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Quantitative inheritance of red eye pigment in *Drosophila melanogaster*

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1. INTRODUCTION

Genetic variation in *Drospohila* eye pigments has provided classical material for studies on gene action (Beadle & Tatum, 1941) and, more recently, for numerous studies of biochemical differences caused by changes at many loci in *Drosophila melanogaster* (Hadorn & Mitchell, 1951; Forrest & Mitchell, 1954*a*, *b*; Viscontini & Möhlmann, 1959; Zeigler, 1961, etc.). Chemical study of the usual eye pigments has shown that they are composed of two distinct types (Mainx, 1938), comprising the brown pigments, which are ommochromes (Becker, 1942), and the red pigments which are pteridines. The normal red pigment in *Drospohila* is made up of three closely related compounds known as drosopterins (Hadorn & Mitchell, 1951; Zeigler, 1961). Various additional pteridine compounds which normally accompany the drosopterins are believed to be either precursors or to be less directly related to them. Such compounds can be identified and estimated by virtue of their different R_F values when chromatographed, and also their property of fluorescing at different wavelengths.

Almost all this work has dealt with clearly defined changes, generally at single loci. But there have been a few reports suggesting that polygenic variation in pigment level may be extensive and thereby merit closer attention. Thus Nolte (1955) reported quantitative differences in red and brown pigments between strains of *D. melanogaster* and Honigsberg, Chejne & Castro (1964) have provided similar evidence. Goldschmidt (1954) clearly demonstrated the effect of genetic background on the ratio of pteridine compounds. Robertson & Forrest (1957) reported quantitative variation in isoxanthopterin content, one of the fluorescent pteridine compounds, between various inbred lines of *D. melanogaster*.

If polygenic variation is indeed extensive, and if selection can create substantial differences in pigment content, such biometrical evidence will have a dual significance with respect to the chemistry of pigment production on the one hand, and the properties of polygenic variation on the other, with, perhaps, the possibility of establishing interrelations between the two. The present paper is concerned with a survey of quantitative variation for red eye pigment and the biochemical evidence will be dealt with later.

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2. MATERIAL AND METHODS

(i) *General*

All experiments were carried out on a long-maintained cage population of *Drosophila melanogaster*, known as Pacific, or on three inbred lines, referred to as P_2, P_4 and P_7 which were started from the parent population at the same time. Flies were grown on the usual cornmeal–molasses medium fortified with dried yeast, and the numbers of individuals cultured per vial were such as to minimize variation in body size due to crowding.

Since the quantity of pigment may be expected to vary intrinsically and also to be influenced by the size of the eye and/or the size of the body, both eye and body size were scored to determine genetic and environmental correlations between pigment content and these traits. Thorax length and eye width were chosen as suitable measures of body and eye size. The measurements were carried out on live flies with the aid of a micrometer eyepiece and an adjustable platform fitted to the moving stage of the microscope (Robertson & Reeve, 1952). Only the right eye was measured and, except in selection lines, only females were scored. Unless otherwise stated all tests were carried out at 25° ± 0·5°C.

(ii) *Pigment extraction*

The method recommended by Ephrussi & Herold (1944) was used to extract red eye pigment. This involves extraction of heads from which the clypeus and proboscis have been removed, for 24 hours at 25°C. with 30% ethanol acidified to pH 2 with

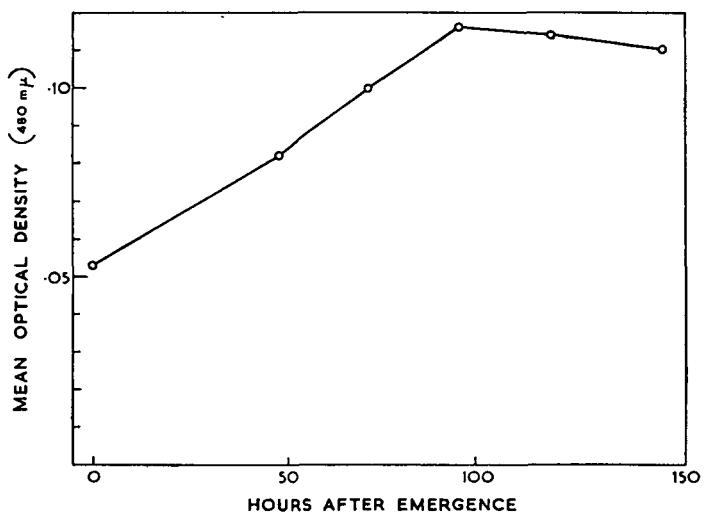

Fig. 1. The change in pigment content after eclosion.

concentrated hydrochloric acid. Absorption was then recorded in 1 or ½ cm. cuvettes in a Beckman spectrophotometer at 480 mμ which was found to be the wavelength of maximum absorption as recorded by Nolte (1952*a*, *b*). The relation between absorption and concentration was checked by extracting ten heads in 1 ml. and

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serially diluting the extract to $\frac{1}{32}$ of the original concentration. The relation was found to be entirely linear over this range, which exceeds that in the various tests described later. There was no evidence that any further pigment could be removed by further extraction after 24 hours at 25°C. It is important that flies are aged for at least 4 days before extracting pigment, which continues to increase for 2 to 3 days after eclosion, as shown in Fig. 1. All the flies studied in the present tests were aged for at least 4 days. Pigment content is generally expressed in terms of optical density.

(iii) *Selection procedure*

Initially nothing was known of the properties of genetic variation in pigment content, and so both family and simple phenotypic selection for high and low pigment was carried out. The procedure was as follows.

(*a*) *Family selection*

Fifty males and fifty virgin females were drawn from the Pacific population and randomly mated in pairs. From each progeny males and virgin females were collected. Five females were scored individually for pigment content, thorax length and eye width and the score for pigment was taken as the ratio of optical density to eye width. The average of the five ratios for each family provided the measure of performance per family and was used to identify the three extreme high and three extreme low families which were referred to respectively as HA, HB, HC and LA, LB, LC. From each of these families eight males and eight females were drawn at random and these were mated in pairs according to a cyclical mating scheme designed to minimize inbreeding, similar to that used by Robertson & Reeve (1952). A males were mated randomly to B females to give offspring denoted A, B males were mated to C females to give offspring denoted B, and C males were mated to A females to give offspring denoted C. Thus for both the high and the low lines there were twenty-four matings.

In each successive generation the single extreme culture in the A, B or C group based on the average scores of pigment to eye-width ratio for five females, was retained to provide eight pairs of flies which were then mated according to the cyclical scheme just described. The procedure therefore involves family selection with minimization of inbreeding and also minimal selection for eye or body size rather than for differences in intrinsic pigment content.

(*b*) *Phenotypic selection*

Here 100 males and virgin females were drawn from the Pacific population and mated in pairs at random to provide 100 families. After the cultures had taken, the parents were removed from each and their heads extracted together without measurement of eye width. Only progeny of the highest or lowest three pairs were taken to establish the high and low lines and, from each such progeny group, ten pairs of virgin flies were drawn at random and combined with the other two sets of ten pairs to give thirty random pair matings. After the cultures had taken the parents were scored and all progeny other than those from the extreme three pairs were discarded, and so on in successive generations.

3. RESULTS

(i) *Independent and correlated variation*

To determine how far the size of the eye and of the body influence pigment content per fly, inter- and intrapopulation comparisons have been carried out; the latter will be described first.

Table 1. *Comparison of average pigment content, eye size and body size in eight wild stocks*

Stock	Optical density	Eye width (1/100 mm.)	Thorax length (1/100 mm.)
Gabarros	0·066 ± 0·002	39·1 ± 0·30	104·1 ± 1·30
Pavia	0·085 ± 0·004	39·8 ± 0·15	105·9 ± 0·40
Pacific	0·088 ± 0·001	40·3 ± 0·10	106·4 ± 0·21
Caribbean	0·092 ± 0·003	40·6 ± 0·27	103·6 ± 0·80
Kaduna	0·093 ± 0·002	39·5 ± 0·20	103·0 ± 0·40
Holte	0·094 ± 0·002	39·6 ± 0·12	107·1 ± 0·37
Sao Paolo	0·098 ± 0·001	40·8 ± 0·18	108·7 ± 0·60
Renfrew	0·109 ± 0·004	41·8 ± 0·28	109·1 ± 1·20

(a) *Wild populations*

Eight strains derived from widely separated localities, and kept generally for many generations under laboratory culture, were grown under optimal conditions and scored for pigment content, eye width and thorax length. For each strain ten females were scored from each of three cultures. Standard errors were estimated from within- and between-culture components of variance; between-culture effects were generally quite insignificant, as is generally found to be the case in all our tests. Table 1 shows the means for the three traits, tabulated in order of increasing optical density per fly. Clearly the pigment content is influenced by the size of the fly. However, the multiple regression analysis, set out in Table 2, shows that 70% of the sums of squares due to variation between the strains is accounted for by correlated changes in eye size and no further significant reduction in variance is

Table 2. *Regression analysis for optical density in relation to eye and body size in wild stocks*

Source of variance	d.f.	Sum of squares	Mean squares
Between stocks	7	1056	151
Linear regression of optical density on eye width	1	673	673**
Multiple regression of optical density on eye width and thorax length	2	681	340·5**
Difference	1	8	8
Residual	5	375	75**
Error (within genotype)	16	42	3

** indicates significance at the 0·01 level of probability.

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effected by holding thorax length constant. The residual variance of pigment content between the means of the strains is highly significant when tested against the error variance derived from the pooled between-culture intra-line variance.

(*b*) *Intrapopulation variance*

The most direct way to estimate the intrapopulation variance is to compare the variation of genetically heterogeneous flies from the wild population with that of genetically uniform individuals grown under similar conditions, provided they react to environmental variation in the same way as the wild flies. The F_1 of crosses between inbred lines provides suitable material for estimating environmental variance (σ_e^2), since there will be no genetic segregation and hence we can subtract the variance from that of the wild populations ($\sigma_g^2 + \sigma_e^2$) to estimate σ_g^2, the purely genetic contribution, assuming that genetic and environmental components combine additively. This

Table 3. *Comparison of average pigment content, eye size and body size in Pacific wild population, inbred lines and crosses between inbred lines*

Genotype	Optical density	Eye width (1/100 mm.)	Thorax length (1/100 mm.)
Pacific population	0·088 ± 0·001	40·3 ± 0·10	106·4 ± 0·21
Inbreds:			
P_2	0·088 ± 0·002	41·0 ± 0·28	111·0 ± 0·91
P_7	0·067 ± 0·002	39·1 ± 0·41	105·7 ± 0·91
P_4	0·008 ± 0·002	40·7 ± 0·27	100·7 ± 0·85
Crosses:			
$P_2 \times P_7$	0·087 ± 0·001	39·9 ± 0·16	109·1 ± 0·80
$P_2 \times P_4$	0·084 ± 0·002	40·9 ± 0·14	107·0 ± 0·65
$P_4 \times P_7$	0·064 ± 0·001	40·4 ± 0·10	104·1 ± 0·66

procedure has been shown to provide meaningful estimates for other characters in *D. melanogaster*, such as body size, egg production, development time etc. (Robertson, 1957) and it is equally applicable in the present instance. Before applying the method we must first be satisfied that the genotypes used to estimate σ_e^2 are suitable. Inbred lines are not, since they are generally more subject to environmental variation. Crosses between such lines generally return to the mean level of the foundation population for characters subject to inbreeding decline. In the present case, in the inbred lines P_7 and P_4 there is a substantial reduction in pigment content, independent of eye size, below the normal level, while in P_2 the pigment content is about the same as in the foundation stock (Table 3). Two of the crosses, $P_2 \times P_7$ and $P_2 \times P_4$ clearly resemble the latter, while the third, $P_7 \times P_4$, has a lower level, and so this cross was not used to provide estimates of σ_e^2, which is thus derived from the pooled within-culture variation for the other two crosses.

Table 4 shows the partition of variance into genetic and environmental components, while Table 5 shows the further partition of the variance of eye pigment into independent and correlated components, which are estimated from the genetic and environmental components of variance and coveriance for optical density and eye width.

Table 4. *Analysis of variance of optical density, eye width and thorax length in the wild population*

		Variance in terms of individuals			Correlation coefficients	
Source of variation	d.f.	$\sigma^2_{O.D.}$	$\sigma^2_{E.W.}$	$\sigma^2_{T.L.}$	O.D./E.W.	E.W./T.L.
Variable population	136	60·0	87·8	710·9	0·51	0·74
Uniform F_1	72	24·0	43·6	337·9	0·52	0·66
Difference (genetic)	—	36·0	44·2	373·0	0·51	0·81
Percentage genetic	—	60·0	50·4	52·5	—	—

O.D., E.W. and T.L. refer respectively to optical density, eye width and thorax length—variances have been multiplied by 10^6 to eliminate zeros.

The tables indicate that more than 70% of the genetic or environmental variance of pigment content is independent of eye size and also that, of the total variation of pigment content independent of eye size, about 60% can be attributed to genetic segregation. This value is rather similar to the corresponding estimates for eye width and thorax length; the latter agrees well with earlier estimates for this trait in other wild strains (Robertson, 1957). The non-genetic variation will include uncontrollable variation in environment, even under favourable conditions, developmental 'noise', errors of estimation etc. The high level of genetic variation in the wild population is quite compatible with the inter-strain differences already noted.

Table 5. *Correlated and independent variation of pigment content*

Variance	Genetic	Environmental
Correlated with eye size	9·4	6·5
Independent	26·6	17·5
Ratio: independent/total	0·78	0·73

Variances are multiplied by 10^4.

(ii) *Temperature and nutritional differences*

The foregoing comparisons were made at 25°C. under optimal nutritional conditions. We need to know whether major environmental differences affect the relation between pigment content and eye and body size, and for this purpose flies were cultured at different levels of crowding and at different temperatures.

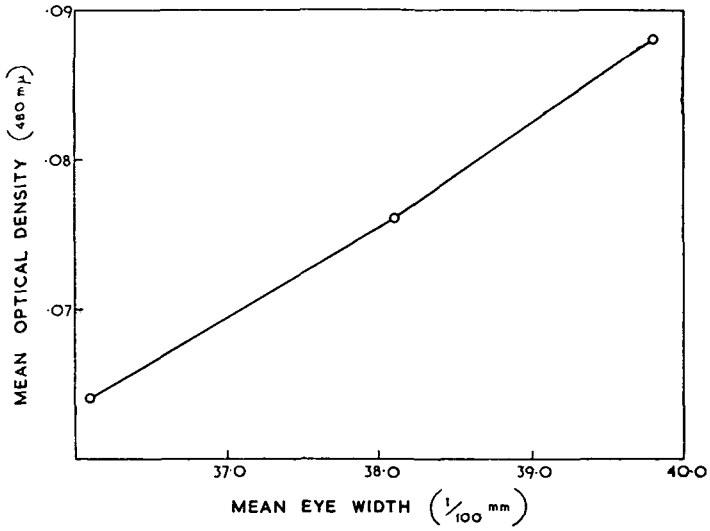

Fig. 2. The relation between pigment content and eye size in flies cultured under different conditions of crowding.

(*a*) *Crowding*

Figure 2 shows the relation between average optical density and eye width, while Table 6 shows the regression analysis for each level of crowding, which extended from 70 to 300 individuals per vial. There is evidently a very high correlation between eye size and pigment content. At 70 and 150 eggs per vial correlated variation in optical density is entirely accounted for by the regression on eye width since the effect of holding thorax length constant is negligible. But at 300 eggs per vial, in comparatively small-sized flies, there remains a significant regression on body size, after correlated changes in eye width have been allowed for. The reason

Table 6. *Regression analysis of pigment content in flies grown at different levels of crowding*

		70 eggs/vial		150 eggs/vial		300 eggs/vial	
Source of variation	d.f.	Mean square	*b*	Mean square	*b*	Mean square	*b*
Total	21	55	—	35	—	16	—
Linear regression on eye width	1	245	0·42*	57	0·25	67	0·15*
Joint regression on eye width and thorax length	2	130	—	29	—	55	—
Difference	1	15	—	2	—	42*	—
Residual	19	47	—	35	—	12	—

b refers to the linear regression of pigment content on eye width.
* indicates significance at the 0·05 level of probability.

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for this is unknown but it could be due to changes in eye shape which make the simple measure of eye width a less accurate measure of eye volume or surface area in small flies.

It will also be noted that the regression of pigment content on eye width is progressively lower at higher levels of crowding, i.e. 0·42, 0·25 and 0·15 for 70, 150 and 300 individuals per vial, respectively. A possibly relevant feature is shown in Table 7, which includes total and independent variance of optical density, the independent and correlated variance of eye width and also the variance of thorax length. To allow for the differences in mean, values are expressed as squared coefficients of variation. As usual, increased crowding leads to greater variability of body size, reflected in scores for both eye and thorax. The variance of eye width, independent of thorax length is not affected by the increased level of crowding, since the values for the squared coefficients work out at respectively 3·0, 3·0 and 2·9, for progressively higher levels of crowding. But the independent variation of pigment content shows a remarkable reduction from 65 to 12 when the number of individuals per vial is increased from 70 to 300. Apparently, therefore, culture under conditions of food shortage leads to a reduction in the effects of segregation and other causes of independent variation in pigment. This cannot be attributed to inadequate detection of pigment, since, even in the smallest individuals, the pigment concentration falls well within the range of the linear relationship between optical density and pigment level.

Table 7. *Squared coefficients of variation of pigment content, eye size and thorax length at different levels of crowding*

Variation	70 eggs per vial	150 eggs per vial	300 eggs per vial
Pigment			
Total	77·4	46·2	38·4
Independent of eye width	64·0	43·6	11·6
Eye width			
Total	4·0	4·0	10·2
Independent of thorax length	3·1	3·1	2·9
Thorax length	3·2	5·3	13·0

(*b*) *Temperature*

In this experiment flies of the Pacific population and of the three inbred lines were grown under optimal conditions at 18 and 25°C. Table 8 shows that at the lower temperature flies reach a larger size, although there is evidence of differences between the inbred lines in this respect; P_2 is most and P_7 least affected by the change. Differences in eye size show parallel changes, but in no case is the difference in optical density significant statistically. Hence, variance sufficient to cause 20–30% increase in body size leaves pigment content virtually unchanged.

Table 8. *The effect of growth at different temperatures on average pigment content, eye size and body size*

	Optical density			Eye width (1/100 mm.)			Thorax length (1/100 mm.)		
Genotype	18°C.	25°C.	D	18°C.	25°C.	D	18°C.	25°C.	D
Pacific population	0·090	0·088	0·002	41·4	40·6	0·8**	112·5	107·1	5·4**
P_2	0·085	0·084	0·001	40·6	40·4	0·2	113·4	107·3	6·1**
P_4	0·010	0·009	0·001	41·2	40·5	0·7**	104·4	100·3	4·1**
P_7	0·076	0·073	0·003	39·6	39·4	0·2	108·3	106·6	1·7**

D refers to the difference between the temperatures.
** Indicates the significance at 0·01 level of probability.

Comparison of the evidence from crowding and temperature changes indicates that, provided optimal nutritional conditions are supplied, pigment content per individual is stable for a given genotype, and the virtual absence of between-culture effects is consistent with this conclusion.

Table 9. *Comparison of average pigment content, eye size and body size in lines selected for differences in body size*

				Percent deviation from expected	
Genotype	Thorax length (1/100 mm.)	Eye width (1/100 mm.)	Optical density	Optical density	Eye width
SB	102·2 ± 0·31	38·5 ± 0·28	0·080 ± 0·002	+5·3	−1·0
Unselected	106·8 ± 0·22	40·2 ± 0·21	0·084 ± 0·001	—	—
LA	108·3 ± 0·22	41·0 ± 0·29	0·085 ± 0·003	−3·4	+1·0
LB	109·9 ± 0·37	41·2 ± 0·15	0·093 ± 0·002	+4·5	+0·3
LG	110·3 ± 0·51	40·8 ± 0·41	0·102 ± 0·002	+17·2	−1·0
LC	111·8 ± 0·35	40·7 ± 0·24	0·097 ± 0·003	+12·8	−2·0

The expected values are derived from the estimated genetic regression, in the Pacific wild stock, of optical density on eye width and of eye width on thorax length.
LA, LB, LG and LC refer to lines selected for large body size on different diets, SB refers to line selected for small body size.

(c) *Changes in body size*

If the genetic correlation between pigment content and eye size is additive we expect that genetic changes in body size will involve corresponding changes in eye width and pigment content. This was tested by comparing the pigment content, eye width and thorax length, in the unselected population and in several lines selected for large or small body size. The selection procedure for these lines, which have been selected on various axenic diets, has been described elsewhere (Robertson, 1964). In the largest line the flies were some 40% larger than in the small line, and the total range of body size was of the same order as in the extremes of the series grown at different levels of crowding.

The comparisons of genetic and environmental variation in Table 4 enable us to compute the genetic regression coefficients of pigment content on eye width and of eye width on thorax length from the differences in variance and covariance between the genetically variable population and the genetically uniform F_1; the coefficients are respectively 0·46 and 0·28. From the appropriate regression equations we can estimate the expected value of eye width from a genetically determined change in thorax length and also the expected value of pigment content from a given difference in eye width. The values of the optical density and eye width expected for each of the selected lines, by extrapolation from the relations in the foundation population, have been compared with the actual values and the percentage deviation from the expected values are shown in Table 9.

For eye width there is excellent agreement between observed and expected values; none of the difference exceeds 2%. For pigment, however, the deviations from expectation are much less consistent. Thus the lines SB, LA and LB show quite good agreement with expectation since the differences are in the range 3–5%, but in lines LG and LC the pigment content is respectively 17 and 13% higher than expected—deviations which are several times greater than the within-culture standard deviation. Such differences indicate independent genetic control of pigment production, or, possibly pleiotropic effects of the particular genetic changes which have been prodcued by selecting for body size on different diets.

(iii) *Selection*

The response to selection, expressed as a percentage deviation from the average value of the unselected stock, is shown in Figs. 3 and 5, while Figs. 4 and 6 show the total percentage deviation is optical density between the high and low lines. In view

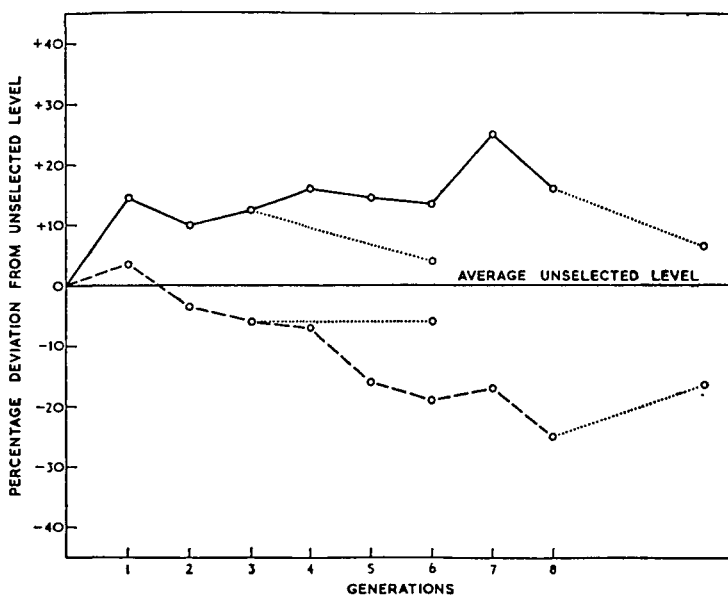

Fig. 3. The response to family selection for high and low pigment/eye-width ratio. The dotted lines indicate the effect of relaxing selection.

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of the labour, controls were scored every second or third generation, and, since the means agreed closely, they have been averaged to provide the base line from which the selection responses are measured. The response to selection over a period of

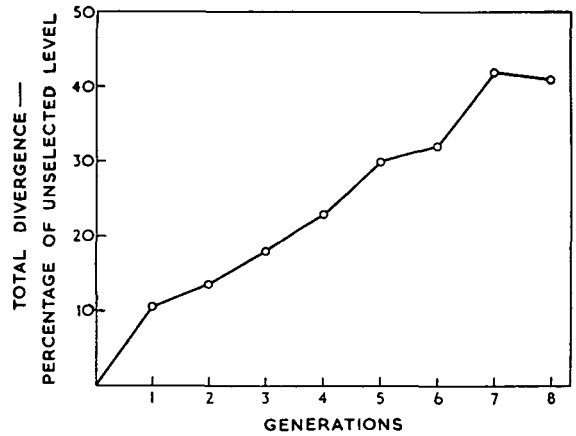

Fig. 4. The divergence between lines derived by family selection for high and low pigment/eye-width ratio.

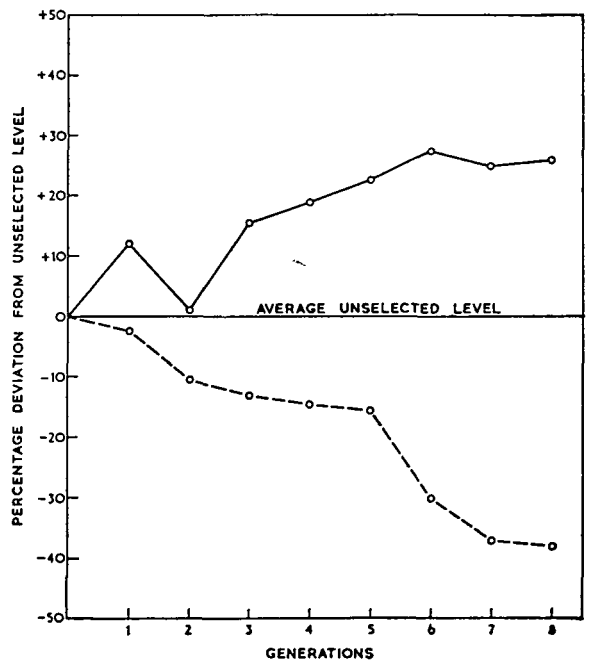

Fig. 5. The response to phenotypic selection for high and low pigment content.

eight generations was similar with both family and phenotypic selection. In the high line of the family selection series there was a 14·5% increase in generation 1 and little evidence of further change thereafter; the higher value at generation 7 is probably not of genetic origin, since the value declined again at generation 8. Selection for low pigment led to a steady decline over the entire period and there

was no evidence that the response has ceased by generation 8, by which time the pigment content had declined by about 25%. Thus, after eight generations, the two lines differed by about 40% in total pigment content (Fig. 4).

In the phenotypic selection lines, the response was higher than in the family series, and in the high line the final value after eight generations was about 26% greater than in the unselected (Fig. 5). In the low line, response continued over the entire period of the experiment; after generation 5, the rate of response apparently increased. By generation 8 the high and low lines differed by about 54% (Fig. 6). Thus there is well-defined asymmetry in the response to selection for high- and low-line pigment content in both experiments.

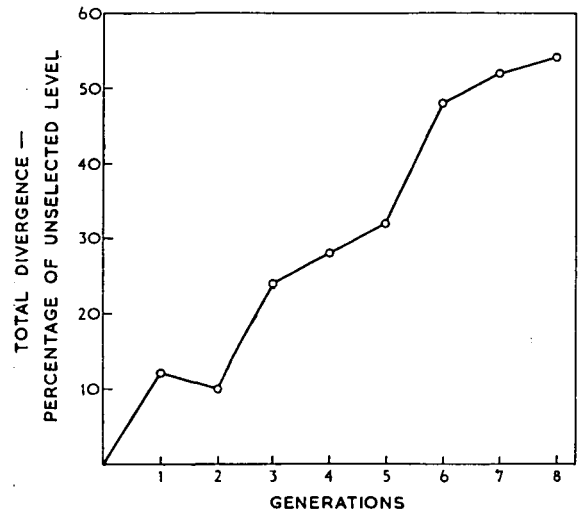

Fig. 6. The divergence between lines derived by phenotypic selection for high and low pigment content.

The data from the phenotypic selection experiment can be used to provide an estimate of the additive genetic variance, which can be compared with the earlier estimates of the total genetic variance of pigment content. For this purpose only the early generations of selection are relevant, since the longer selection is carried on the greater the divergence in genetic composition between original population and selected lines. Using the regression of response on accumulated selection differential for the first four generations, the heritability works out at $0{\cdot}26 \pm 0{\cdot}04$, implying that about one-quarter of the phenotypic variance is additively genetic. It will be recalled that the earlier estimate of total genetic variance was about 0·60 and so, within the limitation of these rather crude comparisons, about half the genetic variation behaves as additive.

In the family selection lines selection was relaxed in generations 3 and 8. The flies were allowed to mate at random for three generations and then compared with the unselected population. In the high line there was a decline from an initial value about 16% greater than the controls, to a value which was only 6% greater. In the

low line relaxation of selection had less effect, although in the later generations, the line tended to regress somewhat towards the unselected levels.

Variability was also followed during selection. There was no evidence of any systematic trend in variation in any of the lines except with family selection for high pigment content. Here the coefficient of variation between the means of the families fell progressively from 9·4 at the beginning to 3·2 at generation 8. There is little doubt that this extraordinary decline in between-family variation and the lack of evidence for progress in selection, are interrelated.

Comparison of eye width and thorax length in the selected lines in generation 8 are shown in Table 10. Although in the phenotypic selection lines eye size was ignored, there is no evidence that selection for pigment content alone has led to any greater change in eye or body size than in the family selection, in which the ratio of pigment content to eye width was the criterion. However there is a significant tendency for the eye width to be slightly reduced in both low lines. Thorax length shows no consistent change in relation to selection, and all the lines, high and low, are significantly smaller. However, compared with the differences in pigment content, such changes in eye width and thorax length are quite trivial.

Table 10. *Comparison of average pigment level, eye width and thorax length in selected lines at generation* 8

	Percent deviation from unselected					
	Optical density		Eye width		Thorax length	
Selection	High	Low	High	Low	High	Low
Family	13·4**	−22·0**	−0·5	−4·3**	−5·2**	−1·3**
Phenotypic	26·2**	−38·1**	0·0	−4·8**	−1·4*	−2·2*

* and ** indicate significance at the 0·05 and 0·01 level of probability.

At generations 3, 4 and 6 in the family selection the high and low lines were crossed and the F_1 compared with the parent values. Thirty to forty females drawn equally from three to four vials were scored for each genotype. To minimize possible correlated effects, parents and progeny have been compared in terms of the ratio of optical density to eye width.

In each cross between high and low lines there was a significant positive deviation in favour of higher pigment content (Table 11). Thus, in generation 8, the values for high and low parents, mid-parent and F_1 were respectively 0·262, 0·170, 0·216 and 0·235, so that the deviation from intermediacy was some 9% of the mid-parent value.

At generation 8 males and females of the selected lines and also the F_1 flies from reciprocal crosses between either the two low or the two high lines were mated individually to flies of the unselected Pacific population to test for fertility. Each pair mating was left in a vial for 4 days after which the parents were removed. The Pacific parent was discarded and the parent from the selected line or cross was scored for pigment content. All the flies hatching from each mating were counted. Since

Table 11. *Comparison of optical density/eye width ratio for* F_1 *and mid-parent values in crosses between high and low selected lines (family selection)*

	Ratio optical density/eye width		
Generation	F_1 of high × low	F_1—mid-parent	% increase of F_1 over mid-parent
4	0·227	0·006*	3
6	0·235	0·017**	8
8	0·235	0·019**	9

* and ** indicate significance at the 0·05 and 0·01 level of probability.

the flies scored for pigment were derived from two to three separate cultures it was possible to test for correlation between the number of progeny and parent optical density, expressed as a deviation from the culture mean. If there were a tendency for the flies which deviated most in the direction of selection to have a lower gamete viability or gamete production, we should expect a positive correlation between optical density and progeny number in the low and a negative correlation in the high lines.

Table 12. *Sterility in selection lines*

	Males		Females	
Genotype	Number tested	% sterile	Number tested	% sterile
L_1	37	22	39	25
L_2	22	23	27	21
H_1	38	13	35	16
H_2	33	9	34	3
$L_1 \times L_2$	31	3	29	6
$H_1 \times H_2$	30	13	15	19

The numbers 1 and 2 refer respectively to family and phenotypic selection.

There was a considerable incidence of complete sterility in all lines, especially the low lines (Table 12) in which more than 20% of flies of either sex were completely sterile. Sterility was less in the larger lines, especially in the line in which selection was according to phenotype. Reciprocal crosses between the selected lines did not differ and have been combined. There was no evidence of any reduction of sterility in the high-line cross but a strong indication of improved fertility in both sexes in the crosses between the low lines.

In the records of total progeny produced from eggs laid over the 4-day period, only in the males of the two low lines was there evidence for reduced fertility. In males of crosses ($L_1 \times L_2$) fertility was restored. There was no evidence of such differences in females nor in the high lines of either sex.

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Table 13. *Progeny per test mating and correlation between fertility and pigment content*

Genotype	N	Progeny number (mean)	r	N	Progeny number (mean)	r
L_1	31	123 ± 9	$-0{\cdot}05$	28	142 ± 9	$-0{\cdot}16$
L_2	17	97 ± 13	$-0{\cdot}12$	22	139 ± 7	$0{\cdot}15$
H_1	35	129 ± 11	$-0{\cdot}07$	28	143 ± 8	$0{\cdot}20$
H_2	30	132 ± 7	$0{\cdot}20$	32	147 ± 8	$-0{\cdot}12$
$L_1 \times L_2$	29	154 ± 12	$0{\cdot}00$	27	125 ± 14	$-0{\cdot}03$
$H_1 \times H_2$	26	145 ± 15	$-0{\cdot}24$	16	153 ± 17	$-0{\cdot}14$

The numbers 1 and 2 refer respectively to family and phenotypic selection.

None of the correlations between progeny number and optical density of the tested parent was significant and, in addition, the sign of the correlations do not show a consistent trend, to be expected with an inverse relation between fertility and deviation from the mean. Thus, in the low lines, three out of the four coefficients are negative, and in the large lines two out of the four are positive.

Hence, this evidence, together with the failure to detect any association between the incidence of complete sterility and optical density, provides no support for the view that the response to selection is opposed by the lower fertility of the individuals which deviate most from the mean of the population. The sterility and also the lower fertility of low-line males would appear to be incidental and due to inbreeding and/or linked changes rather than to the changes in pigment content.

Table 14. *Viability of high and low lines; family selection*

		Percent hatch		
			Deviation from Pacific	
Culture conditions	Number of cultures	Pacific	High line	Low line
Uncrowded on usual medium	4	90	−26	−12
Crowded on usual medium	4	89	−26	−18
Uncrowded on deficient medium	4	79	−7	−5
Crowded on deficient medium	4	73	−9	−4

There is also some evidence of lower viability in the selected lines. At generation 8 of the family selection series, replicated cultures of the two lines were set up under optimal and different sub-optimal conditions. Equal numbers of eggs of the selected lines were cultured with eggs of the unselected control stock marked with the white eye mutant, which has been shown in other tests to be quite neutral under such conditions. Table 14 shows the general tendency for the hatch of adults to be lower in the selected lines than in the unselected controls, especially under uncrowded conditions. At higher levels of crowding the differences are negligible. Also the high line, which has responded less to selection, is apparently less viable

than the low line. Such differences suggest the existence of unfavourable gene-environment interaction on the part of the selected lines, probably due to changes not directly related to alteration of pigment content.

(iv) *Analysis of the inbred lines*

It was shown in Table 3 that the inbred lines differ greatly in pigment content. P_2 does not differ significantly from the unselected stock, P_7 shows 24% reduction and P_4 about 90% reduction below the level of the foundation population. Differences of this order can hardly be attributed to the general effect of inbreeding—one of the lines (P_2) shows no change—but suggest that major gene differences have been fixed in the lines P_7 and P_4. Also, since in crosses between the lines, the F_1 always coincides with the mean value for the parent with a higher pigment content, the differences behave as entirely recessive.

To analyse the situation further all possible combinations of homologous pairs of chromosomes from lines P_2 and P_4 have been constructed. Using inversions in chromosomes 2 and 3 marked by dominants, alternative combinations of markers for the second and third chromosome inversions were transferred to either P_2 and P_4 background by repeated backcrossing of marked males. Crosses between the appropriate types with different combinations of markers lead to an array of genotypes whose constitution was known by virtue of the markers present. The procedure was essentially the same as that described by Robertson & Reeve (1955). The following array of genotypes were finally produced for comparison: 222, 422, 242, 224, 442, 424, 244 and 444 in which each number refers to a homologous pair of chromosomes derived from either line 2 or line 4. Since the inversions suppress recombination there is little doubt as to the final constitution of the array of genotypes. Flies were all grown under optimal conditions and scored for optical density. The average pigment values are listed in Table 15 for the eight genotypes.

Table 15. *Comparison of the average pigment levels for genotypes constructed from homologous pairs of chromosomes derived from lines P_2 and P_4*

	Optical density		
Genotype	Observed	Expected	Deviation
222	0·082	0·081	0·001
422	0·078	0·079	−0·001
242	0·053	0·057	−0·004
224	0·036	0·038	−0·002
442	0·054	0·055	−0·001
424	0·034	0·036	−0·002
244	0·015	0·014	0·001
444	0·011	0·012	−0·001

The numbers 2 and 4 represent homologous pairs of chromosomes from lines P_2 and P_4 respectively, while the particular chromosome pair is indicated by the order of the number (I, II and III).

Expected values are derived from fitting the average effects of the chromosome substitutions derived from the least-squares procedure.

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To estimate the average effects of the substitutions of the homologous pairs of chromosomes and test for the presence of interaction the least-squares analysis described by Robertson & Reeve (1955) was used. If we represent the mean value of the genotypes 222, 422, 242, 224, 424, 442, 244 and 444 as respectively A, $A+a$, $A+b$, $A+c$, $A+a+c$, $A+a+b$, $A+b+c$, $A+a+b+c$, where A is the expected mean of P_2 (222) and a, b and c represent the average effects of substituting the chromosome derived from the first, second and third pair of chromosomes from P_4 respectively, we can solve the following equations to arrive at the estimates for A, a, b and c.

$$A = \tfrac{1}{2}\Sigma A - \tfrac{1}{4}[\Sigma a + \Sigma b + \Sigma c]$$
$$a = -\tfrac{1}{4}[\Sigma A - 2\Sigma a]$$
$$b = -\tfrac{1}{4}[\Sigma A - 2\Sigma b]$$
$$c = -\tfrac{1}{4}[\Sigma A - 2\Sigma c]$$

where ΣA equals the sum of all the genotype means, Σa equals the sum of the means of genotypes where the first pair of P_4 chromosomes are substituted, etc.

Table 16. *Average effects of substituting homologous pairs of P_4 for P_2 chromosomes*

P_4 chromosome pair	Substitution effects: Optical density	Substitution effects: Percentage of P_2
I	−0·002	−3
II	−0·024	−30
III	−0·043	−53
Interaction mean square	0·00076	
d.f.	4	
Error mean square	0·00290	
d.f.	7	

Table 16 shows the least-squares estimates of the effects of substitution of chromosome pairs I, II and III of P_4 for P_2 homologues. Evidently chromosomes II and III have major effects, while there is no evidence for any difference in the X chromosomes between P_2 and P_4. The average effects of substituting the IInd and IIIrd chromosomes of P_4 are equivalent to a reduction of respectively 30 and 53% in pigment content.

Interaction can be tested by substituting the four constants, derived from the least-squares analysis, for the types with two or more substitutions and seeing whether the variance of the deviations from the observed values is statistically significant. The sum of squares due to interaction has four degrees of freedom and is tested against the error variance of a genotype mean. To allow for the considerable differences between means, the data were transformed to a logarithmic scale for this test. The analysis set out in Table 16 shows that interaction variance is negligible. Hence, the individual effects of substituting chromosome pairs II and III combine additively. The absence of any sex-linked difference was confirmed from reciprocal

crosses between P_2 and P_4. The optical density of the males was the same, irrespective of the direction of the cross, nor was there any evidence of maternal effects in females.

(v) *F_1, F_2 and backcross*

We have already noted in Table 3 that the F_1 of the cross between P_2 and P_4 resembles the former, indicating that both the separate effects in chromosomes II and III in P_4 behave as recessive. To test the situation further, a large F_2 was raised from the F_1 and the F_1 was backcrossed to the P_4 line with the lower pigment content. If we accept the evidence that the effects of chromosomes II and III from

Table 17. *Comparison of the expected and observed average pigment levels of the* F_2 *from* $P_2 \times P_4$ *and also the backcross to* P_4

	Mean optical density		
	Expected	Observed	Difference p
F_2	0·060	0·058	$-0{\cdot}002 > 0{\cdot}3$
Backcross	0·044	0·046	$+0{\cdot}002 > 0{\cdot}3$

P_4 behave as entirely recessive to their P_2 homologues, we can use the estimates of the chromosome substitutions to predict the average pigment value for the F_2 and the backcross, in the expectation of respectively a 9:3:3:1 ratio in the F_2 or a 1:1:1:1 ratio in the backcross. The values shown in Table 17 indicate excellent agreement between observed and predicted values, providing therefore additional support for the hypothesis that the effects of chromosomes II and III are probably due to single recessive substitutions or at least to very closely linked effects.

Finally, we have evidence that the second chromosome effect in P_4 is homologous to the effect responsible for reducing pigment content in P_7. The second chromosome difference in P_4 reduces pigment content by 30%—about the same amount by which P_7 falls short of the control level—while, when P_7 is crossed to P_2, the F_1 resembles the latter, so this effect is recessive. Finally, when P_4 is crossed to P_7 the pigment level is the same as in P_7. It seems likely therefore that the same mutant gene is fixed in chromosome II of both P_7 and P_4.

4. DISCUSSION

Variation in total pigment content per individual is a function of intrinsic differences in the rate of pigment production and of variation in eye size. Different kinds of environmental change, which influence body and eye size, are not alike in their effects on the pigment content, witness the unchanged content when eye and body size were increased by growing the flies at a lower temperature and the proportional reduction in pigment content when eye and body size were diminished by growing the larvae under crowded conditions. Reduction in eye size may be due chiefly to a reduction in the number of ommatidia, while increase in eye size at lower temperatures may be due to larger ommatidium size. Alpatov (1930) and Robertson (1959) showed that the increase in wing size at lower temperatures is expressed by an

increase in cell size, and ommatidia may resemble the wing in this respect. If so, then the pigment content per ommatidium would be determined almost entirely by genotype, whatever the nature of the environmental variation, and the covariance between pigment content and eye size would be chiefly due to variation in the number of ommatidia.

The inter- and intra-population analysis suggested that under optimal conditions, about 60% of the variance in intrinsic pigment production is genetic in origin. The estimate is rather similar to the estimates for other traits in *Drosophila*, such as body size, egg production, development time etc. (Robertson, 1957). This is perhaps a little surprising since these traits, although they may be similar in this respect, have different genetic properties and respond differently to selection and inbreeding. It is possible that the apparent similarity reflects a reduction of the combined phenotypic effects of segregation, in the adapted population, to about the same level as that due to the combined effects of the irreducible environmental variation and developmental instability, which set a practical limit to how far the phenotypic effects of segregation can be effectively reduced.

The selection response was asymmetrical, recalling the results often encountered in characters which show inbreeding decline and heterosis in crosses and which contribute more or less directly to fitness. There is no reason, however, to classify pigment content in this category. Inbreeding does not necessarily lead to a decline in pigment content; in line P_2 the content is normal. It is more likely that more or less recessive alleles were segregating in the population and contributed to the selection of the lower pigment content. The crosses between high and low lines showed a substantial positive deviation from intermediacy which would be consistent with the above view. It has been well enough demonstrated, by the biochemical studies referred to earlier, that the red pigment is at the end of complex pathways with a number of steps mediated by wild-type alleles at different loci. Alleles responsible for lower or blocked activity at any of these points will be expected to diminish the quantity of the final product. The same end result can probably be effected by numerous alternative kinds of change and the substitutions responsible for reduced pigment will be expected to behave as partly or completely recessive. On the other hand, for the pigment level to be increased, none of the crucial intermediate stages must be blocked and, in this sense, increase in pigment content requires more exacting biochemical and genetic conditions. Asymmetry in the response to selection is therefore hardly surprising.

Changes in eye or body size were trivial when selection was merely for high or low pigment content. This was rather unexpected in view of the readiness with which changes in body size can be selected for (Robertson, 1959), and no reason for this particular result can be suggested. The proportionate increase in pigment level in two of the lines, selected for large body size on different diets, were about as great as that achieved by several generations of selection for high pigment content alone. If such a difference is due to chance fixation it is likely that only a few loci are involved and that their phenotypic effects are comparatively large. It is, of course, possible that changes in pteridine metabolism have been involved in the selection on

different diets and that these have secondarily involved changes in pigment content.

The chromosome analysis of the inbred lines supports the general impression that genetic differences with comparatively major effects on eye pigment content are or have been segregating at appreciable frequency in the foundation population, since otherwise it would be rather unlikely to fix the same second chromosome effect in two lines derived from different parents and also fix an additional major effect on the third chromosome. Since these substitutions, or, at least, chromosome differences, behave as entirely recessive in crosses and combine additively in chromosome interchanges, they probably influence different steps in the pathways of pigment production. The linkage relations of these differences and their biochemical effects will be dealt with in a later paper.

The high level of genetic variance, the effectiveness of selection and the lack of consistent evidence of inbreeding decline arising from non-additive interaction at many loci (Robertson & Reeve, 1955) together suggest that individual variation in pigment content may be unimportant with respect to fitness generally. In the selection experiments there was lower viability in the selected lines, partly due to a sporadic incidence of sterility and partly due to lower viability generally. There was no evidence of lower gamete production or gamete viability in the selection lines. Hence, although it is possible, we have no evidence of an inverse relation between fitness and deviation from the mean in pigment content sufficient to influence the course of selection noticeably, and such differences in viability as have been noted could be secondary rather than correlated with pigment content *per se*.

However, differences in pigment content may influence fitness under natural conditions by altering visual acuity, in which case we should need to know the relation between visual acuity and positive or negative deviation from the mean, whether such a relationship is symmetrical with respect to the direction of the deviation and also whether there is a range of variation which is more or less neutral, giving way to increasing effects of progressive change when certain limits are exceeded. Such information would shed light on whether the differences in intrinsic pigment production between the wild populations are in part adaptive or merely the result of random drift.

SUMMARY

1. The genetic and environmental variation of red eye pigment in individuals of a wild population of *Drosophila melanogaster* has been studied by extracting and measuring the pigment content of individual flies, which were also scored for eye and body size.

2. Comparison of such variability in the wild population with the individual variation in crosses between inbred lines suggested that 60% of the phenotypic variance is genetic. About 75% of both genetic and environmental variance is due to intrinsic variation of pigment content while the remainder is correlated with eye size, which shows appreciable variation, independent of general body size, as measured by thorax length.

3. Selection for high and low pigment content by both phenotypic and family selection led to 40–50% differences between high and low lines after eight generations. The response was asymmetrical and proceeded further and faster with selection for lower pigment content. Crosses between high and low lines showed positive departure from intermediacy, suggesting that more or less recessive effects had contributed to the selection for lower pigment content.

4. There was some evidence of lower viability in the selected lines but no evidence of lower fertility or gamete viability in more extreme individuals of either sex.

5. Comparison of pigment content, eye and body size at different temperatures and under different levels of crowding suggested that the pigment content per ommatidium is subject to a high degree of genetic determination.

6. The average pigment content in strains derived from widely separated localities showed substantial variation, independent of both eye and body size.

7. Inbred lines, derived from the same population, were found to differ greatly in pigment content. Crosses and the exchange of homologous pairs of chromosomes between two of these lines suggested that one or more completely recessive genes were fixed in both the second and third chromosome of one line, while the same second chromosome effect was also fixed in another line. The second and third chromosome difference reduced pigment content by respectively 30% and 50% and combined additively, judging by the effects of single and joint substitutions of homologous pairs.

8. The possibility of combining genetic and biochemical analysis of pigment content is discussed.

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