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Gene-environment interactions of the *eyeless* mutant in *Drosophila melanogaster*

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1. INTRODUCTION

Mutation at the *eyeless* locus on the fourth chromosome of *Drosophila melanogaster* causes reduction in the size of the compound eye which is sometimes associated with tandem or mirror image duplication of the antenna. The mutant phenotype shows a striking variability not only between different individuals within the same inbred line, but also between the left and right sides of the same individual. Morgan (1929) showed that the expression of *eyeless* is influenced by genetic background, since selection for more, or less, extreme expression is attended by changes in penetrance and expressivity of the mutant gene, and the importance of genetic background as a source of at least part of the variability in *eyeless* has subsequently been confirmed by Hinton (1942), Spofford (1956) and more recently Ogaki (1966).

The expression of the mutant is also influenced by environmental variables, and in his original studies Morgan noted that the first emergents from a culture bottle tend to have smaller eyes than those emerging several days later. The effect suggests that gene expression is sensitive to some change in the properties of the environment induced by the larvae themselves rather than to age-dependent changes in maternal physiology, because the progeny of older females which have been transferred to fresh culture bottles also show the effect. The first larvae to hatch in the culture bottle complete the whole or greater part of development under optimal conditions, whereas those hatching later must cope with an increasing intensity of competition for an attenuated food supply in the presence of accumulated waste products. In a search for the relevant environmental factors affecting gene expression, Sang & Burnet (1963) studied the effects of nutritional deficiencies on a highly inbred strain, $eyeless^{K}$, using aseptic culture techniques and a defined synthetic larval culture medium. They showed that a deficiency of ribonucleic acid causes an increase in eye size whereas high concentrations reduce eye size appreciably, and suggested that the culture age effect on live yeast medium may be due to reduced availability of ribonucleic acid resulting from depletion of the available yeast population.

Earlier studies of Baron (1935) and Kamshilov (1939) show that the genetic background exerts an influence on the relationship between *eyeless* expression and

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environmental variables, notably temperature and the humidity of the larval culture medium. The direction of response can be controlled by selecting for an appropriate constellation of modifier genes. Although the environmental variables which have been used in these instances are of a rather non-specific nature, the observations raise the question of how far the array of gene-environment interactions of *eyeless*^{K} described by Sang & Burnet (1963) is determined by the genetic background of the highly inbred strain used by these authors. They also suggest that the differences in the effect of environmental variables upon the expression of different *eyeless* alleles, indicated, for example, by non-correspondence between the reported temperature effective periods for *eyeless*^{2} and eyeless^K (Baron, 1935; Sang & McDonald, 1954), may be due to lack of standardization of the genetic background between strains rather than to actual differences in the properties of heteroallelic mutations at the *eyeless* locus itself.

The object of the present study is to examine whether the relationship between gene expression and availability of dietary ribonucleic acid illustrated by *eyeless*^{K} is general for other mutant alleles at the same locus, and whether there are differences in the form of the gene-environment interactions of the different alleles.

2. MATERIALS AND METHODS

(i) *Strains*

The strains used in this investigation are *eyeless* (*ey*), *eyeless*^{2} (*ey*^{2}), *eyeless*^{4} (*ey*^{4}) (Bridges & Brehme, 1944), and *eyeless*^{K} (*ey*^{K}) (Sang & McDonald, 1954). The *ey*^{K} strain was formed by combining three highly inbred *ey*^{K} lines. Each of the four strains was cultured on a yeast–oatmeal–treacle–agar medium, in mass culture for several generations before the start of the experiments. As the *eyeless* gene is located on the dot-like fourth chromosome, this entire chromosome was transferred from each of the four original strains into the genetic background of an inbred Pacific wild strain. Use was made of the multiple inversion chromosomes: SM5 marked with *Curly*, and TM3 marked with *Serrate*, which prevent crossing over in the second and third chromosomes respectively. A second chromosome carrying marker genes *B1* and L^2, and third chromosome carrying *Sb* were used as markers in males only. The resulting Pacific *eyeless* strains in which, apart from *eyeless* fourth chromosome, the whole Pacific genome including the *Y* chromosome had been reconstituted, were backcrossed three times to the inbred Pacific strain and re-isolated. They were subsequently maintained in mass culture.

(ii) *Culture*

The effect of culture age on the expression of the *eyeless* phenotype was examined from daily collections of the emerging offspring from 15 pairs of flies cultured in half-pint milk bottles on live yeast medium.

Nutritional tests were carried out using the technique for rearing germ-free *Drosophila* larvae on a synthetic diet at 25 °C as described by Sang (1956). Eight to ten replicate cultures, each containing 5 ml. of medium, and inoculated with

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50 first-instar larvae were used at each treatment level. Lecithin was replaced by choline in all media used to test the effect of cholesterol or biotin deficiencies, since commercially available lecithins normally contain appreciable quantities of these substances.

To determine the period of sensitivity to a specific dietary treatment, first-instar larvae were inoculated on to media deficient in the metabolite under test and 0·3 ml. of a sterile solution or suspension added under aseptic conditions and at successively later intervals to bring the concentration to the control or excess level. The cultures were rolled to disperse the solution. A number of cultures were set aside for determination of the mean larval development time (in $\log_{10}$ days) on the deficient medium, and the larval period and times of addition converted to a standard 96 h scale (Bodenstein, 1950).

(iii) *Quantization of the* eyeless *phenotype*

A detailed description of the variable reduction in eye size caused by the *eyeless* mutation is given by Sang & Burnet (1963). The variation in eye size is continuous in that a given eye may have any value on the phenotypic scale between total absence and a size and shape which approaches the wild type. As found by Sang & McDonald (1954), the left and right eyes tend to vary independently within individuals of an untreated population, and in the present instance a non-significant within-individual left–right correlation coefficient of +0·07 was found. The left and right sides of the head are classified separately, and groups of n individuals are treated as populations of $2n$ eyes which are assigned by a subjective classification to one of six grades similar to those illustrated by Sang & McDonald (1954). Assuming an underlying unit normal distribution, the frequency distribution can be treated as a trichotomy using any two of the thresholds between eye grades, from which the mean (m) and standard deviation (s) may be calculated in threshold units as described by Sang (1963) and Sang & Burnet (1963).

Table 1. *Facet counts for eyes selected at random from each eye grade*

Eye grade ...	0	1	2	3	4	5
No. measured	26	25	24	26	26	23
Mean facet number	0·8	83	216	301	422	570
Range	0–5	5–155	127–281	262–413	351–512	462–733

Threshold ...	t_0	t_1	t_2	t_3	t_4
Threshold facet number	5	140	270	385	487

A difficulty inherent in this method of analysis arises when two groups or populations to be compared have widely divergent means on the phenotypic scale, so that it is no longer possible to use identical threshold units for estimation of m and s. This difficulty can be overcome if the thresholds are assigned values that correspond to a real measure of eye size. It is then possible to obtain comparable measures of phenotypic expression even when the frequency distributions are widely divergent, because we are liberated from the restrictive requirement for identical thresholds in each case. Accordingly, the number of facets in eyes selected

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at random from each grade was determined using a stereomicroscope and with the aid of camera lucida drawings. From the distribution of facet numbers within each grade, the cut-off points or thresholds can be assigned facet number values as shown in Table 1.

A check on normality can be readily made by plotting the normal equivalent deviate (probit − 5, Fisher & Yates, 1953, table IX) of the cumulative frequency of eyes in each grade against each successive facet number threshold. The close approach to linearity is shown in Fig. 1. No evidence for systematic departure from linearity was found for any strain so that the assumption of an underlying normal distribution can be regarded as valid.

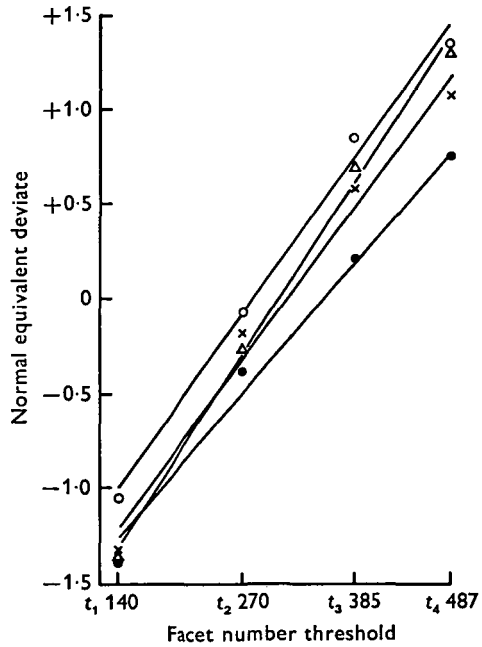

Fig. 1. The relation between successive eye grade thresholds and the normal equivalent deviate of the cumulative percentage of eyes falling below thresholds $t_1 - t_4$ for Pacific ey (●), ey^2 (○), ey^4 (×) and ey^K (△).

The method of analysis based on a gauged trichotomy follows that of Stevens (1948):

$$s = \frac{t_2 - t_1}{u_2 - u_1} \quad \text{and} \quad m = \frac{t_1 + t_2}{2} - \frac{(u_1 + u_2)s}{2},$$

where u_1 and u_2 are the normal equivalent deviates at thresholds t_1 and t_2 respectively. Symmetrically placed thresholds generally give the most efficient estimates of m and s and these have been used wherever possible.

The significance of the difference between two means estimated for different groups or populations is assessed from

$$D = \frac{m_1 - m_2}{\sqrt{[V_{(m_1)} + V_{(m_2)}]}},$$

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where m_1 and m_2 are the respective means for the groups or populations and $V_{(m_1)}$ and $V_{(m_2)}$ are sampling variances of m_1 and m_2 where

$$V_{(m)} = v_m \times s^2/n,$$

n = number of eyes classified, and

$$v_m = \frac{pqr}{\sigma^2 z_1^2 z_2^2 (u_1 - u_2)^2}\left[\frac{u_1^2 z_1^2}{p} + \frac{(u_1 z_1 - u_2 z_2)^2}{q} + \frac{u_2^2 z_2^2}{r}\right],$$

where z_1 and z_2 = ordinates of the normal distribution for u_1 and u_2 respectively and p = proportion of eyes lying below t_1, q = proportion below t_2, and r = proportion above t_2. For a unit normal distribution $\sigma^2 = 1$.

The calculated value of D was compared with tabulated values for the normal distribution (Fisher & Yates, 1953, table I). A value of D exceeding 2·58 is considered significant at the 1 % probability level.

The reduced efficiency of gauged estimates of m and s compared with estimates from direct measurements is more than compensated by the large samples that can be routinely classified by gauging, whereas the time taken for individual facet counts is extremely limiting. A further advantage of this method is that the estimates of m and s can now be expressed in terms of facet number rather than in arbitrary threshold units, as previously used by Sang & Burnet (1963), which give no indication of absolute eye size.

The number of treatments which can be tested within a single experiment is limited. For concise presentation and to facilitate comparisons, mean eye size in Table 3 is expressed as the deviation from the within-experiment control, and the average for the series of within-experiment control values is shown at the head of the table.

Although significant differences in mean facet number between the sexes are present, there is no evidence for any differential response, and sex differences tend to remain constant for each strain. Therefore, except where significance tests are applied, a weighted mean for sexes is presented.

3. RESULTS

The relationship between gene expression and the availability of dietary ribonucleic acid

In their studies on the inbred ey^K strain Sang & Burnet (1963) described the quantitative relationship between gene expression and the concentration of ribonucleic acid in the larval culture medium. They showed that the optimum level for eye development differs appreciably from the larval growth rate optimum, which indicates that an effect of the *eyeless* mutation is to put the growth requirements of the mutant eye disks out of balance with those of the organism as a whole.

The quantitative dose responses illustrated in Fig. 2 show that each of the four non-standardized *eyeless* strains has a well-defined optimal larval growth rate requirement for about 0·4 % ribonucleic acid in the synthetic medium. The form of the relationship between RNA concentration and mean eye size in the same

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experiments illustrated in Fig. 3 shows no general similarity between strains, and the levels of RNA at which eye size is largest are not obviously related to the larval growth rate optimum. The change in eye size with RNA concentration in the ey^4 strain is nearly the inverse of that in the *ey* strain, whilst eye size in the ey^2 strain is relatively stable over most of the dose range. A surprising feature here is that the response of the massbred ey^K strain differs markedly from that described by Sang & Burnet (1963) for the highly inbred Oregon ey^K which is closer to that shown by the *ey* strain in Fig. 3. In the present experiments eye size in the massbred ey^K is more stable at lower RNA concentrations than in the inbred Oregon ey^K

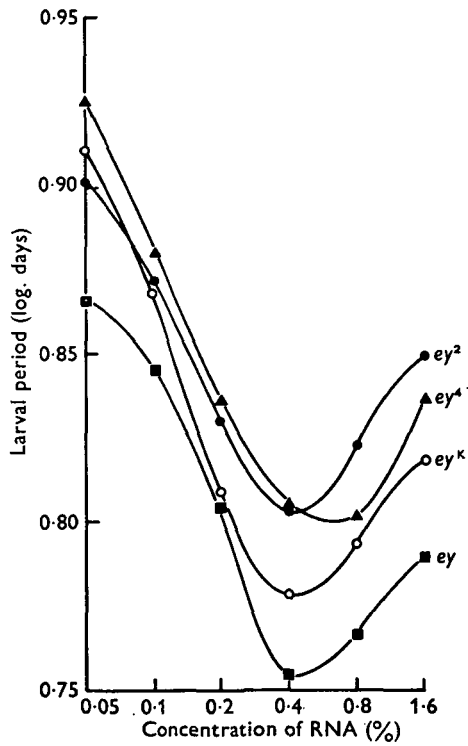

Fig. 2. The relation between the duration of the larval period ($\log_{10}$ days) and the concentration of dietary RNA for *eyeless* mutants in non-standardized genetic backgrounds.

and also shows a well-defined inflexion in the upper part of the dose range, the greatest instability being in the region of the larval growth rate optimum. These effects indicate that there is a substantial difference between the properties of the genetic backgrounds of the inbred and massbred strains and that modifier background is capable of altering the relation between RNA supply and the expression of the ey^K allele. This interpretation finds confirmation in the response to RNA of the Pacific ey^K strain illustrated in Fig. 4 which is much closer to that for the inbred Oregon ey^K strain described by Sang & Burnet.

The effect of modifier genes on the form of the RNA dose response is most

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strikingly demonstrated by the trend in mean eye size with ey^4 on the Pacific background which, in the higher portion of the dose range, is the reverse of that in the original strain. The difference cannot, of course, be a property of the fourth chromosome which both strains have in common. On the Pacific background the response for ey^4 closely resembles that for ey^K, both strains showing an inflexion at 0·1 % RNA, which is the concentration most favourable for the development of the mutant eye disks. Indeed, unlike the original *eyeless* strains, the four Pacific

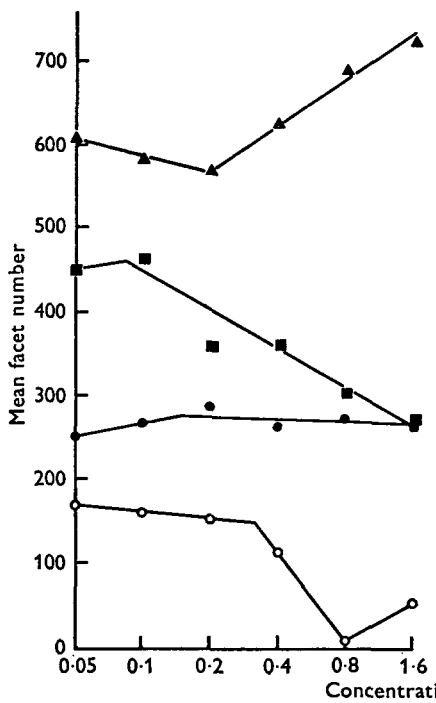

Fig. 3

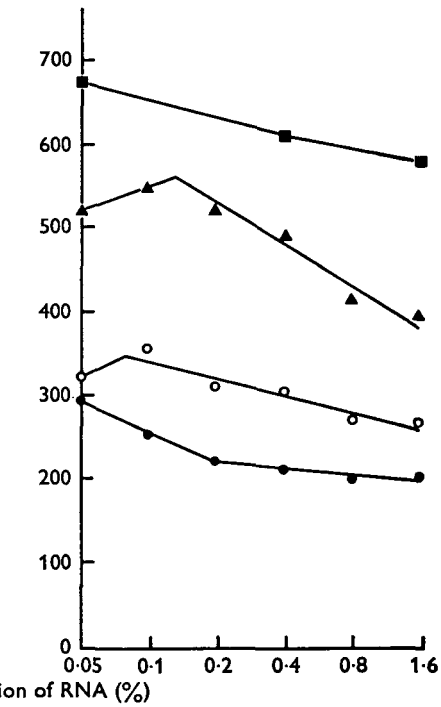

Fig. 4

Figs. 3, 4. The relation between RNA concentration and mean facet number for *eyeless* mutants. Fig. 3. Non-standardized genetic backgrounds. Fig. 4. Pacific background. *ey*, ■; ey^4, ▲; ey^2, ●; ey^K, ○.

strains are concordant in showing an increase in eye size with deficiency levels of RNA. The marked differences in response displayed by the original strains (Fig. 3) can be attributed therefore to the effect of the unique genetic constitution of each strain on the sensitivity of eye development to RNA concentration. The response of Pacific ey^2 differs from that of Pacific ey^4 and ey^K in showing a change of slope in the middle rather than in the lower portion of the dose range, a feature which may provisionally indicate that the properties of ey^2 are qualitatively different from those of the other alleles in respect of RNA sensitivity. The response of Pacific *ey* was examined at only three concentrations but the data are sufficient to show that, like the other alleles, on the Pacific background *ey* also shows a greater mean eye size at suboptimal levels of dietary RNA.

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Other nutritional components of the gene-environment interaction

The mean facet number for each of the four Pacific strains reared on live yeast and control synthetic culture media are shown in Table 2. The Pacific *ey* strain consistently yields the highest estimate of mean eye size, while Pacific ey^2 ranks lowest in both cases. On aseptic synthetic media Pacific *ey* is more clearly differentiated, exceeding the second ranked Pacific ey^K strain by over 150 facets. The Pacific *ey* strain differs therefore in its reaction to the generalized differences in the properties of the live yeast and aseptic synthetic larval growth media.

Table 2. *Mean facet numbers for the four Pacific* eyeless *strains reared in live yeast and aseptic synthetic culture*

	Live yeast culture		Synthetic culture	
	♂♂	♀♀	♂♂	♀♀
ey	362	349	549	615
ey^2	292	228	242	228
ey^4	333	269	338	331
ey^K	315	258	385	397

A comparison of the effects of an array of different suboptimal nutritional conditions on the expression of each of the four *eyeless* alleles on the Pacific background is shown in Table 3. A deficiency of thiamine produces a significant increase in eye size in each of the four strains, and the dose response for the Pacific ey^K strain illustrated in Fig. 5 shows that the relationship between mean eye size and thiamine concentration is linear over a comprehensive dose range, in contrast to the situation with respect to RNA. Deficiencies of cholesterol, lecithin and pyridoxine reduce eye size, but it is only in the case of cholesterol that there is a significant difference in all four strains.

Deficiencies of sucrose, folic acid, and riboflavin and an excess level of sucrose all reduce eye size in the Pacific ey^K strain, which shows the greatest range of significant treatment effects. Variation in the availability of dietary protein to deficiency or excess levels tends to improve eye size in Pacific ey^2, and higher protein levels also tend to have this effect in the Pacific ey^4 strain.

Biotin deficiency reduces eye size in the Pacific ey^K and ey^2 strains, but this reduction was not associated with a significant increase in the incidence of antennal duplications comparable to that found by Sang & Burnet (1963) for the Oregon ey^K strain. This is probably because antennal duplication occurs at an appreciable frequency only when there is a high incidence of grade 0 flies, whereas all of the Pacific *eyeless* strains used in the present study have a much lower expressivity than the inbred Oregon ey^K strain and, consequently, a relatively low proportion of grade 0 individuals. The implication here is that mean eye size must fall below a threshold before biotin deficiency has an effect on the incidence of antennal abnormalities associated with *eyeless*.

Although there is a concordance in the direction of response of mean eye size in

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Table 3. *Effects of nutritional treatments on mean facet number in the Pacific* ey, ey^2, ey^4, *and* ey^K *strains, expressed as the deviation from the within-experiment control mean facet number*

* and ** denote significant deviations at the 5 and 1 % probability levels respectively. Significance tests were carried out as described in §2 (iii). Vitamin concentrations are expressed in μg per replicate.

	ey^K		*ey^2*		*ey*		*ey^4*	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
Control	385	397	242	228	549	615	338	331
Major nutrient deficiency								
Casein 2·5 %	−19	−33*	+36**	+42**	−32	+35	−60	−29
Cholesterol 0·00156 %	−188**	−148**	−37**	−36**	−89**	−108**	−66**	−81**
Lecithin 0·0125 %	−148**	−102**	−13	−11	−80**	−104**	−136**	−38
Sucrose nil	−66**	−25	+24	+14	+50	+4	−58	+42
Major nutrient in excess								
Casein 8·5 %	−23	−4	+49*	+52*	−104*	−53	+91*	+94*
Cholesterol 0·128 %	−68**	−12	+12	−12	−31	+120	−27	+20
Lecithin 0·8 %	−37	−20	+4	+7	+6	−27	+19	+1
Sucrose 3·25 %	−47*	−51**	−24	−13	−24	−16	−16	−30
Vitamin deficiency								
Biotin 0·005 μg	−53**	−43*	−32**	−40**	+42	−19	+53	+13
Folic acid nil	−84**	−54**	+12	+19	+20	−11	+10	+3
Niacin 5·0 μg	+13	+30	−3	+11	−55*	−21	+14	+44
Pantothenate 6·0 μg	+30	−29	+3	−14	+2	+162	+45*	+36
Pyridoxine 0·5 μg	−24	−18	−49**	−40**	−77**	−172**	−17	−21
Riboflavin 1·5 μg	−78**	−50**	−17	−5	+9	−75	−21	−12
Thiamine 0·4 μg	+36*	+67**	+61**	+54**	+146**	+92	+101**	+166**

the four Pacific strains to variations in RNA, thiamine and cholesterol, they differ with respect to certain other nutritional variations. Strain surveys of the type described here are always open to the objection that, if the effect of a nutritional deficiency is tested only at a single arbitrary dosage level, apparently qualitative differences in the responses of the different strains may conceal what are in reality only quantitative differences in sensitivity. Indeed, the response to a pyridoxine deficiency may be of this type since the *ey* and ey^2 respond significantly, whereas reductions in mean eye size for ey^4 and ey^K are not significant, although the direction of change is the same for all strains. The same is also true of the differences in response to lecithin deficiency, where reduction in mean size for ey^4 is significant in only one sex, but the direction of change is the same in all strains.

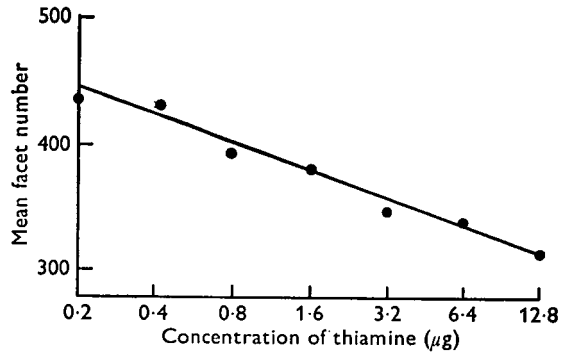

Fig. 5. The relation between thiamine concentration and mean facet number for Pacific ey^K.

More obviously qualitative differences are shown by the response of ey^2 to protein deficiency and of ey^K to riboflavin and folic acid deficiencies. Interesting here is that folic acid deficiency also reduces eye size significantly in the inbred Oregon ey^K strain, whereas there is no evidence that a deficiency of this vitamin has an effect on the expression of any of the other *eyeless* alleles. The data indicate, therefore, that during larval development certain specific nutritional deficiencies create conditions of physiological stress to which some but not all *eyeless* alleles are sensitive, although the effect of variation in the non-standardized fourth chromosomes of the Pacific strains and the presence of some degree of residual variation in the rest of the Pacific genome cannot be ignored. On the basis of differences in their interactions with suboptimal environments the four alleles may be regarded provisionally as heteroalleles, differences between the respective Pacific strains being a reflexion of mutation at different sites within the *eyeless* locus.

The culture ageing effect

As might be expected, the change in mean eye size with culture age is not identical for *eyeless* strains with different genetic backgrounds. The relevant environmental factors must be, to a great extent if not entirely, density dependent, and there is likely to be a variety of interacting components resulting in a complex

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array of possible genotype-environment interactions. On the Pacific background ey^K differs from ey^2, not only in certain features of the RNA dose–response relationship, but also qualitatively in sensitivity to other nutritional components including protein and phospholipid, and at least three vitamins: folic acid, riboflavin and pyridoxine. It is, therefore, worth while to ask how far these alleles are concordant with respect to the way in which phenotypic expression changes with increasing culture age, since differences in the intensity of density-dependent variables affected by fecundity, viability and development rate are largely removed by standardization of the rest of the genotype.

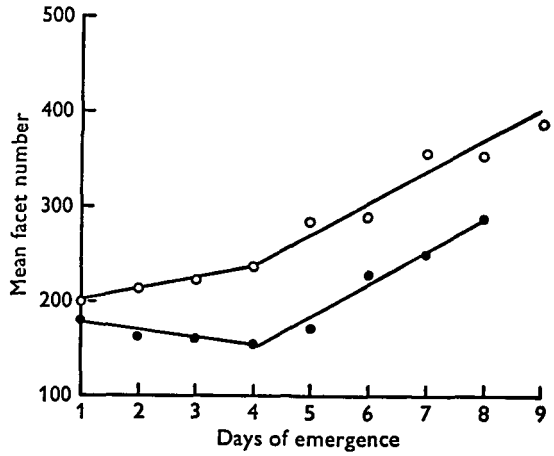

Fig. 6. Mean facet numbers for individuals emerging on successive days from live yeast culture. Pacific ey^2 (●), Pacific ey^K (○).

The change in mean eye size with culture age in the Pacific ey^K and Pacific ey^2 strains examined simultaneously on standardized yeast–oatmeal–molasses–agar medium at 25 °C is shown in Fig. 6. The change in mean eye size for ey^K is the opposite of that for ey^2 during the first 4 days. Both strains show a change in slope at the fourth day, followed by an increase in eye size over the rest of the emergence period. It is the similarity in the response after day 4 which is striking here, and which must be due to some factor or factors to which both alleles are sensitive. This may be the relative depletion of the yeast population and hence of the nutritional resources available to the larvae in ageing cultures (Sang, McDonald & Gordon 1949), resulting in nutritional deficiencies of RNA and/or thiamine.

The increase in eye size on thiamine-deficient synthetic medium indicates that the development of the mutant eye disks may be responsive to a change in carbohydrate metabolism and to the balance of metabolites entering the tricarboxylic acid cycle. On yeasted culture medium alcohol is one of the products of yeast fermentation likely to accumulate as the culture increases in age. The relevance of this to the culture-ageing effect in *eyeless* is that the metabolism of increasing levels of dietary alcohol by the larvae is likely to result in a change in the levels of tricarboxylic-acid mediated metabolites and an increased demand for thiamine pyrophosphate.

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Sensitive period determinations

The duration of the sensitive period during which the expression of the mutant is influenced by dietary deficiencies of RNA, thiamine, or cholesterol was examined using the Pacific ey^K strain. First-instar larvae were inoculated on to deficient media and additions made after successive time intervals as described in § 2. The RNA concentration was raised from 0·05 % initially to 1·6 % finally, that is, to four times the control level. Thiamine was raised from the initial deficiency level of 0·4 μg per tube to 10 μg per tube, and cholesterol from 0·00156 % initially to the growth optimal level of 0·03 % finally.

As illustrated in Fig. 7 there appear to be three sensitive phases or periods for each dietary treatment for which the mid-points are summarized in Table 4.

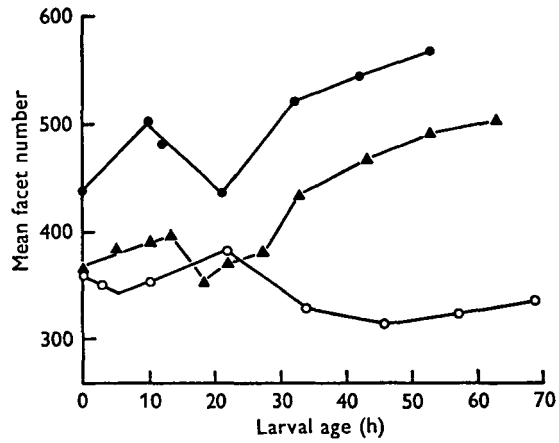

Fig. 7. Sensitive period determinations in Pacific ey^K for RNA (●), cholesterol (○), and thiamine (▲). The metabolites were added to deficient diets at successively later intervals to bring the final concentration to the control or excess level (Materials and Methods). Time is measured in hours from larval eclosion adjusted to a standard 96 h larval period.

During the first 10 h of larval life, deficient RNA and thiamine diets, as expected, produce an increase in eye size. After 10 h, however, the effect is reversed and the deficient media reduce eye size until about 20 h. A third sensitive period begins after 20 h and eye size is again increased. A similar 'three-phase' sensitive period to a deficient cholesterol diet is found, although the respective mid-points are somewhat earlier. Moreover, the effect of a cholesterol deficiency is the exact reverse of a RNA or thiamine deficiency during each successive sensitive phase, in agreement with the opposite effect of a cholesterol deficiency on eye development. The relatively small differences in the timing of the respective sensitive phases between treatments probably reflect differential effects of each specific dietary stress on growth rate during successive larval instars. The estimated sensitive period mid-points for each metabolite may not, therefore, represent identical physiological age on the deficient diets. The spread in physiological age within

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cultures will tend to give an over-estimation of the relative lengths of each sensitive phase although, during the first period, the larvae should still be well synchronized. The later periods are probably shorter than is indicated and a period of insensitivity may intervene between the first and second and the second and third cut-off points.

Table 4. *Mid-points of the successive sensitive phases of the Pacific* ey^K *strain to deficient RNA, thiamine and cholesterol diets*

Deficiency	Mid-points (h) Phase 1	Phase 2	Phase 3
RNA	5·0	15·0	37·0
Thiamine	6·5	16·0	40·0
Cholesterol	2·5	12·5	31·5

The thiamine, cholesterol, and RNA sensitive period determinations for the Pacific ey^K strain show a quite striking similarity, and it seems likely that these three nutrients are closely similar in the timing of their effect on the development of the mutant eye disks.

4. DISCUSSION

The results of the present analysis confirm that the effect of different levels of dietary ribonucleic acid upon the expressivity of mutant alleles at the *eyeless* locus is strongly influenced by the properties of the genetic background and are therefore, in this respect, in agreement with conclusions based on the effect of more generalized environmental variables, notably the effect of temperature on the expression of *cubitus interruptus dominant* (Scharloo, 1962). The direction of change in eye size in response to RNA is not a property of *eyeless* and consequently the characteristics of the dose–response relationship reveal nothing relevant to the action of the mutant. RNA is a non-essential nutritional requirement for *Drosophila* larvae (Sang, 1957), but on nucleic acid-deficient diets, nucleotide biosynthesis has a limiting effect on growth rate. RNA as such is not used directly by the larvae, but serves as a source of preformed ribonucleotides (Burnet & Sang, 1963). Thus the form of the interaction of gene expression with RNA is likely to be influenced by variation in any of the reactions regulating the rates of endogenous ribonucleotide biosynthesis, interconversion, and utilization in different metabolic pathways, and it is sensitivity to RNA rather than the direction of response which is a property of the *eyeless* mutant.

Sang & Burnet (1963) were able to distinguish two distinct and consecutive phases of sensitivity within the RNA sensitive period for the Oregon ey^K strain, the first ending after 24 h of larval life, the second at 42 h on the standardized 96 h time scale for larval development (see § 2). During both periods the deficient diet tended to increase eye size. The Pacific ey^K strain shows at least three post-embryonic phases of RNA sensitivity, as shown in Table 4, and the direction of

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response during the second phase is the reverse of that in the first and third periods. This type of oscillation in the direction of response to environmental variables at successive developmental stages is by no means unique to the present system and is particularly well illustrated by the effects of temperature on heterochromatization described by Hartmann-Goldstein (1967). In contrast, for example, to the stability of the temperature effective periods for different alleles at the *cubitus interruptus* locus (Scharloo & Nieuwenhuijs, 1964), both the sensitive period and the direction of response to treatment during its constituent sensitive phases are markedly influenced by the genetic background associated with the *eyeless* mutant, and this most probably explains the difference between the temperature effective periods for ey^2 and ey^K observed by Baron (1935) and Sang & McDonald (1954). Detailed studies on the *eye-gone* mutant with similar phenotype effects to *eyeless* lead to the same conclusion (Hunt, in preparation).

If the importance of the genetic background is general for the other nutritional environmental components influencing gene expression, any comparison of gene-environment interactions designed to detect underlying differences in the action of mutant genes at the metabolic level requires prior standardization of the residual genotype. The comparison of response profiles on the Pacific background suggests that the four *eyeless* alleles may be heteroalleles differing in the extent of mutational damage within the structural gene and each causing the synthesis of a uniquely defective polypeptide. Slight differences in the properties of the respective mutant enzymes may lead to inequality in their performance under different conditions of physiological stress and to different intensities of expression at the phenotypic level. The four alleles are concordant with respect to sensitivity and direction of response to RNA, thiamine and cholesterol deficiencies on the Pacific background, and this poses the question of whether the nature of the nutritional components influencing gene expression leads to any valid inference concerning gene action in terms of the relevant metabolic processes. Because of the necessarily indirect effect of nutritional manipulations and the lack of detailed information concerning metabolic pathways in larval and imaginal disk tissues, such inferences are at best likely to be very speculative and further work is projected to define the relationship between these nutrients and their effect on the expression of *eyeless*, but studies on the growth of normal and mutant eye disks in a tissue culture system (Schneider, 1964, 1966) should help to narrow the range of possibilities.

SUMMARY

A method is described for quantifying the phenotypic expression of *eyeless* using gauged estimates of the mean and standard deviation of the distribution of facet numbers.

Gene-environment interactions of four *eyeless* alleles *ey*, ey^2, ey^4 and ey^K are compared in different genetic backgrounds and on a standardized Pacific background. The original strains differ in mean phenotypic value and in direction of response to variations in the ribonucleic acid content of the larval culture medium,

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whereas the four Pacific strains are more concordant. Consequently these differences are in part attributable to modifier genes present in the different genetic backgrounds of the original strains. Modifier genes also influence the periods in development at which *eyeless* is sensitive to nutritional treatments.

On the standardized Pacific background deficiencies of cholesterol cause an increase, whereas deficiencies of RNA or thiamine cause a decrease in the expressvity of all four *eyeless* alleles, but non-identity of their interaction profiles over an array of different suboptimal environments suggest that they may form a group of heteroalleles.

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