GENETICS OF THE EYES-REDUCED MUTANT OF *DROSOPHILA MELANOGASTER*, WITH SPECIAL REFERENCE TO HOMOEOSIS AND EYELESSNESS[1]

JAMES W. EDWARDS[2] AND ELDON J. GARDNER

Utah State University, Logan

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MUTANT genes whose phenotypic manifestations result in structural abnormalities are of potential importance in understanding morphogenesis, regionalization, histogenesis, and pattern formation (WADDINGTON 1962). Likewise, traits of variable expression permit conclusions relative to the nature of genetic modifiers and total genetic variability of chromosomes in natural and laboratory populations.

Our interest in such phenomena was aroused during experiments in which copper sulfate was added to the food medium (TURNER and GARDNER 1960) of a melanotic tumor strain, *tu*^{150}, and the F_1 generation exhibited structural eye abnormalities. By inbreeding these flies, a new mutant stock was developed which EDWARDS and GARDNER (1962) named eyes-reduced. The locus of the major gene, *eyr*, was tentatively designated at 3–97$^{\pm}$. When eyes-reduced flies were crossed with a wild-type stock (Cockaponsett), 64.8% of the heterozygotes (*eyr*/+) exhibited abnormal nicks or abnormal growths in the eyes.

The investigation reported here was designed to characterize the homozygous and heterozygous expressions of *eyr*, to study the relationship between genetic modifiers and *eyr*/+ penetrance, to ascertain the nature of the abnormal growths, and to demonstrate any interaction of eyes-reduced and eyeless[4] (*ey*^{4}/*ey*^{4}) genes.

MATERIALS AND METHODS

Experiments designed to evaluate the expressivity of *eyr*/*eyr* were initiated by randomly selecting flies from eyes-reduced stock cultures. Initially ten cultures were prepared, with eight females and eight males per culture. The eyes of the progeny were scored according to the following criteria: no ommatidia (class 0), less than one third of a wild-type eye (class 1), one third to two thirds of an eye (class 2), and more than two thirds of an eye (class 3). Values were recorded separately for each eye and the symbol for the left eye of an individual was always written first (e.g., 01 was the symbol for a fly with no ommatidia on the left side and with less than one third of an eye on the right side of the head). Progeny from each culture were scored during ten consecutive days.

The eight series of crosses designed to study the influence of the genetic background on penetrance of the *eyr* heterozygote involved five stocks: a wild-type (Cockaponsett); eyeless[4] (*ey*^{4}/*ey*^{4}); eyes-reduced (*eyr*/*eyr*); Cubitus-interruptus-dominant, Bent-dominant (*ci*^{D}/*bt*^{D});

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[2] National Defense Education Act Fellow and Public Health Service Fellow (1-F1-GM-19, 468-01). Present address: Salem College, Winston-Salem, North Carolina.

and the H-41 stock (*sc^{S1} B In-S w^a sc^8; Ins SM1, al^2 Cy sp^2/dp b Pm ds^{33k}; C Sb/Ubx^{130} e^8; pol*). The following shorthand identified the origin of the chromosomes: chromosomes from the eye-reduced stock were designated by an E, thus, EEEE/YEEE represented an eyes-reduced male (Y for Y chromosome); chromosomes from the eyeless[4] stock were designated 4; chromosomes from the wild type were designated W; and the chromosomes carrying Curly, Stubble, and Cubitus-interruptus-dominant were designated C, S, and I, respectively. Thus, a fly designated as ECS4/YEEE was a male with one genome from an eyes-reduced parent, one second-chromosome bearing the Curly marker, one third-chromosome bearing the Stubble marker, and one fourth-chromosome from the eyeless[4] stock.

The details of the crosses giving rise to the eight series are as follows. Series 1: H-41 × *ci^D/bt^D*; F_1 BCSI/Y++++ males were crossed with eyes-reduced females; the resulting ECSI/YEEE males were crossed with EEEE/EEEE females. Series 2: H-41 × *ci^D/bt^D*; F_1 BCSI/Y++++ males were crossed with eyes-reduced females; the resulting ECSI/YEEE males were crossed with WWWW/WWWW females. Series 3: H-41 × *ci^D/bt^D*; F_1 BCSI/Y++++ males were crossed with EEEE/EEEE females; the resulting ECSI/YEEE males were crossed with 4444/4444 females. Series 4: H-41 × *ci^D/bt^D*; F_1 BCSI/Y++++ males were crossed with WWWW/WWWW females; WCSI/YWWW males were crossed with EEEE/EEEE females. Series 5: H-41 × *ci^D/bt^D*; F_1 BCSI/Y++++ males were crossed with 4444/4444 females. The resulting 4CSI/Y4444 males were crossed with EEEE/EEEE females. Series 6: WCEI/YWWW males obtained from the final cross in Series 2 were crossed with WWWW/WWWW females. Series 7: 4CEI/Y4444 males obtained from Series 3 were crossed with 4444/4444 females. In this series no attempt was made to classify the abnormal growths in flies homozygous for the eyeless[4] genes. In Series 6 and 7, one third-chromosome was not marked with a dominant gene, thus the actual penetrance values obtained should be doubled to correct for the data including flies not heterozygous for the *eyr* gene. Series 8: this series duplicated Series 2 except that in four of the final crosses the ECSI/YEEE males exhibited abnormal eye structure, whereas in the second four crosses, the ECSI/YEEE males were phenotypically wild type. Eight females and a minimum of four males per cross were used in the final 70 crosses, and the numbers of crosses were 10, 10, 10, 10, 10, 4, 8, and 8, respectively, for the eight series. Parents were removed from the bottles after five days, and progeny were counted over a period of seven days.

The Dichaete chromosome employed in the study on eyes-reduced and eyeless[4] homozygotes was obtained from the following stock: *Ins(2L + 2R)Cy, In(2LR)Pm, In(3L)D/ru h* DC × F *ca Sb In(3R)*.

Third instar *eyr/eyr; ey^4/ey^4* larvae were obtained by mass mating adults in freshly yeasted bottles for three-hour periods with subsequent transfer to new media. Larvae were harvested approximately 103 hours after the mid-point of the egg deposition period, and three larvae were placed in each 1 inch × 4 inch vial containing yeasted medium. Larval viability was scored by noting how long larval mobility was evident. Examinations were made every 12 hours and inviability was assumed to have occurred at the midpoint of the final time period.

Histological studies were made of the abnormal growths from adults in the Series 8 crosses. Tissues were prepared as follows: fixed in alcoholic Bouin's (80°C, 3 hr), imbedded in Tissuemat, sectioned at 10 microns, stained with Delafield's hematoxylin, and counterstained with eosin. The standard Drosophila culture medium composed of cornmeal, molasses, agar, and live yeast was used in all experiments reported herein, and the crosses were maintained at 25 ± 0.5°C.

EXPERIMENTAL RESULTS

Revised location of the eyr *gene:* When flies homozygous for both claret and eyes-reduced genes (*ca eyr/ca eyr*) were mated with wild-type flies (Cockaponsett), and the F_1 females were backcrossed with *ca eyr/ca eyr* males, a frequency of 0.021 crossovers was classified from a total of 5514 progeny. This was interpreted to indicate that *eyr* is approximately 2.1 map units from claret (3–100.7). When homozygous brevis, eyes-reduced flies were mated with wild-type flies,

TABLE 1

Expressivity in the eyes-reduced (eyr/eyr) *and eyeless*^{4} (ey^4/ey^4) *inbred stocks*

Class	*eyr/eyr*		*ey^4/ey^4*	
	% Males	% Females	% Males	% Females
00	21.9	33.4	3.6	15.4
01	9.9	8.4	1.7	6.2
02	6.8	7.7	3.1	5.5
03	0.8	0.5	4.8	5.5
10	9.8	9.1	2.1	4.7
20	10.0	8.6	3.7	4.6
30	0.4	0.1	4.2	7.0
11	10.4	10.4	2.8	1.8
12	9.7	6.9	2.4	3.0
13	0.5	0.7	1.6	1.3
21	8.2	7.2	4.1	1.5
22	9.2	6.1	5.6	4.2
23	0.8	0.4	10.9	5.3
31	0.7	0.5	2.5	1.5
32	0.9	0.1	8.0	5.2
33	0.1	0.0	38.8	27.5
Total (*n*)	1348	1527	945	987

and the F_1 females were backcrossed to *eyr bv/eyr bv* males, 4852 progeny resulted, and *eyr* was calculated to be 0.4 map units from *bv* (3–103.5). The close agreement between the positions estimated by these independent experiments (102.8 and 103.1) indicates that the *eyr* locus is at 3–103± crossover units rather than the previously estimated 3–97± (EDWARDS and GARDNER 1962).

Homozygote expressivity: Degrees of expression (expressivity) characteristic of *eyr/eyr* flies based on ten cultures are given in Table 1. Variations ranged from flies with no ommatidia on either side of the head (class 00) to one fly with both eyes more than two thirds of wild-type eye size (class 33).

For comparison with the eyes-reduced results, a comparable classification was made among flies homozygous for the fourth-chromosome gene *ey*^{4}. The results of this experiment are also summarized in Table 1. Sixty-six percent of the 1932 flies from eight crosses were classified as 33 reflecting an absence of rigid selection for reduced eye size during the recent history of this particular eyeless^4 stock.

One apparent difference between the eyes-reduced and eyeless^4 stocks merits consideration. Previous research (see DISCUSSION) has established that differences in eye size exist between males and females homozygous for the eyeless genes. In contrast, eyes-reduced males and females have similar phenotypes, with perhaps the exception of the 00 class. The 00 class exception must be regarded as tentative until a larger sample and a detailed consideration of the influence of an aging culture medium on eclosion phenotypes can be evaluated. The data now available (EDWARDS 1964) suggest an increase in the frequency of class 00 flies in aging cultures, being of relatively higher magnitude in the females, which is in direct contrast to the situation for eyeless flies (MORGAN 1929).

Inbreeding of flies with no eyes (class 00), flies with less than one third of an eye on one or both sides of the head (classes 01, 10, and 11), and flies with one third of an eye or greater (classes 22, 23, 32, and 33) demonstrated remarkable similarities in the results despite the differences in parental phenotypes (EDWARDS 1964). These results were not unexpected, since they paralleled the results obtained by crossing flies of different expressivities in the eyeless stocks of *D. melanogaster*.

Inviable pupae were observed in several of these experiments. Nine bottles from the claret, eyes-reduced backcross experiment were used in testing the hypothesis that this inviability was associated with the *eyr/eyr* genotype. Two general classes of inviability were distinguished among 289 inviable pupae, which represented 23% of the total organisms. The first class included 212 apparently normally differentiated organisms with very small heads. The 77 pupae of the second type did not show the characteristic darkening of the wings and the general pigmentation characteristic of mature pupae. They did, however, have a melanized area of either unilateral or bilateral expression in the head region.

The phenotypes of flies homozygous for the eyes-reduced genes were profoundly influenced by the Dichaete chromosome (Table 2). The frequency of appearance of eye structures classed 0 and 1 in the controls (i.e., non-Dichaete eyes-reduced homozygotes) was 0.87 for flies also carrying the Curly inversion and 0.82 for non-Curly, claret, eyes-reduced flies. When Dichaete was present, the frequency of organisms with classes 0 and 1 dropped to 0.05. Similar results were seen in flies homozygous for the eyeless[4] genes (Table 3). The Dichaete

TABLE 2

Results of crosses among Cy/+;D ca eyr/+ ca eyr *flies**

Progeny phenotypes	*Cy D ca eyr* / *+ + ca eyr*		*+ D ca eyr* / *+ + ca eyr*		*Cy + ca eyr* / *+ + ca eyr*		*+ + ca eyr* / *+ + ca eyr*	
	Males	Females	Males	Females	Males	Females	Males	Females
00	.	.	.	.	8	11	.	3
01	.	.	1	.	6	2	1	.
02	1	2	.	.	6	6	1	4
03	.	.	.	2	.	.	.	.
10	.	.	.	.	9	8	1	4
20	1	.	.	1	4	4	3	6
30	1	.	.	.	.	1	.	.
11	.	.	.	.	13	1	6	6
12	2	.	2	1	8	5	7	5
13	.	.	.	.	.	.	.	.
21	3	1	1	.	7	6	5	4
22	42	41	51	39	6	10	5	7
23	13	19	7	10	.	.	.	.
31	.	.	.	.	.	.	.	.
32	18	18	6	8	.	.	.	.
33	9	45	4	7	.	.	.	.

* Number of crosses = 2.

TABLE 3

Results of crosses between D/Sb;ey^4/ey^4 *and* +/+;ey^4/ey^4 *flies**

Progeny phenotypes	*D/+;ey^4/ey^4*		*+/Sb;ey^4/ey^4*	
	Males	Females	Males	Females
00	.	.	8	45
01	.	.	3	12
02	.	.	13	17
03	.	.	13	32
10	.	.	1	8
20	.	.	14	10
30	.	1	15	29
11	1	.	6	9
12	1	.	3	10
13	.	1	11	7
21	1	.	9	8
22	3	5	25	21
23	11	8	39	45
31	.	.	9	15
32	14	12	40	39
33	242	238	263	191

* Number of crosses = 6.

chromosome thus effectively suppresses either *eyr/eyr* or *ey^4/ey^4* homozygotes with phenotypically no eyes or extremely reduced eye sizes.

Genetic modification of homoeotic expression of eyr *heterozygotes:* Figures 1 A, B, and C illustrate three expressivities of the eyes-reduced gene when heterozygous. The complete range from a wild-type eye to large growths of bilateral expression has been observed. Figures 1 D, E, and F illustrate normal wing tissue and the tissue of one abnormal growth, which is conclusively wing tissue, thus establishing the homoeotic nature of the *eyr/+* expression. The phenotype of one member of an effectively balanced lethal stock (*eyr/Sb;ey^4/ey^4*) is shown in Figures 1 G, H, and I, wherein the large bizarre growths of wing tissue are readily apparent.

The detailed results of the eight series of crosses used to investigate the influence of chromosomes of different origin on *eyr/+* penetrance (i.e., abnormal growths) are recorded in Table 4. Penetrance of the *eyr* gene is given as percent abnormal. This type of experimental design allows identification of all chromosomes. Comparisons of two genotypes differing only in one pair of chromosomes then allow conclusions to be drawn relative to the effect of specific chromosome substitutions on the penetrance of abnormal growths.

Comparisons among fourth-chromosome substitutions are tabulated in Table 5. The comparisons between IE and EE show that in all four cases the differences are not significant ($P>0.05$) but the comparisons between WE and IE show that two are highly significant ($P<0.01$), two significant, and four not significant. In the case of these differences, the second chromosome carried the Curly inversion; the resultant interaction between these nonhomologous chromosomes complicated

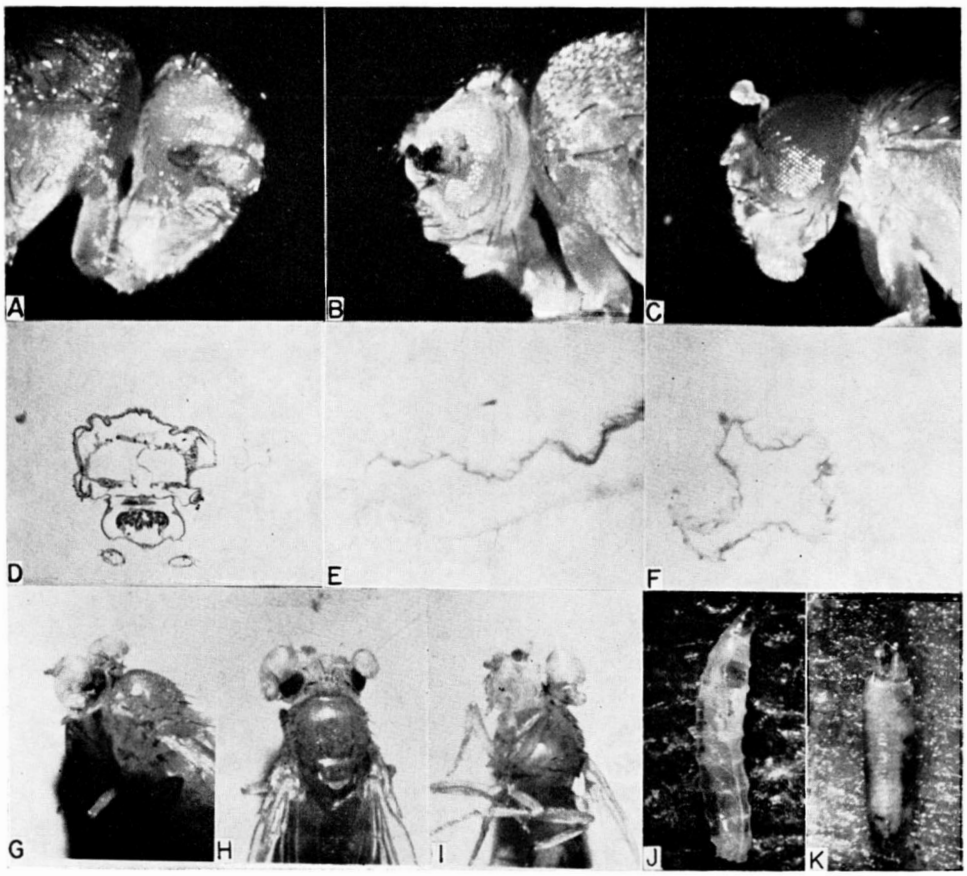

FIGURE 1.—A, B, C. Three expressions of the heterozygous eyes-reduced gene (melanin deposition is exhibited in the distal portion of the abnormal growth shown in B); D. Transverse section through the anterior portion of the head of a fly exhibiting an abnormal growth; E, F. A comparison of normal wing tissue (E) and the tissue of the abnormal growth (F); G, H, I. Three different views of one of the extreme manifestations of wing tissue in the eye region; J. An *eyr/eyr; ey^4/ey^4* "fourth" instar; K. An *eyr/eyr;ey^4/ey^4* abnormal "puparium."

the data. Comparisons between IW and EW resulted in highly significant differences in each case wherein the second chromosome carried the Curly inversion, and in two of four cases wherein non-Curly second chromosomes were present. Comparisons between E4 and I4 yielded four values which were highly significant. In each of these cases, the presence of a fourth chromosome from the eyes-reduced stock, as compared with one bearing the Cubitus-interruptus-dominant gene, resulted in a slightly higher percent of abnormal growths. The most dramatic contrast in fourth chromosome comparisons, however, is shown in the last subdivision of Table 5. In these 4E and IE comparisons, chi-square values ranged from 81.9 to 246.8. This definitively shows that in reference to the production of abnormal growths, the *ey^4*-bearing fourth chromosome is physiologically important in the heterozygous condition.

TABLE 4

Results of the eight series of crosses in the genetic analysis of penetrance

Series	Genotype	N	Percent abnormal	Series	Genotype	N	Percent abnormal
1	YCSI/EEEE	80	71.3	5	YC4I/EEEE	221	58.8
	ECSI/EEEE	127	50.4		4C4I/EEEE	239	17.6
	YCSE/EEEE	233	67.0		Y4SI/EEEE	270	20.7
	ECSE/EEEE	256	55.9		44SI/EEEE	334	2.7
	YESI/EEEE	206	20.4		YC44/EEEE	291	94.9
	EESI/EEEE	232	20.7		4C44/EEEE	249	86.8
	YESE/EEEE	236	24.2		Y4S4/EEEE	291	81.4
	EESE/EEEE	223	24.2		44S4/EEEE	309	28.2
2	YCEI/WWWW	177	89.3		Y44I/EEEE	308	31.5
	ECEI/WWWW	162	84.6		444I/EEEE	311	3.2
	YCEE/WWWW	289	74.1		Y444/EEEE	297	82.8
	ECEE/WWWW	307	59.3		4444/EEEE	281	46.3
	YEEI/WWWW	352	60.8	6	YC?I/WWWW*	128	52.3
	EEEI/WWWW	378	58.2		WC?I/WWWW	147	50.3
	YEEE/WWWW	344	49.7		YC?W/WWWW	210	43.3
	EEEE/WWWW	342	48.5		WC?W/WWWW	232	38.4
3	YCEI/4444	274	26.3		YC?I/WWWW	127	52.8
	ECEI/4444	251	60.2		WW?I/WWWW	149	51.0
	YCEE/4444	283	47.0		YW?W/WWWW	244	48.0
	ECEE/4444	274	72.3		WW?W/WWWW	256	43.0
	YEEI/4444	329	8.0	7(A)†	YC?I/4444	127	24.4
	EEEI/4444	320	24.0		4C?I/4444	151	6.6
	YEEE/4444	292	14.0		Y4?I/4444	162	13.0
	EEEE/4444	358	39.1		44?I/4444	142	4.9
4	YCSI/EEEE	218	64.2	(B)‡	YC?I/4444	152	21.0
	WCSI/EEEE	172	45.4		4C?I/4444	149	6.0
	YCSW/EEEE	289	79.9		Y4?I/4444	182	18.7
	WCSW/EEEE	297	62.6		44?I/4444	148	5.4
	YCWI/EEEE	230	80.0	8(A)†	YCEI/WWWW	64	98.4
	WCWI/EEEE	245	80.4		ECEI/WWWW	59	96.6
	YWSI/EEEE	290	31.7		YCEE/WWWW	116	81.9
	WWSI/EEEE	313	35.8		ECEE/WWWW	128	64.8
	YCWW/EEEE	343	87.8		YEEI/WWWW	153	60.8
	WCWW/EEEE	285	71.9		EEEI/WWWW	179	65.4
	YWSW/EEEE	291	36.4		YEEE/WWWW	136	64.0
	WWSW/EEEE	275	36.4		EEEE/WWWW	157	65.6
	YWWI/EEEE	327	58.1	(B)‡	YCEI/WWWW	98	95.9
	WWWI/EEEE	330	58.5		ECEI/WWWW	65	90.8
	YWWW/EEEE	338	57.1		YCEE/WWWW	114	74.6
	WWWW/EEEE	358	57.8		ECEE/WWWW	143	62.2
5	YCSI/EEEE	165	61.8		YEEI/WWWW	154	54.6
	4CSI/EEEE	196	16.3		EEEI/WWWW	175	62.9
	YCS4/EEEE	276	97.1		YEEE/WWWW	131	42.8
	4CS4/EEEE	278	88.5		EEEE/WWWW	159	57.2

* The probability that one third-chromosome was *E* was assumed to be 0.5.
† Parental males exhibited abnormal eye structure.
‡ Parental males exhibited normal eye structure.

TABLE 5

The influence of different fourth chromosomes on eyr/+ *penetrance*

Comparison	Genotype	Percent abnormal
I *vs.* E	YCS_/EEEE	7'.3 *vs.* 67.0
	ECS_/EEEE	50.4 *vs.* 55.9
	YES_/EEEE	20.4 *vs.* 24.2
	EES_/EEEE	20.7 *vs.* 24.2
W *vs.* I	YCS_/EEEE	79.9 *vs.* 64.2**
	WCS_/EEEE	62.6 *vs.* 45.4**
	YCW_/EEEE	87.8 *vs.* 80.0*
	WCW_/EEEE	71.9 *vs.* 80.4*
	YWS_/EEEE	36.4 *vs.* 31.7
	WWS_/EEEE	36.4 *vs.* 35.8
	YWW_/EEEE	57.1 *vs.* 58.1
	WWW_/EEEE	57.8 *vs.* 58.5
I *vs.* E	YCE_/WWWW	89.3 *vs.* 74.1**
	ECE_/WWWW	84.6 *vs.* 59.3**
	YCE_/WWWW	98.4 *vs.* 81.9**
	ECE_/WWWW	96.6 *vs.* 64.8**
	YCE_/WWWW	95.9 *vs.* 74.6**
	ECE_/WWWW	90.8 *vs.* 62.2**
	YEE_/WWWW	60.8 *vs.* 49.7**
	EEE_/WWWW	58.2 *vs.* 48.5**
	YEE_/WWWW	60.8 *vs.* 64.0
	YEE_/WWWW	54.6 *vs.* 42.8*
	EEE_/WWWW	65.4 *vs.* 65.6
	EEE_/WWWW	62.9 *vs.* 57.2
E *vs.* I	YCE_/4444	47.0 *vs.* 26.3**
	ECE_/4444	72.3 *vs.* 60.2**
	YEE_/4444	14.0 *vs.* 8.0**
	EEE_/4444	39.1 *vs.* 24.0**
4 *vs.* I	YCS_/EEEE	97.1 *vs.* 61.8**
	4CS_/EEEE	88.5 *vs.* 16.3**
	YC4_/EEEE	94.9 *vs.* 58.8**
	4C4_/EEEE	86.8 *vs.* 17.6**
	Y4S_/EEEE	81.4 *vs.* 20.7**
	44S_/EEEE	28.2 *vs.* 2.7**
	Y44_/EEEE	82.8 *vs.* 31.5**
	444_/EEEE	46.3 *vs.* 3.2**

* $P<0.05$. ** $P<0.01$.

The results obtained in the investigation of the genetic modifiers present in X chromosomes of different origin are perhaps the most important findings of all. Three comparisons among EY and WY chromosomes were tabulated in Table 6. The 57.1% represents the same observed result. Each of the three comparative values cited for WEEE/YWWW flies, however, was obtained in a slightly different way. Consequently the orders of magnitude for the differences among these three results are not unexpected. The 49.7% datum comes from Series 2 observations, wherein no regard was paid to the parental phenotype.

TABLE 6

The influence of different X chromosomes on eyr/+ *penetrance*

Comparison	Genotype	Percent abnormal
E *vs.* W	_EEE/YWWW	57.1 *vs.* 49.7
	_EEE/YWWW	57.1 *vs.* 64.0
	_EEE/YWWW	57.1 *vs.* 42.8**
W *vs.* E	_CSI/EEEE	45.4 *vs.* 50.4
E *vs.* 4	_444/YEEE	82.8 *vs.* 14.0**
E *vs.* 4	_CSI/EEEE	50.4 *vs.* 16.3**
Y *vs.* 4	_CSI/EEEE	61.8 *vs.* 16.3**
	_CS4/EEEE	97.1 *vs.* 88.5**
	_C41/EEEE	58.8 *vs.* 17.6**
	_4SI/EEEE	20.7 *vs.* 2.7**
	_C44/EEEE	94.9 *vs.* 86.8**
	_4S4/EEEE	81.4 *vs.* 28.2**
	_44I/EEEE	31.5 *vs.* 3.2**
	_444/EEEE	82.8 *vs.* 46.3**
E *vs.* Y	_CEI/4444	60.2 *vs.* 26.3**
	_CEE/4444	72.3 *vs.* 47.0**
	_EEI/4444	24.0 *vs.* 8.0**
	_EEE/4444	39.1 *vs.* 14.0**
Y *vs.* 4	_CEI/4444	48.8 *vs.* 13.2**
	_CEI/4444	42.0 *vs.* 12.0**
	_4EI/4444	26.0 *vs.* 9.8*
	_4EI/4444	37.4 *vs.* 10.8**
Y *vs.* E	_CSI/EEEE	71.3 *vs.* 50.4**
	_CSE/EEEE	67.0 *vs.* 55.9*
	_ESI/EEEE	20.4 *vs.* 20.7
	_ESE/EEEE	24.2 *vs.* 24.2

* $P<0.05$. ** $P<0.01$.

The penetrance values of 64.0 and 42.8 were obtained, respectively, from phenotypically abnormal and wild-type parents in Series 8. Thus, the most valid conclusion is that the EY *vs.* WY comparison results in similar penetrance values. Likewise, the WE *vs.* EE comparison yielded nonsignificant differences.

The sex-linked modifiers from the eyeless[4] stock acted differently. EY *vs.* 4Y yielded a chi-square value of 276.0, with penetrance values of 82.8 and 14.0, respectively. Also, the EE *vs.* 4E comparison resulted in a highly significant reduction in penetrance in the 4E class. Subsequent comparisons of penetrance values in males and females with X chromosomes of contrasting origin showed highly significant differences in fifteen of the sixteen comparisons. Apparently the presence of one X chromosome from the eyeless[4] stock reduced penetrance, while the presence of two X chromosomes from the eyeless[4] stock reduced penetrance even below the 4Y level. In only one of four EY *vs.* EE comparisons is the difference significant at the 0.01 level (Table 6). This suggests that males and females hemizygous and homozygous for sex-linked modifiers from the eyes-reduced stock have similar penetrance values.

Similar comparisons were made for the third chromosome substitutions. The third chromosome bearing the Stubble inversion and the third chromosome from the eyeless[4] stock had similar modifying properties, while the wild-type third chromosomes contained modifiers which increased penetrance values (i.e., minus modifiers).

Eyelessness in the double homozygote: Crosses between *eyr/eyr;ci^{D}/ey^{4}* males and females yielded 731 progeny. Of these, 43 were non-Cubitus-interruptus-dominant, and, thus, double homozygotes. Pupal inviability was observed in these crosses but was not studied.

Preliminary results of six crosses among *eyr/eyr;ey^{4}/ey^{4}* flies which were all class 00 yielded 311 progeny, of which 297 or 95.5% were without ommatidia (00). The results of a more detailed analysis of the double homozygotes from class 00 parents yielded 1710 progeny, of which 95.5% exhibited eyelessness. Considerable pupal inviability was also observed. The ratio of female to male adults was approximately 2:1, which may presumably be related to sex-linked genetic modifiers. To initiate a study on selection for increase eye size in the double homozygotes (i.e., a select up strain), flies possessing ommatidia were crossed. Of the 109 F_1 progeny, 99 or 90.8% exhibited eyelessness.

Pupal inviability was analyzed in cultures of double homozygotes selected at random from the above studies. The inviable pupae were similar to those observed in the *eyr/eyr* flies, and 761 "light" and 3162 "dark" pupae were observed. In contrast to the eyes-reduced results, however, 16 abnormal larvae and 68 abnormal "puparia" were also observed in the cultures of *eyr/eyr;ey^{4}/ey^{4}* double homozygotes. The abnormal larvae (Figure 1J) were larger than third instar larvae, moved sluggishly, and eventually turned black on the sides of the bottles without forming puparia. Another class formed structurally abnormal "puparia" (Figure 1K). One diagnostic feature was an absence of rigidity which eventually led to contact between the dorsal and ventral surfaces and a more or less planar "puparium." The anterior hooks of the organisms within such "puparia" were never shed. To verify these results, 1156 third instar *eyr/eyr;ey^{4}/ey^{4}* larvae were collected and placed in vials. Of the 18 (7 larvae and 11 puparia) exhibiting prepupal inviability, the average retention of mobility was 238 hours with a range from 156 to 339 hours after the time of egg deposition.

In normal pupation, the cuticle of the third instar forms the puparium *per se*, and a prepupal molt occurs within the puparium. Although the data are incomplete, it does not seem unreasonable to suppose that these abnormal larvae and abnormal puparia represent the so-called prepupal instar, the latter being represented as a fourth instar as the result of stretching the cuticle of the third instar. The frequency of the fourth instars was 84/4007 inviable *eyr/eyr;ey^{4}/ey^{4}* genotypes. From these cultures 1297 adults eclosed, giving a frequency of 84/5304 or 1.58%.

DISCUSSION

The term homoeosis was coined by BATESON in 1894 to describe situations wherein one member of a homologous series assumed characteristics normally

associated with another member of that series. The finding that some of the so-called "abnormal growths" characteristic of *eyr*/+ heterozygotes were definitively wing tissue established that we were dealing with the phenomenon of homoeosis, although unanimity has not been reached on such a conclusion (see HERSKOWITZ 1949; ROBERTS 1964). LEDERMAN-KLEIN (1962) presented the most recent general discussion of this phenomenon as it is associated with eyelessness. She studied the morphology and genetics of a homoeotic mutant, eyeless ophthalmoptera (*ey-opht*). Certain parallels can be established between this mutant and the eyes-reduced heterozygotes. One prerequisite for the appearance of wing tissue in *ey-opht* was homozygosity of the fourth-chromosome eyeless genes, ey^2. In the case of *eyr* heterozygotes, the abnormal growths appeared in the absence of a fourth chromosome carrying an eyeless allele, although the presence of the ey^4 chromosome in the heterozygous state enhanced the expression of abnormal growths. Penetrance of *ey-opht* in selected lines had a mean value of approximately 85%, which paralleled that of the *Sb/eyr;*ey^4/ey^4 strain in our investigation (EDWARDS 1964).

The modifier system associated with eyelessness is complex, and much more research will be required to locate the various modifiers within the chromosomes and to define their effects. Some generalizations can be made on the basis of present evidence. When the X chromosomes from the eyeless^4 stock were associated with the *eyr* heterozygote, the penetrance of abnormal growths showed a greater reduction in females than in males. Previous research has revealed significant sex differences in phenotypes of eyeless homozygotes (MORGAN 1929; SPOFFORD 1956; SANG and BURNET 1963), however, no explanation for these differences was given. These observations bring to mind the concept of dosage compensation (STERN 1960) with the exception that only the modifiers are sex-linked, the major genes being located in the fourth chromosome. The sex-linked eyeless modifiers, therefore, did not exhibit dosage compensation. When the X chromosomes from the eyes-reduced stock were associated with the *eyr* heterozygote, in only one of four cases was $P < 0.01$ in comparisons of penetrance values in males and females. This suggest that sex-linked modifiers from the eyes-reduced stock exhibited dosage compensation. Concomitantly, there should have been no phenotypic differences in eyes-reduced homozygotes relative to sex, which was substantiated by the data with the possible exception of *eyr* homozygotes without ommatidia (class 00), wherein a differential influence of an aging food medium was noted. With regard to the difference between *eyr* and ey^4, it is interesting to recall the conclusions of KRIVSHENKO (1959) and FUNG and GOWEN (1960) indicating that the fourth chromosome of *D. melanogaster*, which contains ey^4, may have originally been derived from the X chromosome.

These data on sex-linked modifiers have not taken into account the level of selection of the homozygotes, i.e., relative abundance of plus and minus sex-linked modifiers, and final analysis must await such a distinction.

One criticism of this experimental design for detecting modifiers is perhaps important. No attempt at isogenicity was made within the initial stocks employed in this investigation. Such a design seemed most appropriate in this introductory

study since some estimate of genetic variability within these stocks should precede an elimination of that variability. And, presumably, in those cases wherein statistically significant differences were observed in only a few of the total comparisons, this source of variability was a major factor.

The genomes of eyes-reduced and eyeless^4 stocks may be compared with respect to their influence on *eyr*/+ penetrance in three ways. (1) Sex-linked modifiers differed significantly, notably by the absence of compensation in the X chromosomes from the eyeless^4 stock. (2) The third chromosome bearing the Stubble inversion and the third chromosome from the eyeless^4 stock had similar modifying properties. The wild-type third chromosome modified the penetrance of the trait in a minus direction as compared with the influence of the Stubble and eyeless^4 third chromosomes. (3) The *ey*^{4} bearing fourth chromosome significantly increased penetrance.

This study represents only a beginning toward comparing the genetic backgrounds of these two stocks. Eventually it may be possible to determine whether the same loci on a given chromosome modify *eyr* in one case and *ey*^{4} in another case in a similar manner. The recent analyses of the genetic modifiers associated with the crossveinless phenotypes (see MILKMAN 1965; MOHLER 1965) have substantially contributed to the knowledge of such modifiers or "major gene modifier systems" (MOHLER 1965). The results reported here relative to sex-linked modifiers suggest that studies involving three homozygotes (*eyr/eyr*, *ey*^{4}/*ey*^{4}, and *eyr/eyr;ey*^{4}/*ey*^{4}) would yield definitive results.

The problem of adult asymmetric eye size in eyeless flies has been investigated by GUTHRIE (1925), MEDVEDEV (1935), STEINBERG (1944), and DEMARINIS (1959), but no satisfactory explanation has resulted (see VAN VALEN 1962). The appearance of mobile prepupal instars may facilitate an explanation. It does not seem unreasonable to suppose that hormonal imbalance characterized these larvae. Certainly the relationships between hormones and gene activation deserve the current level of attention (CLEVER 1963; SCHNEIDERMAN and GILBERT 1964; WIGGLESWORTH 1964; BURDETTE and ANDERSON 1965). Whether the explanation for the double homozygotes can be applied to the homozygotes is unknown, but the observation that continued selection for reduced eye size leads to inviability in *ey*^{2}/*ey*^{2} organisms (BARON 1935) and the observation that from the genetic standpoint markedly asymmetric flies are most similar to symmetric *ey*^{4}/*ey*^{4} flies of medium eye size (SPOFFORD 1956), reinforces the opinion that the hormonal environment of the developing eye disc merits special consideration. Such an interpretation suggests that optic asymmetry results from unequal stimulation or inhibition by hormones present in the internal environment of the developing eye discs.

The chromosome bearing Dichaete greatly decreased the appearance of eyeless and eyes-reduced homozygotes with reduced eye structure, or, conversely, this chromosome enhanced the appearance of organisms with increased numbers of ommatidia. It is therefore possible that the influence of this chromosome is by means of a modification of the internal hormonal environment of the developing eye discs.

SUMMARY

The locus of *eyr* was established at 3–103±. This is the major gene which when homozygous results in asymmetrically reduced eye size. The chromosome bearing the Dichaete inversion greatly decreased the occurrence of flies with no ommatidia or less than one-third the wild-type number in both eyes-reduced (*eyr/eyr*) and eyeless[4] (*ey*^{4}/*ey*^{4}) flies. Abnormal eye growths characteristic of *eyr*/+ heterozygotes were composed of wing tissue; thus, *eyr* when heterozygous can be regarded as a homoeotic mutant. A detailed study of genetic modifiers of homoeosis resulted in penetrance modifications of from 2.7 to 100%. Sex-linked modifiers from the eyeless[4] stock did not show dosage compensation, and the *ey*^{4} gene when heterozygous significantly increased *eyr*/+ penetrance. The *eyr/eyr; ey*^{4}/*ey*^{4} double homozygotes were characterized by an absence of ommatidia in 95.5 percent of eclosed adults. The frequency of inviable double homozygotic pupae was 75.5%, of which, 1.58% was associated with the occurrence of mobile prepupal larvae which were actually "fourth" instars.

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