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Gene-environment interactions of the *eye-gone* mutant in *Drosophila melanogaster* and a comparison with *eyeless*

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1. INTRODUCTION

The joint participation of the phenotypically identical eye colour mutants *rosy* and *maroon-like* of *Drosophila melanogaster* in the production of the enzyme xanthine dehydrogenase (Glassman, 1965) has shown that such non-allelic genes may indeed control a single step in metabolism. The eye mutants *eyeless* (*ey*) and *eye-gone* (*eyg*), located respectively at 4–0·2 and 3–35·5 (Bridges & Brehme, 1944) similarly yield indentical phenotypic effects. In both cases, an extreme variability in adult eye size is apparent. A study of the gene-environment interactions of these two genes in a standardized genotype may be expected to delimit their interrelations in eye development.

The important dietary variables in the control of *eyeless* expression have already been established by Hunt & Burnet (1969). This publication will be concerned therefore with an analysis of the dietary interactions of *eye-gone* and a comparison with *eyeless*.

2. MATERIALS AND METHODS

The strains used in this investigation are *eyg* (obtained from the California Institute of Technology, Pasadena) and a Pacific *eyg* line. The same inbred Pacific wild-type stock as employed for the synthesis of the Pacific *eyeless* strains described by Hunt & Burnet (1969) was used for the synthesis of Pacific *eyg*. To ensure complete replacement of the original genome, the multiple inversion chromosomes SM5 and TM3 *Ser* and the dominant marker genes L^2 and *Sb* were used and the derived Pacific *eyg* was finally backcrossed to the wild type and re-isolated for three generations. The two strains were maintained on a yeast–oatmeal–treacle–agar food medium in mass culture at 25 °C.

Nutritional tests were performed with germ-free larvae in sterile synthetic culture at 25 °C (Sang, 1956). Further details are as previously described (Hunt & Burnet, 1969). Sterile synthetic culture was also used in the determinations of the sensitive periods to a cholesterol-deficient diet. Germ-free first-instar larvae were inoculated on to cholesterol-deficient (0·00156 %) media and at successively later intervals a sterile suspension of cholesterol was added to bring the final concentra-

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tion to the control (0·03 %) level. For purposes of comparison, the larval period and times of addition were converted to a standard 96 h scale (Bodenstein, 1950).

Mean expression of the original *eyg* strain is estimated by the method of gauging (Stevens, 1948) and the application of this analysis is discussed by Hunt & Burnet (1969). However, the expression of the Pacific *eyg* strain is too extreme to allow any reliable gauged estimate of mean facet number, the range of eye sizes occupying the lower end of the phenotypic scale. Phenotypic expression is measured therefore as the frequency (percentage) of eyes in a population or treatment group, and frequency differences are tested against the distribution of χ^2 by means of 2×2 contingency tables (Woolf, 1951).

Mean eye size in Tables 1 and 2 is expressed as the deviation from within-experiment control values and an average control value is presented at the head of each table. A significant deviation from control is indicated by the superscript* for the 5 % probability level and ** for the 1 % probability level.

Table 1. *Effects of nutritional treatments on mean facet number in the original* eyg *strain, expressed as the deviation from the within experiment control mean facet number.*

(* and ** denote significant deviation at the 5 % and 1 % probability levels respectively. Significance tests were carried out as described by Hunt & Burnet (1969). Vitamin concentrations are expressed in μg per replicate.)

	♂♂, *m*	♀♀, *m*
Control	305	257
Major nutrient deficiency		
Casein 2·5 %	−121**	−139**
Cholesterol 0·00156 %	−262**	−229**
Lecithin 0·0125 %	−145**	−156**
Sucrose nil	−71**	−71**
RNA 0·05 %	−40*	−44**
Major nutrients in excess		
Casein 8·5 %	+13	+23
Cholesterol 0·128 %	−16	−10
Lecithin 0·8 %	−56	+12
Sucrose 3·25 %	−14	+21
RNA 1·6 %	−13	−19
Vitamin deficiency		
Thiamine 0·4 μg	+160**	+87**
Pyridoxine 0·5 μg	+20	+22
Niacin 5·0 μg	−15	+43*
Pantothenate 12·0 μg	−20	−19
Biotin 0·005 μg	−169**	−92**
Riboflavin 1·5 μg	−41	−15
Folic acid nil	−16	+39**

3. RESULTS

Dietary surveys

Vitamin deficiencies and deficient and excess levels of the major nutrients were tested on the original *eyg* and Pacific *eyg* strains (Tables 1, 2). An aberrant sex ratio is present in the original strain (approximately twice the number of females to males) and attention should be centred on the female estimates (Table 1).

Table 2. *Effects of nutritional treatments on eye size and antennal duplication in the Pacific* eyg *strain*

(Eye size is expressed as the deviation from within experiment control values. * and ** denote significance at the 5% and 1% probability levels respectively. Significance tests were carried out as described in Materials and Methods. Vitamin concentrations are expressed in μg per replicate.)

	♂♂, % eyes	♀♀, % eyes	Antennal duplications %
Control	44·0	35·0	0·3
Major nutrient deficiency			
Casein 2·5%	+4·8	−1·2	2·2*
Cholesterol 0·00156%	−20·5**	−9·1*	1·7*
Lecithin 0·0125%	+2·7	−5·1	0·8
Sucrose nil	+1·4	+7·2	0·3
RNA 0·05%	−22·7**	−22·8**	10·3**
Major nutrients in excess			
Casein 8·5%	−4·8	+0·1	0·8
Cholesterol 0·128%	+3·9	−3·4	0·9
Lecithin 0·8%	+5·7	+5·2	0·7
Sucrose 3·25%	+2·5	+11·0**	1·5
RNA 1·6%	0	−6·6	1·8
Vitamin deficiency			
Thiamine 0·4 μg	−0·8	+1·5	4·0**
Pyridoxine 1·0 μg	+0·7	+1·4	0·8
Niacin 5·0 μg	+11·5	+0·2	1·5*
Pantothenate 6·0 μg	−5·3	−6·4	0·7
Biotin 0·005 μg	−5·3	+1·1	0
Riboflavin 1·5 μg	−21·8**	−24·1**	8·5**
Folic acid nil	−28·3**	−24·8**	3·0

A suboptimal level of dietary thiamine significantly increases both the male and female estimates of mean eye size for the original strain, whereas deficiency levels of folic acid and niacin are similarly effective on females only. Mean eye size is reduced by deficiency levels of RNA, biotin, sucrose, lecithin, casein and cholesterol. In contrast, deficiency levels of riboflavin, folic acid, cholesterol, and RNA augment the expression of the *eyg* gene in the Pacific background while only a high carbohydrate diet (in females) produces a significant increase in eye size (Table 2). Therefore, only deficient RNA, cholesterol, and folic acid diets significantly alter expression of *eyg* in both the original and Pacific backgrounds and the direction of change in eye size in response to a folic acid deficiency for the Pacific strain is the inverse of that for the original strain.

The effects of varying thiamine and cholesterol concentration on expression in the *eyg* and Pacific *eyg* strains are further demonstrated by the comprehensive dose responses illustrated in Figs. 1 and 2. For both strains there appears to be a threshold concentration for cholesterol above which little or no improvement in eye size is achieved. In contrast to *eyg*, the Pacific *eyg* strain is insensitive to thiamine concentration throughout an extensive dose range, and it is perhaps significant

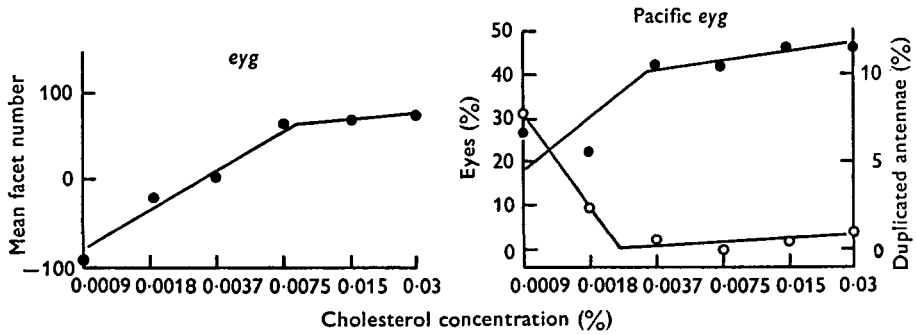

Fig. 1. The effect of dietary cholesterol concentration on the expression of the *eyg* mutant in the original and Pacific wild genotypes. Eye size, solid circles; antennal duplication, open circles.

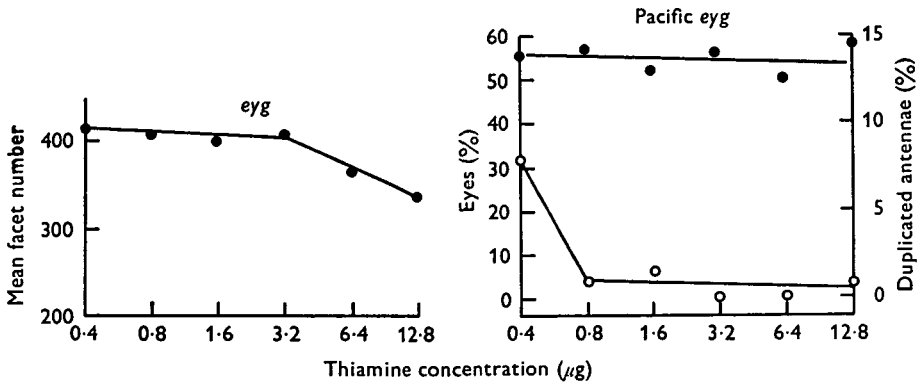

Fig. 2. The effect of dietary thiamine concentration on the expression of the *eyg* mutant in the original and Pacific wild genotypes. Eye size, solid circles; antennal duplication, open circles.

therefore that a low level of thiamine increases the incidence of antennal duplications in this strain. The eye and antennal imaginal primordia arise early in development from a common evagination of the dorsal wall of the pharyngeal cavity, and division of this complex into separate eye and antennal components does not occur until the late second instar. It is not surprising, therefore, that both the *eyeless* (Sang & Burnet, 1963) and *eye-gone* mutants exhibit pleiotropic effects on antennal development, usually in the form of a duplicated antenna on one side of the head, although low mean eye size, as found in Pacific *eyg*, does appear a prerequisite for an appreciable frequency of antennal duplications.

A highly significant rise in the number of duplications is observed with flies

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reared on deficient thiamine, riboflavin, and RNA diets and a smaller increase with deficiencies of niacin, casein, and cholesterol (Table 2). Such antennal abnormalities are almost invariably associated with a missing eye on the same side of the head, although a change in mean eye size does not necessarily result in a corresponding change in duplication frequency. For example, a folic acid deficiency markedly increases the expressivity of the *eyg* gene without any accompanying change in the frequency of antennal duplications and, conversely, a deficiency of thiamine affects only antennal development. Similar results are obtained with flies reared on casein and niacin-deficient diets.

Cholesterol sensitive period determinations

The original *eyg* strain is sensitive to a cholesterol-deficient diet throughout the first and second larval instars, with a possible second sensitive phase beginning at 55 h of larval life (Fig. 3). In contrast, as least three distinct sensitive phases are

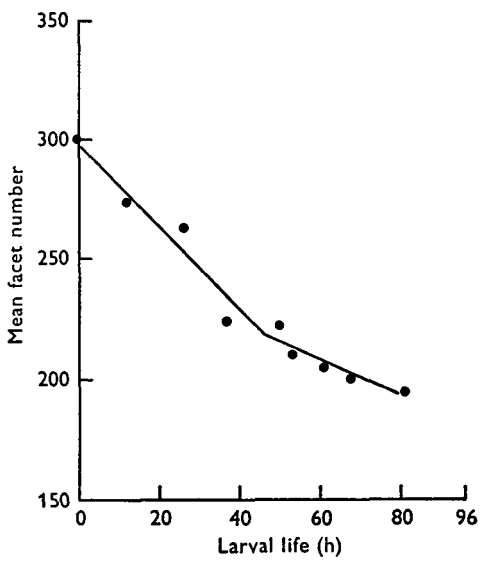

Fig. 3

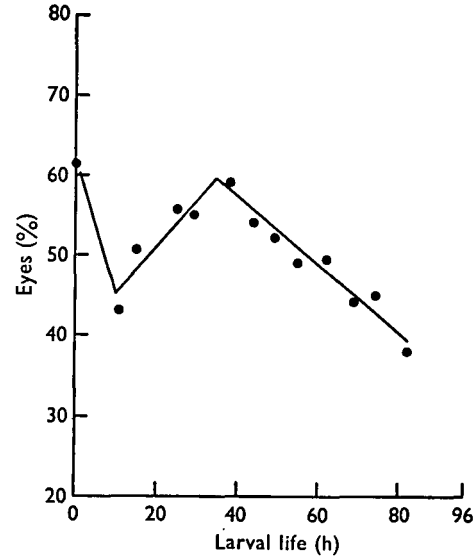

Fig. 4

Fig. 3. Cholesterol sensitive period determination for the original *eyg* strain. Larval life is measured in hours from eclosion and adjusted to a standard 96 h scale at 25 °C.

Fig. 4. Cholesterol sensitive period determination for the Pacific *eyg* strain. Larval life is measured in hours from eclosion and adjusted to a standard 96 h scale at 25 °C.

apparent for Pacific *eyg* (Fig. 4). During the first 10 h of larval life, a deficient cholesterol diet decreases eye size and this is followed by a second period from 10 to 35 h during which eye size is increased. Finally, a third period during which eye size again declines continues until the late third instar. The mid-points of these successive sensitive phases are at 5, 22, and 58 h, respectively, and the direction of response to the sterol-deficient diet during each sensitive phase is identical to that reported by Hunt & Burnet (1969) for the Pacific ey^K strain.

4. DISCUSSION

The observation that the original and Pacific *eyg* strains give rather dissimilar arrays of sensitivities to a variety of dietary changes is in accordance with the accumulating evidence for an important role of genetic background in the control of the gene-environment interactions of a number of different mutant systems (Baron, 1935; Scharloo, 1962; Sang & Burnet, 1967; Hunt & Burnet, 1969). Of greater significance perhaps is the effect of modifier background on the sensitive developmental stages to a low dietary cholesterol concentration. Although both *eyg* strains show a reduction in eye size when reared on a cholesterol-deficient diet, the form and timing of this sensitivity is markedly different; the original strain gives only a relatively simple 'two-phase' sensitive period, whereas eye development in Pacific *eyg* is cholesterol dependent during three distinct postembryonic periods. Moreover, there is a striking concordance in the cholesterol sensitive periods for the Pacific *eyg* and Pacific ey^K (Hunt & Burnet, 1969) strains. Both Pacific *eyg* and ey^K show an identical 'three-phase' sensitivity but the mid-points of the successive sensitive phases for Pacific *eyg* at 5, 22, and 58 h are somewhat later than the corresponding mid-points for Pacific ey^K (at 2·5, 12·5, and 31·5 h respectively). It is possible, therefore, that the action of the *eyg* mutation during each successive sensitive phase occurs at a later developmental stage than *eyeless*.

Hunt & Burnet (1969) have shown that the expression of the Pacific *eyeless* strains is sensitive to deficiency levels of thiamine, lecithin, cholesterol, and RNA. Of these dietary treatments, only cholesterol and RNA deficiencies produce significant treatment effects on Pacific *eyg*, although the effect of a thiamine deficiency on eye size in original *eyg* and on antennal development in Pacific *eyg* is perhaps significant. A limiting thiamine supply on Pacific *eyg* may result in the overgrowth of the antennal primordium rather than an increase in the size of the eye disk. Certainly other dietary manipulations can specifically influence either eye or antennal development and the *eyg* mutation apparently renders both eye and antennal development sensitive to dietary changes. A similar situation arises with the inbred ey^K strain when reared on a biotin-deficient diet (Sang & Burnet, 1963).

It is not possible to come to a decision as to the metabolic inter-relations of the effective dietary treatments for *eye-gone* and *eyeless*. However, an important observation is that the properties of the Pacific wild genotype do not exert identical controls over the gene-environment interactions of the *eyeless* and *eye-gone* genes. Evaluation is complicated by the apparent heteroallelic behaviour of the *eyeless* alleles, and a detailed analysis of the role of the more important primary metabolites in mutant development is necessary. Nevertheless, the cholesterol sensitive period determinations for Pacific ey^K and *eyg* indicate that these two genes may act consecutively on a single developmental process.

The highly significant effect of a cholesterol deficiency on all *eye-gone* and *eyeless* strains so far examined suggests that sterol metabolism may play a direct role in the elaboration of the mutant phenotype. One important site for sterol utilization

in insects is hormone synthesis (Karlson, Hoffmeister, Hoppe & Huber, 1963; Kobayashi, Saito, Ishitoya & Ikekawa, 1963) and there is some evidence, albeit indirect, that humoral balance is extremely critical for eye development (Vogt, 1943, 1946; Edwards & Gardner, 1966). However, such an interpretation can be at present only extremely tentative.

SUMMARY

A comparison of the gene-environment interactions of the *eyg* mutant in two different genetic backgrounds clearly demonstrates that the properties of the genetic background play a major role in the control of the gene-environment interactions of this mutant. Similarly, modifier background is important in the determination of the sensitive stages in eye development to a cholesterol-deficient diet.

The phenotypic identity of the *eyeless* and *eye-gone* mutants suggests a close underlying metabolic and developmental relationship. Possible inter-relations of these two mutant genes are discussed in the light of their gene-environment interactions in a standardized genotype.

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REFERENCES

Baron, A. L. (1935). Facet number in *Drosophila melanogaster* as influence by certain genetic and environmental factors. *J. exp. Zool.* **70**, 461–489.

Bodenstein, D. (1950). The postembryonic development of Drosophila. In *Biology of Drosophila* (ed. M. Demerec), pp. 275–367. New York: John Wiley and Sons, Inc.

Bridges, C. B. & Brehme, K. S. (1944). The mutants of *Drosophila melanogaster*. *Publs. Carnegie Instn.* no. 552.

Edwards, J. W. & Gardner, E. J. (1966). Genetics of the *eyes-reduced* mutant of *Drosophila melanogaster*, with special reference to homoeosis and eyelessness. *Genetics* **53**, 785–798.

Glassman, E. (1965). Genetic regulation of xanthine dehydrogenase of *Drosophila melanogaster*. *Fedn. Proc.* **24**, 1243–1251.

Hunt, D. M. & Burnet, B. (1969). Gene-environment interactions of the *eyeless* mutant in *Drosophila melanogaster*. *Genet. Res., Camb.* (in the Press).

Karlson, P., Hoffmeister, H., Hoppe, W. & Huber, R. (1963). Zur Chemie des Ecdysons. *Ann. Chem.* **662**, 1–20.

Kobayashi, M., Saito, M., Ishitoya, Y. & Ikekawa, N. (1963). Brain hormone activity in *Bombyx mori* of sterols and physiologically vital active substances. *Proc. Soc. exp. Biol. Med.* **114**, 316–318.

Sang, J. H. (1956). The quantitative nutritional requirements of *Drosophila melanogaster*. *J. exp. Biol.* **33**, 45–72.

Sang, J. H. & Burnet, B. (1963). Environmental modification of the *eyeless* phenotype in *Drosophila melanogaster*. *Genetics* **48**, 1683–1699.

Sang, J. H. & Burnet, B. (1967). Physiological genetics of melanotic tumors in *Drosophila melanogaster*. IV. Gene-environment interactions of *tu-bw* with different third chromosome backgrounds. *Genetics* **56**, 743–754.

Scharloo, W. (1962). The influence of selection and temperature on a mutant character (ci^D) in *Drosophila melanogaster*. *Archs néerl. Zool.* **14**, 431–512.

Stevens, W. L. (1948). Control by gauging. *Jl R. statist. Soc.* **10**, 54–98.

VOGT, M. (1943). Zur Produktion und Bedentung metamorphosefördernder Hormone während der Larvenentwicklung von *Drosophila*. *Biol. Zbl.* **63**, 395–446.

VOGT, M. (1946). Zur labilen Determination der Imaginalscheiben von *Drosophila*. I. Verhalten verschiedenaltriger Imaginalanlagen bei operativer Defektsetzung. *Biol. Zbl.* **65**, 223–238.

WOOLF, B. (1951). Calculation of χ^2 for a 2×2 Table. *Nature, Lond.* **168**, 1087.