, give a value of *P* which exceeds 0.05. It must be emphasized, however, that even in these two cases *r* is greater for the eye discs than for the imaginal eye. Furthermore, as pointed out in

the previous section, the estimated value for r for the imaginal discs is certainly lower than its true value and therefore the observed differences between the values of r for the facet numbers and the eye disc sizes are in every case lower than the true differences.

TABLE 2

CORRELATION VALUES FOR FACET NUMBERS OF THE LEFT AND RIGHT EYES AND FOR THE AREAS OF THE LEFT AND RIGHT EYE DISCS, AND THE VALUE OF D/σ_D FOR THE COMPARISONS BETWEEN THE STATISTICS OF THE FACET NUMBERS AND THE EYE DISC AREAS

GENOTYPE	SEX	FACETS r	N	SEX	EYE DISCS r	N	D/σ_D*
L^2	♂	0.03	35	♂	0.58	25	2.25
	♀	0.41	31	♀	0.67	24	1.27
L^4	♂	0.36	26	♂	0.65	25	1.33
	♀	0.16	37	♀	0.68	23	2.39
L^5	♂	0.35	26	♂	0.74	25	1.93
	♀	0.09	22	♀	0.66.	25	2.26
ey^2	♂	−0.25	19	♂	0.84	25	4.42
	♀	0.04	16	♀	0.86	25	3.75

* Since the question asked is, "is the coefficient of correlation for the eye discs *greater than* that for the facets?" only one tail of the normal curve is used, hence $P = 0.05$ at $D/\sigma_D = 1.64$ and $P = 0.01$ at $D/\sigma_D = 2.33$ (comparisons made by converting r to Z).

TABLE 3

COEFFICIENTS OF VARIATION FOR THE FACET COUNTS AND EYE DISC AREAS AND THE VALUES OF D/σ_D FOR THE COMPARISONS OF THE STATISTICS OF THE CORRESPONDING FACET NUMBERS AND EYE DISC AREAS

GENOTYPE	SEX		FACETS $CV \pm \sigma_{cv}$	EYE DISCS $CV \pm \sigma_{cv}$	D/σ_D*
L^2	♂♂	Left	52.6 ± 6.4	21.1 ± 3.2	4.4
		Right	57.9 ± 7.0	19.5 ± 3.0	5.1
	♀♀	Left	52.7 ± 6.8	23.7 ± 3.6	3.8
		Right	40.9 ± 5.3	20.6 ± 3.2	3.3
L^5	♂♂	Left	41.8 ± 5.9	18.1 ± 2.8	3.6
		Right	32.9 ± 4.7	25.9 ± 3.8	1.1
	♀♀	Left	31.9 ± 4.9	20.9 ± 3.1	1.9
		Right	33.4 ± 5.2	18.6 ± 2.8	2.5
L^4	♂♂	Left	46.4 ± 6.5	17.2 ± 2.6	4.1
		Right	43.5 ± 6.2	15.9 ± 2.5	4.2
	♀♀	Left	44.5 ± 5.3	16.6 ± 2.7	4.8
		Right	35.3 ± 4.3	20.2 ± 3.2	2.8
ey^2	♂♂	Left	34.9 ± 5.8	22.7 ± 3.4	1.8
		Right	27.8 ± 4.9	21.6 ± 3.2	1.05
	♀♀	Left	31.3 ± 5.6	34.2 ± 5.0	−0.37
		Right	30.6 ± 5.5	28.7 ± 4.2	0.28

* Since the question asked is, "is the coefficient of variation for the eye discs *less than* that for the facets?" only one tail of the normal curve is used, hence $P = 0.05$ at $D/\sigma_D = 1.64$ and $P = 0.01$ at $D/\sigma_D = 2.33$.

Table 3 contains the data pertinent to a comparison of the coefficients of variation (Cv) of the larval eye discs and of the facet numbers of the imaginal eyes. Of the twelve comparisons among the Lobe alleles (i.e., facet number of the left imaginal eyes against the area of left larval eye discs, and right eyes against right eye discs of the males and females separately, making four comparisons within each genotype) only the one concerned with the right eyes and discs of the Lobe[5] males results in a value of P greater than 0.05. This seems to be due to the relatively large coefficient of variation (Cv) shown by right eye discs of the Lobe[5] male larvae. The raw data of these eye disc measurements show one eye disc which measured 77 and another which measured 88; the next closest value to these is 61. If these two measurements are tested as possible samples of a population composed of the remaining 23 measurements it is found that neither of them fit, i.e., $P < 0.01$. Therefore, there is considerable justification for excluding these measurements from the sample. If these two values are excluded $\overline{X} = 48.3 \pm 1.64$ and $Cv = 12.2 \pm 1.8$. If this Cv is compared with that of the facet number D/σ_D is found to be 4.1. It seems valid therefore to conclude that the coefficient of variation of the eye discs of the Lobe[5] males is smaller than that for the facet number, and therefore that the Lobe[5] males conform to the same pattern as the other lobe flies involved in the experiment.

The coefficients of variation derived from the ey^2 data quite clearly do not show the same relationships that those for the various lobe alleles do (table 3). In only one case, the left eyes of the ey^2 males, is P less than 0.05. In the remaining cases it is considerably above 0.05. When the data for each of the four sets of eye discs are plotted in a scatter diagram, no tendency to cluster about a modal value is seen. The 25 values of each group are arranged in an almost unbroken sequence over the entire range with very little repetition of any given value. Thus in the data for the left eye discs of the males, five values occur twice, none more than twice; in the case of the right eye, one value occurs three times and all the others once each; in the data for the left eye disc of the females three values occur twice each, none more than twice; and, finally, only one value occurs twice in the data for the right eye discs of the females. Clearly the high values of the coefficients of variability shown by the eye discs of the ey^2 males and females are not due to one or two exceptional individuals as is the case with the right eye discs of the Lobe[5] male larvae but are a true expression of the variability of the sample. The increased variability of eyeless[2] eye discs relative to that of the eye discs of the Lobe alleles may be, in part or in whole, due to variation in the age of the larvae selected for dissection, for as pointed out above the age control was relatively poor. This problem will be returned to below.

Discussion.—The theory of development of the eye discs reviewed in the

introduction of this paper postulated in part that variation between the left and right eyes of a given individual might result from a variation in the fate of labilely determined cells in the larval eye discs. Such variation would not result in a change in the size of these discs, hence the left-right correlation should under these circumstances be greater for the larval eye discs than for the facet numbers of the imaginal eyes. The data of table 2 show quite clearly that the requirements of the hypothesis are fulfilled in each of the four mutant types studied. Hence in each of these mutant types the facet number of the imaginal eye may be varied during the larval stages by altering the path of development of labilely determined cells. These cells, in the Lobe alleles and eyeless[2], must be much more sensitive to minor variations in the environment than they are in Bar and wild type since in the former Margolis (1936) has shown a significant left-right correlation for the eyes of genetically Bar flies and Margolis and Robertson (1937) have shown that even marked changes in temperature during larval development fail to cause any great shift in the facet number of wild type.

These experiments taken in conjunction with those on Bar and modified Bar (Steinberg 1941a and 1941b) indicate that mutations may influence the labilely determined cells with regard to their sensitivity to environmental changes (ey^2, the Lobe alleles and Bar), and with regard to the path in development which they will take (modifier of Bar). Presumably there are mutants (perhaps among those already studied) which influence the number of labilely determined cells present in an eye disc, but no technique for detecting such effects is available at the moment.

The data in table 3, concerning the variability of the facet number of the imaginal eyes as compared with that of the corresponding eye discs, when considered in conjunction with the data of table 2, afford evidence in regard to the manner in which the range of variation of the facet number is determined in each of the specific genotypes. Variation in the facet number of the imaginal eye may arise as the result of variation in the number of cells entering into the formation of the eye disc (detectable as a variation in the size of the discs) or as a variation in the number of labilely determined cells which finally form facets (detectable by means of a higher left-right correlation for the size of the larval eye discs than for the facet number of the imaginal eye) or through some combination of the two (detectable by a combination of the above methods). The data of table 3 show that the coefficients of variation for the eye discs are in all cases significantly smaller than those for facet number. Nevertheless, the coefficients of variation for the eye discs are rather high, particularly when compared with the values derived for Bar and wild type eye discs in the author's earlier studies. However, it is entirely likely that a large part of the relatively increased variability is due to the much less accurate control of age employed in these

experiments. It will be recalled that in the present experiments age was determined simply by observing which larvae had crawled up the side of the dishes preparatory to puparium formation. In the earlier experiments, newly hatched larvae were collected over a 2-hour period and raised in 3-inch petri dishes. In the mature age group, measurements were made of the eye discs of only those larvae which were unpupated at the time when about one-half the total number of larvae in the dish had pupated. Therefore, it seems valid to conclude that in the Lobe alleles, as in Bar, the variation in facet number occurring from fly to fly is due to the variation in the number of labilely determined cells which go to form facets rather than head chitin.

The story seems to be quite different with eyeless[2] for here there is no difference between the coefficients of variation of the eye discs and of the facet numbers. Comparison of the data for the eye discs of eyeless[2] larvae with those for the eye discs of the Lobe alleles (table 3) shows that the values of the coefficients of variation of the latter are lower than those of the former. A similar comparison of the data for the facets shows that while the coefficients of variation for the facet numbers of Lobe[2] and Lobe[4] eyes are larger than those for eyeless[2], those for Lobe[5] are about the same. There is, then, a disproportionate increase in the variability of the areas of the eye discs of ey^2 larvae. There can be no doubt that the values of the coefficients of variation derived from the facet numbers of imaginal eyes are accurate since the facet counts are accurate. The same is certainly not true of the values derived from the eye disc data, for, as pointed out in the introduction, the eye discs vary in size with age and any inaccuracies in age determination would tend to increase the coefficients of variability and superimposed on this variation is the variation introduced by the technique of measurement. It is possible, therefore, that the absence of a difference between the coefficients of variation of the eye discs and of the facets is more apparent than real. Only further experiments can settle this question. If the data are correct, it would mean that the variation in facet number from fly to fly in eyeless[2] is dependent upon variation in the number of cells entering into the formation of the eye discs. Superimposed upon this would be the variation in the number of labilely determined cells which go to form facets. It is this variation which affects the left-right correlation coefficients. It is unlikely that variation in the number of cells entering into the formation of the eye discs would influence the value of r because from the nature of the development of the eye discs it is highly probable that equal numbers of cells would enter into the formation of each disc.

Summary.—Previous experiments had led to the hypothesis that the facet number of the Bar eye of *Drosophila melanogaster* was determined as follows: (a) a reduced number of cells enter into the formation of the eye

discs thus limiting the range over which the facet number of Bar eyes can vary and (b) a group of labilely determined cells which may form either head chitin or facets depending upon the nature of the extrinsic and intrinsic environment determine where, within the possible range of variation of facet number, the facet number of a given eye will fall. The experiments reported in this paper were designed to test the applicability of this hypothesis to the development of Lobe[2], Lobe[4], Lobe[5] and eyeless[2]. The data show that the development of these mutants may be adequately described by the theory.

Margolis, O. S., "Studies on the Bar Series of Drosophila III. The Facet Relations in Dorsal and Ventral Lobes of the Eye," *Genetics*, **21**, 817–831 (1936).

Margolis, O. S., and C. W. Robertson, "Studies on the Bar Series of Drosophila IV. The Temperature Effective Period for Facet Determination in the Wild Type," *Genetics*, **22**, 319–331 (1937).

Medvedev, N. N., "Genes and Development of Characters. I. The Study of the Growth of the Imaginal Discs of Eyes of the Wild Type Larvae and Three Mutants— Lobe[e], Glass[2] and Eyeless[2] in *Drosophila melanogaster*," *Zeit. ind. Abst. u. Vererb.*, **70**, 55–72 (1935).

Steinberg, A. G. A Reconsideration of the Mode of Development of the Bar Eye of *Drosophila melanogaster*. *Genetics*, **26**, 325–346 (1941a).

Steinberg, A. G., "Studies on Modifier of Bar. A Mutant Which Affects the Expression of the Bar Mutation of *Drosophila melanogaster*," *Genetics*, **26**, 440–451 (1941b).

Steinberg, A. G., "The Development of the Wild Type and Bar Eyes of *Drosophila melanogaster*," *Can. Jour. Research*, **D21**, 277–283 (1943).

Steinberg, A. G., and Abramowitz, M., "The Bar 'Locus' and the v^+ Reaction in *Drosophila melanogaster*," *Proc. Nat. Acad. Sci.*, **24**, 107–111 (1938).

MECHANISM OF ENZYMATIC ADAPTATION IN GENETICALLY CONTROLLED YEAST POPULATIONS

By S. Spiegelman, Carl C. Lindegren* and L. Hedgecock

Department of Zoölogy and The Henry Shaw School of Botany, Washington University

Introduction.—For some time now it has been recognized that the fermentation of galactose by yeast (see Lippmann[1]) differs greatly from that of other hexoses. Armstrong[2] found that some yeasts had and others had not the power of fermenting galactose. Slator,[3] in a quantitative investigation of the same subject, was able to confirm the statement previously made, that certain yeasts which have the power of fermenting galactose, possess it only after the yeast had been acclimatized by culture in the presence of the sugar.