THE RELATION BETWEEN EXPRESSIVITY AND SELECTION AGAINST EYELESS IN DROSOPHILA MELANOGASTER[1]

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MANY mutant stocks of Drosophila mass cultured in the laboratory for generations become phenotypically almost indistinguishable from wild type. Upon outcrossing, the mutant reappears in the F_2 with its pristine strength of expression. Presumably, genes reducing the degree of expression, or expressivity, of the major character of the mutant stock had appeared and accumulated through natural selection during its maintenance in the laboratory. These modifying genes are probably disadvantageous to their possessors in a culture of wild type flies. MARSHALL and MULLER (1917) found more extreme phenotypes in homozygous recessive mutants obtained from stocks maintained in a heterozygous condition for many generations, than in parallel stocks maintained as homozygotes.

Does lower expressivity reduce the rate of selection against a deleterious gene when compared with its wild type allele? If so, is the relation demonstrable within a phenotypically variable isogenic stock or must genetic modifiers exist? The rate of selection is determined by the viability and productivity of flies of the relevant genotypes. The first of these parameters can be measured in crosses giving equal numbers of mutant and wild type zygotes whereas the latter parameter can be estimated from experiments in which mutant and wild type males compete in mating, or from experiments in which mutant and wild type females compete in oviposition. The effect of the degree of expression of the mutant on viability and productivity—and thus on selection—can be studied in crosses involving mutant parents with different grades of expression.

The next question is whether any correlation observed between expressivity and selection is to be attributed to the accumulation of modifiers. If the degree of expression is influenced by modifiers, there should be a positive correlation between the expression of the mutant character in parent and offspring. Furthermore, if the character is bilateral, the grades on the two sides are correlated to a greater extent within the progeny of a group of parents heterozygous for modifiers than of homozygous parents. The reason for this is that with heterozygous parents both local environment and genetic segregation affect the individual offspring whereas with homozygous parents only the local environment determines differences in expression.

The results to be reported demonstrate that modifiers soon disrupted the initial isogenicity of the mutant stock, enhancing its viability and productivity and decreasing its expressivity, so long as the mutant stock was maintained separately from the wild type stock. Once mutant and wild type parents were taken from segregating cultures, the accumulation of modifiers was greatly retarded. Neverthe-

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less, it is clearly demonstrable that mutant males and females of extreme expressivity are less productive than mutants of more nearly wild type appearance. No effect of expressivity on viability was found.

MATERIALS AND METHODS

The eyeless-4 (*ey*^{4}) allele was used because of its variable expression. For brevity, it will be referred to simply as *ey* hereafter. The selective disadvantage of *ey/ey* flies with various degrees of reduction in eye size were compared against a "wild type" standard. Two stocks were derived, differing only in their fourth chromosomes. For the + stock, a *Cy/Pm*; *Mo Sb/H* ♂ was crossed first to a *ClB/dl-49* ♀ and then to one of his Bar Curly Stubble daughters. From the progeny of the latter cross a Curly Stubble male and a Bar Curly Stubble female were selected for mating. Wild type progeny should be isogenic for chromosome X (from the *Cy/Pm*; *Mo Sb/H*♂) and chromosomes 2 and 3 (from the *ClB/dl-49* ♀). Of the several fertile strains of wild type appearance so obtained, one was used in this study.

The *ey* chromosome was introduced from flies received in December 1952 from the California Institute of Technology. An *ey/ey* ♂ was mated to a *ClB/dl-49*; *Cy/Pm*; *Mo Sb/H* ♀. An F_1 *dl-49*; *Pm/+*; *H/+*; *+/ey* ♂ was mated to an F_1 *ClB/+*; *Cy/+*; *Mo Sb/+*; *+/ey* ♀. An F_3 *dl-49*; *Cy/Pm*; *Mo Sb/H*; *ey/ey* ♂ was then selected for mating with a female from the isogenic wild type stock, from which eventually an *ey* stock was extracted whose chromosomes X, 2 and 3 were as isogenic with the + stock as can be insured by the use of stocks bearing these marked inversions. To secure reasonable isogenicity in chromosome 4, a +/+ ♂ was mated successively to an *ey/ey* ♀ and to one of his *+/ey* daughters. The progeny of the latter cross were pair-mated to extract the *ey* strain used in the study. Five generations of brother-sister pair matings of the + strain raise to 60% the chance that the wild type fourth chromosomes were isogenic. The use of marked inversions does not guarantee isogenicity because of the enhancement of crossing over elsewhere in flies heterozygous for rearrangements. The lack of parent-offspring or right-left correlations soon after derivation of the stocks (see below), however, strongly suggests that isogenicity was achieved.

The *ey* stock so obtained had much smaller eyes than the original *ey* stock, varying with temperature but not overlapping wild type. The *ey/ey* flies used as parents were classed by eye size as follows: "small"—with both eyes less than one third normal; "medium"—both approximately half normal; "large"—both over half normal, at least one more than two thirds normal; and "asymmetric"—the two eyes of markedly different size. Intermediates between these categories were not used.

Each eye of the offspring from all crosses was graded according to the arbitrary system:

Grade	Amount of eye
0	no more than one facet
1	less than one third of an eye
2	between one third and two thirds of an eye
3	between two thirds of an eye and a full eye
4	normal wild type eye

In statistical treatment of phenotypic grade, except in correlating right and left eyes, the individual offspring was represented by the sum of the grades of his two eyes. Thus, zero represented a totally eyeless individual and 8, a wild type individual, while 4 represented individuals with both eyes grade 2, with one eye grade 3 and the other grade 1, or with one eye normal and the other missing (these usually breeding as $+/ey$ and thus haplo-IV on one side of the head).

The experimental crosses were performed in half-pint bottles containing the standard cornmeal-agar-Karo-molasses medium, each containing eight pairs of parents. Under these conditions, the eclosion rate drops sharply on the fourth or fifth day. Genetically *ey/ey* flies eclosing later have larger eyes. Eclosion began at approximately the same time in all bottles mated at the same time. In two series, counts were made daily during the entire eclosion period. Both the rate at which the average grade increased and the day on which the highest grade of *ey/ey* occurred differed between the two series. However, within each series the different parental classes and kinds of matings had no influence on either rate of increase or date of maximum eclosion. In cultures producing both $+/ey$ and *ey/ey* offspring, the relative frequencies of these classes did not alter systematically or significantly throughout the eclosion period.

In most of the study, offspring were counted which emerged during maximum eclosion, from approximately 60 to 78 hours after the first eclosion. In the earlier part (series 8–40 as described in the next section), all offspring emerging during this period were classified. Later (series 41–50), no more than 30 flies of each sex were classified, since χ^2 tests on the series 39 counts showed the sampling to be random. When less than 20 emerged, the remainder of the 20 were classified the next day. Cultures producing fewer than 20 offspring during two days of counting were omitted.

EXPERIMENTAL DESIGN

Selection acts through both viability and productivity, or in other words, for an insect, through both survival from zygote to imago, and contribution of either egg or sperm to the zygotes of the next generation. The relative viabilities of mutant and wild type genotypes can be expressed by a viability coefficient s: if the zygotic ratio is 1 mutant: 1 wild type, the final adult ratio is $(1 + s)$ mutants: 1 wild type. The productivity coefficient used here is defined so that $(1 + t)$ eggs are laid (or fertilized) by the mutant for every egg laid (or fertilized) by the wild type of the same sex.

The relative viability of $+/ey$ and each class of *ey/ey* flies was evaluated from the progeny of two systems of mating: 8 *ey/ey* ♀♀ × 8 $+/ey$ ♂♂ per bottle (mating system A) and 8 $+/ey$ ♀♀ × 8 *ey/ey* ♂♂ per bottle (mating system B). From the reciprocal crosses, any maternal influence on viability, or non-virginity of parental females could be assessed. Germinal selection was assumed negligible, i.e., the zygotic ratio was assumed to be $\frac{1}{2}ey/ey$: $\frac{1}{2}+/ey$, since MULLER and SETTLES (1927) demonstrated no selection against even a deficiency for several autosomal loci. Reports of germinal selection (LOBASHOV 1940) might simply result from larval selection.

The relative productivities of *ey/ey* and $+/ey$ ♂♂ were determined from the progeny of mating system C, 8 *ey/ey* ♀♀ × (4 *ey/ey* ♂♂ + 4 $+/ey$ ♂♂) per bottle. The relative productivities of *ey/ey* and $+/ey$ ♀♀ were determined from the

progeny of mating system D, (4 *ey/ey* ♀♀ + 4 +/*ey* ♀♀) × 8 *ey/ey* ♂♂ per bottle. Since progeny from any bottle were sampled just once at maximum eclosion, only one of the two components of productivity was measurable: rate, not duration, of offspring production. A comprehensive estimate of productivity would include both.

A final mating system, E, was 8 *ey/ey* ♀♀ × 8 *ey/ey* ♂♂ per bottle.

Bottles were grouped in series started at different times. The parents in any one series were of the same age from the same sources, handled identically. Most series included:

Phenotype of *ey* parents	Mating system				
	A	B	C	D	E
small	2 bottles	2 bottles	2 bottles	2 bottles	2 bottles
medium	2 bottles	2 bottles	2 bottles	2 bottles	2 bottles
large	2 bottles	2 bottles	2 bottles	2 bottles	2 bottles
asymmetric	2 bottles	2 bottles	2 bottles	2 bottles	2 bottles

In later series, there were three instead of two replicates. Parents were removed and examined again before their offspring eclosed.

For the first 23 series, the +/*ey* parents came from isogenic + ♂♂ × *ey/ey* ♀♀. The *ey/ey* parents were selected from the isogenic stock. All flies used as parents eclosed within 12 to 18 hours after removal of all imagoes from stock bottles. Before mating, males and females were aged in vials containing 20–100 of the same sex. Vials producing eggs or larvae were discarded.

The isogenic + stock was accidentally lost after series 23. For the next 16 series, +/*ey* flies came from mating systems A and B of preceding series. From series 27 on, *ey/ey* parents were obtained from mating systems A through E (E avoided as much as possible to retard any accumulation of modifiers). Meanwhile, a + stock was re-isolated from +/*ey* offspring of series 20. Males from this later + stock, crossed to *ey/ey* ♀♀ of assorted phenotypes, produced +/*ey* parents for the last 11 series.

Series 1 through 7 were run at 29°C for maximal phenotypic variation. The sterility in most of these was excessive, rendering a detailed statistical comparison with later series fruitless. Series 2, however, yielded enough offspring to calculate certain correlations. Fertility was improved by running series 8–50 at 25°C. Series were begun only a few days apart, except for series 21, 57 days after series 20.

FACTORS INFLUENCING EYE SIZE IN EY/EY STOCKS

The stocks did not long remain isogenic. Instead, eye size varied more markedly in the later series.

The eye grades of *ey/ey* offspring in each bottle were averaged. Table 1 presents the mean of these averages for each group of bottles with the same phenotypic class of *ey* parent and mating system (A or E), in series 8–20 (October to December, 1953), in series 21–33 (February to early April, 1954), in series 34–42 (late April and May, 1954), and in series 43–50 (June and July, 1954). The distributions of eye grades are also given.

TABLE 1

Percentage of ey *offspring with each summed eye grade, and average of bottle mean eye grades, in mating systems A and E, grouped by series and parent phenotype*

Mating system	Series	Phenotype of *ey* parent	Male offspring							Female offspring						
			Number offsp.	Summed eye grade					Mean	Number offsp.	Summed eye grade					Mean
				<2	2	3	4	>4			<2	2	3	4	>4	
A	8–20	small	534	10	34	29	20	7	2.78	444	21	38	25	13	3	2.29
ey/ey ♀♀ ×		medium	478	6	36	33	21	4	2.79	466	16	36	30	14	4	2.43
+/*ey* ♂♂		large	325	5	35	33	23	4	2.85	318	16	35	27	15	7	2.65
		asymmetric	462	10	35	30	21	4	2.72	388	23	40	23	12	2	2.31
	21–33	small	450	5	29	31	28	7	3.02	428	18	27	28	20	7	2.72
		medium	439	3	16	30	36	15	3.48	345	9	18	25	33	15	3.35
		large	371	1	15	27	38	19	3.65	339	10	15	23	30	22	3.50
		asymmetric	413	3	21	29	35	12	3.30	356	9	23	29	27	12	3.16
	34–42	small	431	3	31	33	36	6	3.04	428	15	27	30	21	7	2.83
		medium	433	3	15	29	37	16	3.55	378	7	18	29	30	16	3.33
		large	422	1	11	28	38	22	3.78	383	6	12	25	31	26	3.69
		asymmetric	391	2	18	27	35	18	3.51	364	9	21	24	28	18	3.28
	43–50	small	320	4	34	33	24	5	2.95	314	11	31	28	23	7	2.85
		medium	327	3	28	25	31	13	3.29	333	5	21	27	29	18	3.44
		large	343	2	19	23	35	21	3.66	322	5	14	22	33	26	3.75
		asymmetric	302	2	25	33	28	12	3.27	341	9	22	27	29	13	3.19
E	8–20	small	1081	13	50	25	12	0	2.38	1037	34	39	21	6	0	1.94
ey/ey ♀♀ ×		medium	951	6	41	33	19	1	2.71	945	20	39	28	12	1	2.33
ey/ey ♂♂		large	656	4	37	32	25	2	2.87	641	14	33	32	17	4	2.58
		asymmetric	962	8	41	31	19	1	2.65	871	22	40	26	11	1	2.22
	21–33	small	834	5	36	39	17	3	2.96	814	18	33	28	18	3	2.54
		medium	959	3	13	28	41	15	3.62	905	9	20	25	33	13	3.26
		large	596	1	5	18	44	32	4.20	547	3	10	17	37	33	4.04
		asymmetric	721	2	18	32	36	12	3.43	683	10	23	28	28	11	3.08
	34–42	small	790	6	31	33	26	4	2.93	811	17	30	29	20	4	2.64
		medium	772	2	16	25	37	20	3.63	694	5	15	26	34	20	3.55
		large	931	1	8	21	42	28	4.02	851	5	14	19	36	26	3.83
		asymmetric	854	2	20	28	36	14	3.43	804	6	23	27	30	14	3.30
	43–50	small	644	6	38	32	22	2	2.77	657	18	31	30	18	3	2.55
		medium	688	2	17	25	41	15	3.54	676	4	16	27	38	15	3.50
		large	613	0	9	16	44	31	4.14	624	3	8	18	31	40	4.20
		asymmetric	636	4	21	30	33	12	3.32	652	7	21	30	32	10	3.17

After the first group of series, the mean eye grade increased, especially when parents were "medium" or "large". The offspring were more extreme—smaller-eyed from "small" parents and larger-eyed from "large" parents—when both parents were *ey* and alike (mating system E) than when one parent was +/*ey* (mating system A). The "within bottle" variance increased concurrently, possibly because of the appearance and segregation of modifiers after series 20, but possibly simply as an artifact of the grading scale.

The initial isogenicity of the *ey* stock is attested by the offspring of mating system E in series 2. The midparent-son correlation (based on the offspring of "small", "medium" and "large" parents) was −.101, which is not significantly different from zero (d.f. = 163, P = .20). The midparent-daughter correlation was −.018, also not significantly different from zero (d.f. = 159, P = .81).

Before or during series 8–20, modifiers of the *ey* eye size had appeared. The parent-offspring correlations (table 2) are considerably lower in series 8–20 than they are

TABLE 2

Coefficients of correlation between grade of ey *parent* and grade of individual* ey *offspring, mating systems A through E. Equal replication within series*

Mating system	Series	Sons		Daughters	
		Number offsp.	r	Number offsp.	r
A	8–20	686	.1113	639	.1153
ey/ey ♀♀ × +/*ey* ♂♂	21–33	1054	.2299	926	.2570
	34–42	1295	.2702	1207	.2775
	43–50	958	.2597	932	.2921
B	8–20	892	.1960	855	.1320
+/*ey* ♀♀ × *ey/ey* ♂♂	21–33	1176	.1605	1089	.1614
	34–42	1077	.2089	1021	.2426
	43–50	982	.2203	910	.1955
C	8–20	1047	.1335	911	.2195
ey/ey ♀♀ × +/*ey* ♂♂ & *ey/ey* ♂♂	21–33	1436	.2644	1385	.2893
	34–42	1702	.3307	1618	.3725
	43–50	1412	.3448	1374	.3866
				1174	.1580
D	8–20	1324	.0960		
+/*ey* & *ey/ey* ♀♀ × *ey/ey* ♂♂	21–33	1824	.3276	1624	.3003
	34–42	1766	.3280	1668	.3391
	43–50	1445	.3917	1485	.4203
E	8–20	1463	.1793	1467	.2223
ey/ey ♀♀ × *ey/ey* ♂♂	21–33	1914	.4688	1729	.4415
	34–42	2481	.3770	2363	.3660
	43–50	1945	.4584	1957	.4742

* Average grade of:

	ey mothers			*ey* fathers		
	Small	Medium	Large	Small	Medium	Large
series 8–20	1.38	4.0	5.5	1.76	4.0	5.5
series 21–33	1.41	4.0	5.5	1.78	4.0	5.5
series 34–42	1.53	4.0	5.5	1.81	4.0	5.5
series 43–50	1.54	4.0	5.5	1.85	4.0	5.5

later, but are already significantly different from zero. In the interval before series 21–33, heterogeneity due to modifiers was heightened. The genetic variance increased 5.3-fold (= $[.46/.20]^2$) from the first to the fourth group of series; the original isogenic stock had none.

As would be expected if phenotypic grade is influenced by modifiers, the correlations are highest in mating system E and lowest in A and B. The differences in correlations found among the mating systems are less in the earlier than in the later series. Mating system A does not differ significantly from B within a series group, nor C from D. Apparent discrepancies—parent-son correlations in series 8–20 for B and D, and

TABLE 3

Correlation coefficients of right and left eye grades in ey *offspring for each phenotypic class of parent in each mating system. (618 ≤ N ≤ 2104)*

Mating system	Series	Phenotypic class of *ey* parent			
		Small	Medium	Large	Asymmetric
A	8–20	.316	.230	.293	.163
ey/ey ♀♀ × +/*ey* ♂♂	21–33	.283	.330	.397	.335
	34–42	.325	.432	.408	.437
	43–50	.361	.491	.476	.391
B	8–20	.248	.211	.219	.221
+/*ey* ♀♀ × *ey/ey* ♂♂	21–33	.292	.362	.503	.320
	34–42	.385	.417	.436	.397
	43–50	.380	.360	.447	.439
C	8–20	.295	.280	.228	.334
ey/ey ♀♀ × +/*ey* & *ey/ey* ♂♂	21–33	.270	.341	.406	.364
	34–42	.302	.408	.422	.443
	43–50	.302	.440	.474	.557
D	8–20	.199	.222	.228	.227
+/*ey* & *ey/ey* ♀♀ × *ey/ey* ♂♂	21–33	.277	.326	.434	.334
	34–42	.289	.409	.434	.356
	43–50	.357	.409	.478	.405
E	8–20	.222	.215	.279	.243
ey/ey ♀♀ × *ey/ey* ♂♂	21–33	.223	.290	.363	.241
	34–42	.314	.366	.411	.380
	43–50	.276	.384	.469	.351

parent-daughter correlations in series 43–50 for A and B—are of borderline significance only, probably attributable to accidents of sampling.

"Asymmetric" parents, with an average grade somewhat less than 4.0, seemed genetically similar to "medium" parents. The variance of the offspring eye grades between bottles with "asymmetric" parents was not significantly different from that with "medium" parents in the same groups of series. Furthermore, the correlation between right and left eyes in offspring of "asymmetric" parents is about the same as in offspring of "medium" parents (table 3).

A significant right-left correlation does not necessarily indicate segregation of modifiers, since non-genetic factors could conceivably affect both eyes simultaneously. An increase in right-left correlation with time does, however, indicate an increased number of modifiers segregating.

The right-left correlation was negligible—+.08 for males ($.4 < P < .5$) and −.22 for females ($.02 < P < .05$)—in the isogenic *ey* stock when first derived. A significant correlation was found, however, in the offspring from each mating system, for each parent phenotype, and in each series group in the experiment (table 3). Differences in r of 0.12 are significant at least at the 5% level; differences of 0.15, at the 1% level.

The increase in right-left eye correlation with time is pronounced, though somewhat less so in the offspring of "small" parents in mating system E, suggesting that "medium" and "large" parents are more heterozygous for modifiers affecting eye size than "small" parents.

From the pattern of parent-offspring and right-left correlations, the history of the eye-size modifiers of *ey* can be reconstructed. Initially, the *ey* and + stocks differed at most in their fourth chromosomes. The + fourth chromosomes probably came from a single source, but possibly two. Between the time of the marked-chromosome derivation of the first *ey* stock in August 1953 and the first group of series at 25°C (series 8–20, October through December), plus modifiers arose. Genetic diversity increased almost 5-fold following the 57-day interval between series 20 and 21, with no consistent increase during the last two periods (April through July).

Over 9000 flies were used to propagate the stocks after their derivation by the method which GOWEN, STADLER and JOHNSON (1946) found reduced heterozygosity more effectively than 97 generations of brother-sister pairing. Any locus with a mutation rate of 10^{-5}/chromosome-generation had a 9% chance of mutating during the experiment. Several modifying mutations were likely, although probably only a modest fraction of the estimated 5000 loci of *melanogaster* influence the *ey* phenotype. All four chromosomes can carry eyeless modifiers (BARON 1935). Any mutation lengthening the larval period would correspondingly increase facet number (BODENSTEIN 1939).

Until series 27, *ey* parents of all classes came from the same stock bottles. Meanwhile, larger-eyed flies became more common; natural selection was favoring plus-modifiers. From series 27 on, *ey* parents were taken from experimental cultures of mating systems A, B, C and D and randomized as to source. Thus, while few were the result of assortative matings, the earlier selection within *ey* stocks for increased eye-size was replaced by selection within each phenotypic grade of eyeless, from competition with +/*ey*.

RELATIVE VIABILITIES OF EY/EY AND +/EY

Table 4 gives the average percentage of +/*ey* offspring per bottle of mating systems A and B for each class of *ey* parent, the χ^2's of heterogeneity between bottles, their degrees of freedom and the probabilities of obtaining higher χ^2's without real heterogeneity. Table 5 gives the tests of differences between the categories itemized in table 4. A single bottle, mating system B, "asymmetric", series 24, gave a highly aberrant ratio in its sons (5 *ey*/*ey*: 30 +/*ey*), although the ratio in its daughters was normal, with *ey* daughters slightly in excess. This bottle was excluded from the statistical treatments.

Evidently, it is not eye size of the *ey* parents which affects viability. However, in series 8–30, significantly smaller proportions of daughters than of sons are *ey*/*ey*. There is little heterogeneity in the daughters. The mating-system difference in daughters of "small" parents is 2.55 times its standard error, and might have the same cause as the mating-system difference in sons (see below). The daughters of "asymmetric" parents differ significantly from early to late series—$\chi^2 = 7.2$, $.001 < P < .01$, in mating system A and $\chi^2 = 4.2$, $.02 < P < .05$, in mating system B.

TABLE 4

Percentage of heterozygotes in mating systems A and B

Offspring sex	Phenotype of *ey* parent	Series	Mating system A (*ey/ey* ♀♀ × +/*ey* ♂♂)					Mating system B (+/*ey* ♀♀ × *ey/ey* ♂♂)				
			Number offsp.	Average % +/*ey* in offspring	χ^2	d.f.	*P*	Number offsp.	Average % +/*ey* in offspring	χ^2	d.f.	*P*
♂♂	small	8–30	1785	51.88	46.10	45	.3–.5	1668	55.76	48.50	41	.1–.2
		31–50	1922	52.71	51.30	53	.5–.7	1623	53.60	54.34	50	.3–.5
	medium	8–30	1554	52.06	48.55	43	.2–.3	1781	54.74	26.72	44	.95–.98
		31–50	1948	52.98	48.89	54	.5–.7	1669	53.09	66.07	54	.1–.2
	large	8–30	1189	53.15	24.72	29	.5–.7	1602	56.37	39.00	35	.2–.3
		31–50	1902	52.73	57.01	54	.3–.5	1751	54.65	54.02	53	.3–.5
	asymmetric	8–30	1349	51.96	38.78	35	.3–.5	1252	55.43	29.74	30	.3–.5
		31–50	1818	54.07	45.64	53	.7–.8	1854	53.34	66.10	53	.1–.2
	Total	8–30	5877	52.20	158.15	152	.3–.5	6303	55.56	143.96	150	.5–.7
		31–50	7590	53.11	202.84	214	.5–.7	6897	53.68	240.53	210	.05–.1
♀♀	small	8–30	1649	53.37	45.26	45	.3–.5	1715	56.09	49.77	42	.2–.3
		31–50	1830	54.48	59.21	53	.2–.3	1638	58.00	48.21	52	.5–.7
	medium	8–30	1565	55.02	46.05	43	.3–.5	1732	54.79	47.76	45	.3–.5
		31–50	1763	55.64	62.26	53	.1–.2	1645	55.32	68.67	53	.05–.1
	large	8–30	1230	55.37	26.55	29	.5–.7	1452	55.30	47.95	35	.05–.1
		31–50	1810	54.75	64.02	54	.1–.2	1632	54.90	44.22	53	.7–.8
	asymmetric	8–30	1359	57.32	41.56	38	.3–.5	1200	52.58	19.61	31	.9–.95
		31–50	1696	52.48	45.99	53	.7–.8	1735	56.43	50.21	53	.5–.7
	Total	8–30	5803	55.16	159.42	155	.3–.5	6099	54.85	165.09	153	.2–.3
		31–50	7099	54.36	231.48	213	.1–.2	6650	56.17	211.32	211	.3–.5

Between mating systems A and B within the earlier series with "asymmetric" parents, $\chi^2 = 5.78$; within the later series, $\chi^2 = 5.42$, $.01 < P < .02$. However, which mating system has the higher proportion of +/*ey* daughters of "asymmetrics" differs from early to later series, so that, pooling mating systems, the net early-late difference is negligible ($\chi^2 = .23$), and, pooling groups of series, the net mating-system difference is also negligible ($\chi^2 = .03$).

The viability of *ey/ey* sons is very significantly greater in mating system B than in mating system A in series 8–30. The only tenable explanation invokes the genetic heterogeneity demonstrated even in the earlier series. Modifiers favoring viability of the *ey/ey* may well have accumulated during the separate maintenance of *ey* and + stocks before series 24. Some modifiers could act via a maternal effect on egg cytoplasm. Maternal genotype has been shown to influence viability of certain offspring genotypes (LYNCH 1920; REDFIELD 1926). Thus, it is conceivable that *ey/ey* zygotes survive better if their mothers came from the *ey* stock (mating system A) than if their mothers came from a cross to + (mating system B). For the effect to be more pronounced in sons than in daughters, chromosome X must have the same effect on viability in both stocks, but must override the earlier conditioning of the egg cytoplasm only when paired, as in females. By series 27, *ey/ey* mothers (mating system A) and +/*ey* mothers (mating system B) would differ systematically only in chromosome 4. The preponderance of inheritance from the *ey* stock would account for the later series resembling the earlier series of mating system A more than mating system B.

An unlikely alternative explanation is that not all the mothers used were virgin.

TABLE 5

Chi square analysis of proportions of heterozygotes in mating systems A and B

Comparison	Groupings for which estimates of % of + /*ey* are accepted	χ^2	d.f.	*P*
Phenotype, series group, sex and mating system	Total of all 32 classes	50.8	31	.01–.02
Phenotype	A ♂♂ 8–30, A ♂♂ 31–50, B ♂♂ 8–30, B ♂♂ 31–50, A ♀♀ 8–30, A ♀♀ 31–50, B ♀♀ 8–30, B ♀♀ 31–50	19.3[a]	24	.70–.80
Series group, sex and mating system	Total of 32 classes, applied to the 8 groups pooling phenotypes	31.5	7	≪.001
Early vs. late series groups	A ♂♂, B ♂♂, A ♀♀, B ♀♀, 8 groups	8.9[b]	4	.05–.10
♂♂ vs. ♀♀	A 8–30, A 31–50, B 8–30, B 31–50, 8 groups	21.7[c]	4	≪.001
A vs. B	♂♂ 8–30, ♂♂ 31–50, ♀♀ 8–30, ♀♀ 31–50, 8 groups	18.9[d]	4	<.001
(a) Components of phenotype	A ♂♂ series 8–30	0.6	3	.90–.95
	A ♂♂ series 31–50	0.9	3	.80–.90
	B ♂♂ series 8–30	0.9	3	.80–.90
	B ♂♂ series 31–50	1.0	3	.80–.90
	A ♀♀ series 8–30	4.7	3	.10–.20
	A ♀♀ series 31–50	3.7	3	.20–.30
	B ♀♀ series 8–30	3.7	3	.20–.30
	B ♀♀ series 31–50	3.8	3	.20–.30
(b) Components of early vs. late (pooled phenotypes)	A ♂♂	1.2	1	.20–.30
	B ♂♂	4.7	1	.02–.05
	A ♀♀	0.8	1	.30–.50
	B ♀♀	2.2	1	.10–.20
(c) Components of ♂♂ vs. ♀♀ (pooled phenotypes)	A series 8–30	10.3	1	.001–.01
	A series 31–50	2.3	1	.10–.20
	B series 8–30	0.6	1	.30–.50
	B series 31–50	8.5	1	.001–.01
(d) Components of A vs. B (pooled phenotypes)	♂♂ series 8–30	13.8	1	≪.001
	♂♂ series 31–50	0.4	1	.50–.70
	♀♀ series 8–30	0.1	1	.70–.80
	♀♀ series 31–50	4.5	1	.02–.05

If this were so, the non-virginity existed chiefly in the +/*ey* mothers in the earlier series. To account quantitatively for the discrepancy, 10.8% of the sperm fertilizing the eggs of these +/*ey* ♀♀ must have come from their brothers. Such a high value for a single class of females is extremely unlikely, since the procedure for obtaining virgins was uniform throughout the experiment. The only direct evidence of non-virginity of mothers of any class could be obtained from +/*ey* offspring in bottles belonging to mating system E after series 26, when *ey* parents came from bottles also

TABLE 6

Viability coefficients s *for* ey/ey

Sex	Mating system	Series	Number +/*ey* (*n*)	Total number (*N*)	*s**	95% Confidence limits	
						Upper	Lower
♂♂	A	8–30	3068	5877	−.077	−.028	−.123
		31–50	4031	7590	−.109	−.068	−.149
	B	8–30	3502	6803	−.193	−.152	−.232
		31–50	3702	6897	−.129	−.087	−.170
	Pooled		14303	26667	−.128		
♀♀	A	8–30	3201	5803	−.180	−.136	−.221
		31–50	3859	7099	−.153	−.112	−.192
	B	8–30	3345	6099	−.169	−.126	−.210
		31–50	3735	6650	−.212	−.173	−.250
	Pooled		14140	25651	−.179		

* $s = \frac{N}{n(1-a)} - 2$, where a is the proportion of wild type appearing individuals which are genetically *ey/ey*.

producing +/*ey*. Almost any non-virginity would be due to precocity of brothers. In these series (27–50), of 61 bottles with 478 "small" mothers, 5 gave one +/*ey* offspring each, totaling 3 sons and 2 daughters. They occurred in series 31, 35–37 and 50. Of 62 bottles with 496 "large" mothers, one in series 49 produced 6 sons and 3 daughters which proved +/*ey* in test-crosses. In this bottle, more than one mother may have mated previously. Of 62 bottles with 494 "medium" mothers and of 60 bottles with 472 "asymmetric" mothers, none gave + offspring. Thus probably 7 of the 1940 mothers used for mating system E in series 27–50 had mated previously with ey^+-bearing males. In consequence, 14 of their 15,419 offspring, or 0.09%, were +/*ey* because of non-virginity, i.e., roughly 0.36% of the sperm came from prior insemination, in contrast to the 10.8% required to attribute the early difference between mating systems A and B to non-virginity.

Exceptionally large-eyes *ey/ey* flies could be erroneously classed as +. To detect this, 5901 phenotypically + offspring in series 22–26, 38, and 44–50 were testcrossed individually to *ey/ey* in vials at room temperature. In 24 vials only eyeless offspring were obtained, the remainder giving both *ey* and +. The proportion of phenotypically + flies which proved to be *ey/ey* was 0.407% ± 0.082%, unaffected by sex, series, or parent phenotype. Hence, approximately 0.46% of the *ey/ey* ♂♂ appeared +, as did 0.50% of the *ey/ey* ♀♀.

If x represents the proportion of offspring which are +/*ey*, after correcting for phenotypic overlap, the viability coefficient of *ey/ey* is

$$s = \frac{1}{x} - 2.$$

Because of the maternal effect on viability, table 6 gives coefficients for each mating system in both groups of series. The overall average was −.128 for males and −.179 for females.

RELATIVE PRODUCTIVITIES OF EY/EY AND +/EY MALES

Offspring from four types of matings in mating system C yielded evidence concerning the relative productivities of *ey/ey* and +/*ey* ♂♂:

(1) 8 "small" *ey/ey* ♀♀ × 4 +/*ey* ♂♂ + 4 "small" *ey/ey* ♂♂;
(2) 8 "medium" *ey/ey* ♀♀ × 4 +/*ey* ♂♂ + 4 "medium" *ey/ey* ♂♂;
(3) 8 "large" *ey/ey* ♀♀ × 4 +/*ey* ♂♂ + 4 "large" *ey/ey* ♂♂;
(4) 8 "asymmetric" *ey/ey* ♀♀ × 4 +/*ey* ♂♂ + 4 "asymmetric" *ey/ey* ♂♂.

The productivities of the four classes of *ey/ey* fathers can be compared, using that of +/*ey* ♂♂ as standard. The ratio of +/*ey* to *ey/ey* in the progeny depends on two factors: the relative proportions of eggs fertilized by the two kinds of males and the relative viabilities of the *ey/ey* and +/*ey* offspring. The male productivity coefficient, $t^{♂}$, evaluates the first of these factors. Since the latter factor differs in sons and daughters, two estimates of $t^{♂}$ can be made, one based on sons and the other on daughters.

The unit of analysis was the bottle, rather than the individual offspring, since the χ^2 of heterogeneity of proportion of +/*ey* in sons between bottles with "medium" parents was 260.733, d.f. = 97, $P \ll .0001$. The percentages of +/*ey* in the offspring were transformed into arcsines for analyses of variance, to permit valid comparisons when percentages differed widely. Variation between bottles signifies a real variation in productivity. Table 7 presents the correlation between the proportions of +/*ey* in daughters and in sons. All correlations are significantly positive, especially between sons and daughters of "medium" parents, whose heterogeneity was tested.

The major results for mating system C are shown in table 8. The proportion of +/*ey* offspring in series 8–30 was on the average higher than in series 31–50, except for "large" parents. When a four-partite division of the series is made, the shift in proportion obviously is not gradual, but abrupt. Various explanations could be ruled out. The difference was not due to the inclusion of more series with one range of ages of parents in the earlier than in the later series, since an analysis of variance disclosed that, if male productivity depends on age at all, it is equally influenced

TABLE 7

Coefficients of correlation between ♂ and ♀ arcsin $\sqrt{\%+/ey}$ *'s in mating system C*
(ey/ey ♀♀ × +/ey ♂♂ and ey/ey ♂♂)

Phenotypic class of *ey* parents	Number of bottles (N)	Correlation between daughters & sons (r)	95% confidence limits	
			Upper	Lower
Small	96	+.500	+.636	+.332
Medium	96	+.720	+.804	+.607
Large	85	+.617	+.734	+.466
Asymmetric	94	+.692	+.784	+.569
Pooled	371	+.639	+.700	+.575

TABLE 8

Comparison of arcsin $\sqrt{\%+/ey}$ in series 8–30 and series 31–50, mating system C, for sons and for daughters

Phenotype of *ey* parents	Offspring sex	Series 8-30			Series 31-50			S.e. of diff. of means	STUDENT'S *t*	*P*
		No. bottles	Mean % +/*ey*	Mean arcsine	No. bottles	Mean % +/*ey*	Mean arcsine			
"Small"	♂♂	43	38.32	37.99	53	31.90	33.91	1.61	−2.49	.01–.02
	♀♀	43	40.05	39.51	54	35.25	36.02	1.71	−2.04	.02–.05
"Medium"	♂♂	45	33.02	34.53	53	26.45	30.10	1.77	−2.50	.01–.02
	♀♀	43	37.76	37.42	53	27.28	30.60	1.96	−3.48	.001
"Large"	♂♂	32	29.91	32.15	54	32.68	34.01	2.45	+0.76	> .05
	♀♀	31	30.88	33.00	54	32.87	34.61	2.12	+0.76	> .05
"Asymmetric"	♂♂	40	35.71	36.46	55	29.77	32.25	1.46	−2.88	.001–.01
	♀♀	39	37.99	37.68	55	30.67	33.19	1.70	−2.64	.001–.01
Pooled	♂♂	..	...	35.47	..	...	32.57	0.92	−3.17	< .001
	♀♀	..	...	37.20	..	...	33.62	1.00	−3.60	< .001

Comparison	S.e. of diff. of means	STUDENT'S *t*	*P*
♂♂ series 8–30 "small" vs. "medium"	1.71	+2.02	.02–.05
"medium" vs. "large"	2.37	+1.00	> .05
♂♂ series 31–50 "small" vs. "medium"	1.67	+2.28	.02–.05
"medium" vs. "large"	1.88	−2.08	.02–.05
♀♀ series 8–30 "small" vs. "medium"	1.88	+1.11	> .05
"medium" vs. "large"	2.26	+1.96	≃.05
♀♀ series 31–50 "small" vs. "medium"	1.49	+3.64	< .001
"medium" vs. "large"	1.50	−2.67	.001–.01

in *ey/ey* ♂♂ and in +/*ey* ♂♂ between 1 and 19 days after eclosion ($F = 0.3$, d.f. = 3 and 39, $P > .05$). The ratio of *ey/ey* to +/*ey* fathers surviving until their removal from the cultures was also not related to their measured productivities ($F < 1$, d.f. = 2 and 74, $P > .05$). The series with unusually high or low "productivity" of *ey/ey* fathers did not correspond with those displaying the highest or lowest "viabilities" of *ey/ey* offspring in mating systems A and B.

Two possible explanations remain to be discussed. Both presuppose heterogeneity at other loci influencing fertility. The first presumes that factors increasing productivity in *ey/ey* ♂♂ accumulated while the *ey* stock was maintained separately. After the + stock was lost, +/*ey* fathers (series 24–50) were the direct or indirect product of successive backcrosses to the *ey* stock. Hence, modifiers usual to the wild type were replaced by those optimal for *ey/ey*, reducing the productive advantage of +/*ey*. This interpretation is consistent with MATHER's (1943) views, substantiated in HASKELL's (1940) study of polygene complexes affecting bristle number in scute and in wild type.

The second possibility is that after series 27, *ey/ey* ♂♂ had *ey/ey* parents which had competed successfully with +/*ey* flies for leaving offspring. In any event, the greatest increase in productivity occurs for those phenotypic classes of *ey/ey* ♂♂ which were least productive earlier, so the segregating fertility genes apparently have little effect on eye size.

TABLE 9

Effects of "line breeding" on productivity of males

Series	Line	Offspring sex	Phenotype of *ey* parents								Total	
			Small		Medium		Large		Asymmetric			
			No.*	Mean**	No.	Mean	No.	Mean	No.	Mean	No.	Mean
27, 31, 35, 39, 43, 47	*a*	♂♂	14	30.22	15	28.63	15	38.82	15	29.20	59	31.74
		♀♀	15	34.69	15	26.59	15	36.09	15	32.68	60	32.51
28, 32, 36, 40, 44, 48	*b*	♂♂	15	34.37	14	31.27	15	36.13	16	36.69	60	34.71
		♀♀	15	39.08	14	31.80	15	39.58	16	32.81	60	35.83
29, 33, 37, 41, 45, 49	*c*	♂♂	16	35.46	16	33.11	16	31.07	16	33.22	64	33.22
		♀♀	16	37.35	16	31.33	16	31.63	16	35.12	64	33.86
30, 34, 38, 42, 46, 50	*d*	♂♂	15	34.64	15	30.88	16	31.74	15	31.34	61	32.14
		♀♀	15	34.64	15	36.85	16	32.24	14	32.43	60	33.92
Total		♂♂	60	33.76	60	31.00	62	34.34	62	32.69	244	32.96
		♀♀	61	36.46	60	31.52	62	34.79	61	33.30	244	34.03

* Number of bottles.

** Mean of arcsines of the square roots of the percent. of +/*ey* offspring.

Four breeding lines were distinguishable in the later series: *ey* progeny from series 27 were used as parents for series 31, from series 31 for series 35, etc. Table 9 shows consistently greater proportions of +/*ey* offspring when the *ey/ey* fathers were "small" than "medium". A paired comparison test of "small" vs. "medium," pooling sons and daughters, yielded $t = 3.98$, d.f. $= 3$, $.02 < P < .05$. In contrast, the relation between "medium" and "large" differs from line to line. The statistical "line-by-parent phenotype" interaction falls a little short of the 5% significance level for both sons and daughters. Thus, while "small" is always less productive than "medium," the relative productivities of "large" and "medium" vary, "large" being more productive in early series, "medium" in the later series, on the whole. Table 10 gives an analysis of variance on data for daughters, randomly eliminating several series to ensure orthogonality. A paired comparison test of "small" vs. "medium" gave $t = 4.115$, d.f. $= 11$, $.001 < P < .01$; "medium" vs. "large" in groups 1–4, $t = 1.32$, d.f. $= 3$, $.20 < P < .30$; "medium" vs. "large" in groups 5–12, $t = 3.12$, d.f. $= 7$, $.01 < P < .02$. An analysis of sons gave similar results.

It seems warrantable to conclude that extreme smallness of eye itself reduces male productivity, probably by diminishing the frequency of copulation. STURTEVANT (1915) found that success in copulation depended partly on sight. KALMUS (1943) reported that the smaller the eye, the greater a moving object must be to be seen, since the angle between adjacent ommatidia is greater in smaller eyes. Phenotypically "medium" or "large" *ey/ey* flies could not discriminate rotating stripes as narrow as could wild type. Totally eyeless flies displayed no pattern vision at all. Extremely small-eyed males may not only lack optical equipment for locating and pursuing partners, but may behave abnormally due to brain deficiency. The sizes of the three optic glomeruli of the brain depend strictly on the size of the eye itself without obvious alteration of the rest of the brain or compensatory enlargement of the antennal ganglion (POWER 1943).

TABLE 10

Analysis of proportion of +/ey amongst daughters, mating system C. Each entry is the sum of the arcsine $\sqrt{\%+/ey}$'s from 6 bottles.

Group	Including series	Phenotypic grade of *ey* parents				Total
		"Small"	"Medium"	"Large"	"Asymmetric"	
1	10, 11, 12	231.61	194.70	213.83	241.28	881.42
2	14, 15, 19, 20	247.57	235.13	173.16	220.06	875.92
3	22, 25, 26	231.71	207.20	168.30	179.65	786.86
4	27, 28, 29	239.40	226.54	215.68	205.68	887.30
Subtotal		950.29	863.57	770.97	846.67	3431.50
5	31, 32, 33	227.56	146.74	211.57	202.35	788.22
6	34, 35, 36	214.81	193.68	241.60	182.87	832.96
7	37, 38	249.90	190.04	205.09	204.35	849.38
8	39, 40	196.37	185.07	213.43	221.98	816.85
9	41, 42	178.61	189.95	177.77	170.08	716.41
10	43, 45	235.96	177.73	189.81	210.27	813.77
11	46, 47	231.31	162.27	183.88	192.55	770.01
12	48, 49	201.08	179.20	211.18	204.19	795.65
Subtotal		1735.60	1424.68	1634.33	1588.64	6383.25
Total		2685.89	2288.25	2405.30	2435.31	9814.75

Analysis of variance

Source of variance	Groups 1–4			Groups 5–12			Groups 1–12		
	Sum sq.	d.f.	Mean sq.	Sum sq.	d.f.	Mean sq.	Sum sq.	d.f.	Mean sq.
Groups	283	3	94.3	500	7	71.5	1146	11	104.2
Grades	677	3	225.7	1049	3	349.7	1130	3	376.6
Group-by-grade	574	9	63.8	1248	21	59.4	2455	33	74.4
Total between classes	1534	15	...	2797	31	...	4731	47	...
Within classes	6263	80	78.3	11859	160	74.1	18122	240	75.5
Total betw. bottles	7797	95	...	14656	191	...	22853	287	...
Groups/within classes	$F = 1.20, P > .05$			$F < 1, P > .05$			$F = 1.38, P > .05$		
Grades/within classes	$F = 2.88, .01 < P < .05$			$F = 4.72, .001 < P < .01$			$F = 4.99, .001 < P < .01$		

BATEMAN (1948) found that the more often a male copulated, the more numerous his offspring. If a female was mated to different males in close succession, the sperm from the various partners was mixed. KAUFMANN and DEMEREC (1942) found that the sperm deposited in the last of three successive matings by an aged "virgin" male fertilized a female's five-day output of eggs. In their case, whether later sperm replaced or mixed with sperm deposited earlier depended partly on genotype. To discover whether mixing or replacement of sperm was common in mating system C, the surviving *ey/ey* mothers in series 23 were isolated in vials. Sixteen gave no offspring, 21 gave $\frac{1}{2}$ *ey/ey*: $\frac{1}{2}$ +/*ey*, and 4 gave all *ey/ey* offspring. No mixing of

TABLE 11

Male productivity coefficients t^{σ} based on data from sons and daughters

Phenotype of *ey* male	Offspring sex	Series 8-30			Series 31-50		
		t^{σ}	95% conf. limits*		t^{σ}	95% conf. limits	
			Upper	Lower		Upper	Lower
Small	♂♂	−.618	−.462	−.747	−.284	−.045	−.477
	♀♀	−.576	−.397	−.723	−.401	−.185	−.576
	Pooled**	−.600			−.348		
Medium	♂♂	−.387	−.142	−.581	+.089	+.436	−.184
	♀♀	−.482	−.243	−.670	+.102	+.491	−.198
	Pooled	−.436			+.095		
Large	♂♂	−.212	+.266	−.543	−.327	−.036	−.553
	♀♀	−.113	+.325	−.431	−.276	+.030	−.475
	Pooled	−.159			−.301		
Asymmetric	♂♂	−.513	−.351	−.642	−.155	+.084	−.352
	♀♀	−.492	−.268	−.671	−.144	+.089	−.338
	Pooled	−.506			−.149		

* Based on the standard error of the mean arcsine. The mean percentage of $+/ey$ offspring was transformed into an arcsine; 2σ was added or subtracted. The resulting arcsine was transformed back into a percentage, from which the corresponding upper or lower value of t^{σ} was calculated.

** Obtained by weighting the estimates based on sons and on daughters by the reciprocals of the squared differences between the appropriate pairs of 95% confidence limits.

sperm was suggested. The highest proportion of *ey/ey* in the regular counts for series 23 were in the bottles which had the mothers producing only *ey/ey* after isolation. Inasmuch as the copulations resulting in the counted offspring probably occur soon after the parents are placed in the bottles, the test just cited indicates but roughly the vigor of the two kinds of males.

Table 11 gives the productivity coefficients t^{σ} for each class of *ey* father:

$$t^{\sigma} = \frac{1 - y(4 + 3s) + 3a'(1 + s)}{2(y - a')(1 + s)} = \frac{1.012261 - y(4 + 3s)}{2y(1 + s) - .008174}$$

where s is the appropriate viability coefficient of *ey/ey* determined from mating system A, a' is the proportion of *ey/ey* phenotypically wild type, and y is the proportion of phenotypically wild type sons or daughters, averaged by bottle. The productivity coefficients estimated from sons and daughthers agree very well, and were therefore combined, weighting the separate estimates by the inverse of their variances. In series 8–30, "large" *ey/ey* ♂♂ are not significantly less productive than $+/ey$. "Small" males are, however, less than half as productive. "Medium" and "asymmetric" males are intermediate. In series 31–50, "small" males are 65% as productive as +; "medium," fully as productive as +; "large," 70% as productive as +; "asymmetric," 85% as productive as +. Some combinations of modifiers which enhance the fertility of *ey/ey* ♂♂ favor large-eyed males less than medium-eyed males.

RELATIVE PRODUCTIVITIES OF EY/EY AND +/EY FEMALES

Table 12 presents the results of mating system D, concerning the productivity of *ey/ey* ♀ ♀. There are no differences detectable between daughters in series 8–30 and in series 31–50. Because of the maternal influence on viability, some difference between sons in early and late series was anticipated. None of significance was found, comparing sons from a single class of *ey* parents, but, pooling sons from all classes of *ey* parents, $t = 1.957$, $P = .05$ of a merely chance divergence. Separate values of $t^{♀}$ will therefore be presented for earlier and later series.

An approximate analysis of variance disclosed no component of variance of male arcsines assignable to class of *ey* parents ($F = 0.69$, d.f. = 3 and 371, $P > .05$). However, a significant component of the variance of female arcsines was due to maternal grade ($F = 3.02$, d.f. = 3 and 372, $.01 < P < .05$). For the comparison of daughters of "small" vs. "medium" parents, $t = 2.20$; "small" vs. "large," $t = 2.36$; "large" vs. "asymmetric," $t = 2.06$—all significant at the 5% level. The close parallelism between sons and daughters strongly suggests real differences between classes of *ey* mothers, whose borderline significance has been somewhat exaggerated in daughters and depressed in sons by accidents of sampling. A paired comparison test of "medium" vs. "small" in both sexes and series groups (d.f. = 3) yielded $t = 3.30$, $.02 < P < .05$; between "medium" and "asymmetric", $t = 3.42$, $.02 < P < .05$. Productivity is highest in "medium" and "large" mothers, lowest in "asymmetric."

When all offspring eclosing during a specified period were counted (series 7–40), there was no significant relationship between productivity of *ey/ey* ♀ ♀ relative to +/*ey* ♀ ♀ and total number of sons per bottle, except that "small" females had relatively more sons when the total per bottle was low. The incidence of large and small counts was distributed at random over the classes of *ey* parents and through the early and late series (8–30 vs. 31–50).

No effect of "line breeding" after series 30 was found in sons (table 13). However, "large" parents consistently produced higher proportions of *ey/ey* daughters than

TABLE 12

Comparison of arcsin $\sqrt{\%+/ey}$ *in series 8–30 and series 31–50 mating system D, for sons and daughters*

Phenotype of *ey* parents	Offspring sex	Series 8-30			Series 31-50			S.e. of diff. of means	STUDENT'S *t*	*P*
		No. bottles	Mean % +/*ey*	Mean arcsine	No. bottles	Mean % +/*ey*	Mean arcsine			
Small	♂♂	43	33.03	34.72	55	30.69	32.95	1.631	1.087	>.05
	♀ ♀	42	32.33	34.31	55	32.74	34.69	1.290	0.297	>.05
Medium	♂♂	47	30.94	33.04	55	29.28	32.60	1.419	0.312	>.05
	♀ ♀	47	30.27	32.78	55	28.78	32.05	1.486	0.495	>.05
Large	♂♂	32	31.92	33.96	54	29.23	32.47	1.573	0.949	>.05
	♀ ♀	32	30.75	33.18	54	27.92	31.46	1.737	0.986	>.05
Asymmetric	♂♂	37	34.62	35.86	53	30.86	33.08	1.733	1.605	>.05
	♀ ♀	37	34.66	35.66	54	31.47	33.68	1.699	1.167	>.05
Pooled	♂♂	159	32.56	34.33	217	30.01	32.77	0.795	1.957	≃.05
	♀ ♀	158	31.95	33.93	218	30.23	32.97	0.779	1.233	>.05

TABLE 13

Effects of "line breeding" on productivity of females

Series	Line	Offspring sex	Phenotype of *ey* parents								Total	
			Small		Medium		Large		Asymmetric		No.	Mean
			No.*	Mean**	No.	Mean	No.	Mean	No.	Mean		
27, 31, 35, 39,	*a*	♂♂	15	32.90	15	31.89	15	32.89	15	34.93	60	33.15
43, 47		♀♀	15	35.19	15	31.65	15	29.26	15	33.65	60	32.44
28, 32, 36, 40,	*b*	♂♂	16	35.61	16	33.64	15	33.18	15	38.19	62	33.14
44, 48		♀♀	16	35.45	16	34.98	15	32.34	16	39.05	63	35.50
29, 33, 37, 41,	*c*	♂♂	16	31.24	16	32.76	16	31.46	15	33.20	63	32.15
45, 49		♀♀	16	34.00	16	31.98	16	32.84	15	33.27	63	33.02
30, 34, 38, 42,	*d*	♂♂	15	32.83	16	33.36	16	33.10	15	29.00	62	32.11
46, 50		♀♀	15	34.19	16	31.00	16	31.85	15	29.14	62	31.54
Total		♂♂	62	33.15	63	32.93	62	32.65	60	33.83	247	33.13
		♀♀	62	34.71	63	32.42	62	31.60	61	33.86	248	33.14

* Number of bottles.

** Mean of arcsines of the square roots of the percentage of $+/ey$ offspring.

did "small" parents. The daughter difference between lines borders on significance at the 5% level, suggesting genetic differences established during line breeding. The lack of "line-by-phenotype" interaction indicates less divergence in female than in male fertility modifiers. Marked differences between lines existed only for "asymmetric" where $F = 5.99$, d.f. $= 3$ and 57, $P < .01$.

The formula for the productivity coefficient t^{φ} of females must be modified as follows for maternal influence on male viability:

Mother genotypes:	$+/ey$	ey/ey
Offspring zygotes	$1\ +/ey{:}1\ ey/ey$	$(2 + 2t)\ ey/ey$
Offspring adults:	$1\ +/ey{:}(1 + s_B)ey/ey$	$(2 + 2t)(1 + s_A)ey/ey$

$$\text{or, } \frac{1}{4 + (2s_A + s_B) + 2t(1 + s_A)} + /ey{:}\ \frac{3 + (2s_A + s_B) + 2t(1 + s_A)}{4 + (2s_A + s_B) + 2t(1 + s_A)}\ ey/ey$$

where s_A refers to ey/ey offspring of ey/ey mothers and s_B, ey/ey offspring of $+/ey$ mothers. The proportion of adults appearing wild type is

$$y = \frac{1 + a'[3 + (2s_A + s_B) + 2t(1 + s_A)]}{4 + (2s_A + s_B) + 2t(1 + s_A)},$$

where a' is the proportion of ey/ey flies overlapping wild type. Then

$$t^{\varphi} = \frac{1 + a'[3 + (2s_A + s_B)] - y(4 + s_B + 2s_A)}{2(1 + s_A)(y - a')}$$

$$= \frac{1.012261 - y(4 + s_B + 2s_A)}{2y(1 + s_A) - .008174}.$$

Table 14 presents t^{φ} for each class of *ey* mother. All were more productive in the later series; STUDENT's t for the paired comparisons between early and late is 3.28,

TABLE 14

Female productivity coefficients $t^{♀}$ *based on data from sons and daughters*

Phenotype of *ey* female	Offspring sex	Series 8-30			Series 31-50		
		$t^{♀}$	95% conf. limits*		$t^{♀}$	95% conf. limits	
			Upper	Lower		Upper	Lower
Small	♂♂	−.323	−.105	−.500	−.201	+.047	−.403
	♀♀	−.211	+.011	−.395	−.234	−.080	−.369
	Pooled**	−.269			−.224		
Medium	♂♂	−.210	+.062	−.425	−.111	+.026	−.233
	♀♀	−.079	+.191	−.299	+.021	+.256	−.175
	Pooled	−.145			−.076		
Large	♂♂	−.265	+.006	−.478	−.108	+.080	−.269
	♀♀	−.111	+.247	−.386	+.087	+.340	−.124
	Pooled	−.208			−.037		
Asymmetric	♂♂	−.401	−.179	−.577	−.212	+.041	−.417
	♀♀	−.341	−.080	−.548	−.150	+.070	−.348
	Pooled	−.376			−.183		

* Based on the standard error of the mean arcsine. The mean percent of $+/ey^4$ offspring was transformed into an arcsine; 2σ was added or subtracted. The resulting arcsine was transformed back into a percentage, from which the corresponding upper or lower value of $t^{♀}$ was computed.

** Obtained by weighting the estimates based on sons and on daughters by the reciprocals of the squared differences between the appropriate pairs of 95% confidence limits.

d.f. = 3, $.02 < P < .05$. The son and daughter estimates of $t^{♀}$ agree better for "small" and "asymmetric" than for "large" and "medium." The productivity of the last two appears greater when daughters rather than sons are considered; STUDENT's t of the paired comparisons between sons and daughters is 3.97, d.f. = 7, $.001 < P < .01$. Perhaps the rate at which "medium" and "large" females lay eggs increases relative to $+/ey$ during the first days of oviposition. Throughout the experiment, daughters eclosed earlier than sons. The eggs developing into sons may be laid earlier than those developing into daughters included in the same count. (Any difference in development rate between ey/ey and $+/ey$ would be subsumed in s and would not affect t.)

In series 8–30, ey/ey ♀♀ ranged from 62% to 86% as productive as $+/ey$ ♀♀ (using the pooled estimates); in series 31–50, from 78% to 96% as productive.

The circus movements of "asymmetric" females may hinder oviposition. Otherwise, there is no *a priori* reason for presuming that female eye size itself influences either copulation or oviposition. Possibly, the smaller optic ganglia in the small-eyed fly might impair egg-laying behavior—as in choosing an oviposition site. Any other correlation between productivity and eye size must result from a less direct effect of the *ey* allele, or from the segregation of modifiers acting early in the sequence of events initiated by *ey*. When the *ey* locus acts to influence eye size is not known, but the optic disk is smaller in ey/ey than in normal from the beginning (CHEN 1929). The temperature-effective period of *eyeless* (ey^2) extends from before hatching until the optic disk is differentiated (BARON 1935). Eye size can be affected as early as

four hours after the egg is laid, two hours after blastoderm formation (HOWLAND and CHILD 1935), though not before the maturation divisions of the maternal nucleus (HOWLAND and SONNENBLICK 1936). Nevertheless, how much optic disk material will form facets is not completely determined until puparium formation (STEINBERG 1944).

DISCUSSION

Unless modifiers independently alter eye size and viability or productivity in the *ey/ey* fly, the consequences of differential viability or productivity of different phenotypic classes of a single genotype can be predicted. It has been assumed here that the + allele is fully dominant, and, further, that the selection coefficients are independent of the frequency of the major gene (in this case, of *ey*). When there are different viability coefficients for the various phenotypic classes of the recessive genotype, the net viability coefficient for one sex is the weighted average of the coefficients of the different classes; for the genotype as a whole, $\bar{s}$, the unweighted average of the coefficients for the two sexes. The same methods of averaging apply to the productivity coefficient, t. In a random-breeding population, the change in the zygotic frequency of the recessive allele in one generation is given by the approximate expression

$$\delta q = q^2(1 - q)(\bar{s} + \bar{t}),$$

if $\bar{s}$, $\bar{t}$ and q are small enough that their products are negligible.

Thus, any situation which increases either $\bar{s}$ or $\bar{t}$—i.e., decreases their negative value—will favor a slower decline, or an increase, in the proportion of recessive individuals. Modifiers which increase those categories of recessive individuals with higher values of either s or t will be selected for so long as there is an appreciable proportion of recessive individuals. The speed with which such modifiers accumulate can be judged from the various indices of segregation which in the present experiment rose from zero at the beginning to a significant extent in series 8–20 and jumped markedly in the 57-day interval between series 20 and 21.

Eye size in the *ey/ey* fly seems to have no effect on the viability coefficient since the proportion of *ey/ey* progeny did not depend on the *ey* parents' phenotype in mating systems A and B, although the average eye grade of progeny did depend on it. Modifiers of *ey* can therefore be expected to derive their greatest selective advantage from improving male productivity by increasing eye size. Modifiers directly affecting productivity independently of eye size, but as a part of the optimal complex of polygenes in the *ey/ey* genotype, undoubtedly contributed to the general increase in male productivity in the latter half of the experiment.

SUMMARY

Whether a deleterious recessive gene of variable expression is subjected to selection the intensity of which is related to the phenotypic departure from wild type was tested for the ey^4 allele. Selection was analyzed into two components: viability and productivity. Two stocks initially differing only in fourth chromosomes were the source of the ey^+ and ey^4 alleles.

1. The isogenic strain of *ey*^{4} gradually accumulated an abundant supply of modifiers for eye size, as shown by greatly increased variance, the midparent-offspring correlation rising from an initial value of zero to .20 in the first quarter and to .46 in the last quarter of the experiment, and the correlation between right and left rising from zero to .25 and then to .46 in these periods.

2. Eyeless males were at a selective disadvantage of 13% in viability; females, 18%, in both cases independently of eye size of *ey* parents—either mother or father. The viability of eyeless flies can not be affected by their own eye sizes in view of the parent-offspring correlation. There is a slight maternal effect on the viability of eyeless males.

3. Eyeless males were at a severe disadvantage when competing with $+/ey$ ♂♂ to leave offspring. Small-eyed males were at a greater disadvantage (60% in the first half, 35% in the second half of the experiment) than medium-eyed males (44% in the first half, 0% in the second), while asymmetric males were intermediate, probably a direct result of poor vision. Larger-eyed males are in some lines at a greater, and in other lines at a less, disadvantage than medium-eyed males. In general, the larger-eyed males were superior to other *ey* males in the first half but inferior in the second half of the experiment, probably because modifiers were on the whole selected for adjustment to the "medium" type in the breeding system followed in the latter.

4. Eyeless females were at some productive disadvantage to $+/ey$ ♀♀ (ca. 20%). Small-eyed and asymmetric females were at a greater disadvantage than medium-eyed and large-eyed females. There was also improvement during the course of the experiment. All of these effects are less striking than in the case of male productivity. They may reflect more indirect pleiotropic effects of *ey*^{4} and its modifiers.

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