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Lethal interactions of the *eye-gone* and *eyeless* mutants in *Drosophila melanogaster*

By DAVID M. HUNT

M.R.C. Experimental Genetics Unit, Department of Animal Genetics, University College London, Wolfson House, 4 Stephenson Way, London, N.W. 1

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SUMMARY

A synthetic lethal system involving homozygous combination of the non-allelic eye mutants *eye-gone* and *eyeless* is described. The major lethal crisis is towards the end of the pupal instar although a number of double homozygotes die at pupation. The pupal lethal crisis can be overcome in a number of individuals by the experimental removal of the operculum from differentiated pupae. However, only a few of the resulting enclosed double homozygotes proved fully viable, the remainder dying almost immediately after emergence. Dissection of the surviving adults revealed that the brain is located in the anterior thorax.

INTRODUCTION

In a number of instances, the homozygous combination of mutants with similar biosynthetic deficiencies has led to the production of synthetic lethal and semi-lethal systems (Taira, 1960; Goldberg, Schalet & Chovnick, 1962; Lucchesi, 1968). The non-allelic eye mutants *eye-gone* (*eyg*, 3–35·5) and *eyeless* (*ey*, 4–2·0) in *Drosophila melanogaster* produce essentially identical phenotypes of an extreme variability in adult eye size (Lindsley & Grell, 1968) and a study of their dietary interactions in a standardized genotype (Hunt & Burnet, 1969; Hunt, 1969) has revealed close similarities in the sensitivities of both mutants to the availability of certain primary metabolites. Both observations are suggestive of common or closely related underlying disturbances in biosynthesis. Furthermore, it has already been established that both mutants act as semi-lethal factors (Baron, 1935; D. M. Hunt, 1968 unpublished data), with a reduction in mean eye size leading almost invariably to an increase in the number of differentiated pupae that fail to eclose. This communication will be concerned therefore with a study of the combined effects of these two eye mutants on eye development and pupal viability.

MATERIALS AND METHODS

Three alleles at the *eyeless* locus, ey^2, ey^4, and ey^K and one allele at the *eyg* locus have been used in this investigation. The ey^2 strain is also homozygous for the recessive mutant *cubitus interruptus* (*ci*) on the fourth chromosome. The multiple

inversion chromosome TM3 *Ser* and the dominant marker gene ci^D were employed in the synthesis of the double homozygote for *eye-gone* and *eyeless*. To enable the separation of homozygous and heterozygous *eyg* larvae and pupae, the recessive genes *thread* (*th*) and *scarlet* (*st*) were introduced into the *eyg* third chromosome. Homozygous larvae can now be distinguished by malpighian tubule coloration (Beadle, 1937) and homozygous pupae by the *th* aristae. All stocks were maintained in mass culture at 25 °C.

RESULTS

Using different founder stocks, the double homozygote for *eye-gone* and *eyeless* was synthesized on three separate occasions and, in each case, the genotypes of the viable offspring could be established (Fig. 1). In addition, the introduction of *th* and *st* into the *eyg* third chromosome enabled classification of inviable pupae (Fig. 1, scheme ii).

(i) Synthesis of double homozygote for *eyg*; ey^K and *eyg*; ey^4

Parental generation

$$\frac{TM3\ Ser}{eyg}\ \frac{ci^D}{ey}$$

$$\downarrow$$

$$\frac{TM3\ Ser}{eyg}\ \frac{ci^D}{ey},\ \frac{eyg}{eyg}\ \frac{ci^D}{ey},\ \frac{TM3\ Ser}{eyg}\ \frac{ey}{ey},\ \frac{eyg}{eyg}\ \frac{ey}{ey}$$

(ii) Synthesis of double homozygote for *eyg*; *ci* ey^2

Parental generation

$$\frac{TM3\ Ser}{eyg\ th\ st}\ \frac{ci}{ci}\ \frac{ey^2}{ey^2}$$

$$\downarrow$$

$$\frac{TM3\ Ser}{eyg\ th\ st}\ \frac{ci\ ey^2}{ci\ ey^2},\ \frac{eyg\ th\ st}{eyg\ th\ st}\ \frac{ci\ ey^2}{ci\ ey^2}$$

Fig. 1. Genotypes of the parental generation and progeny in the synthesis of the double homozygote for *eye-gone* and *eyeless*.

No eclosed adults lacking both the *Ser* and ci^D phenotypes were found amongst the progeny of scheme i, Fig. 1. Similarly, in scheme ii, offspring of genotype *eyg th st*/*eyg th st*; *ci* ey^2/*ci* ey^2 failed to emerge (Table 1). Therefore, homozygous combination of *eye-gone* and *eyeless* can be considered a synthetic lethal system with death occurring within the puparium. In all but one of the lethal pupae examined, the compound eyes were completely absent and many showed a more or less extreme reduction in head size together with a reduced or absent ptilinum (Fig. 2*a*, *b*). Finally, seven lethal individuals lacked all structures derived from the eye-antennal complex.

Using the pale yellow (*st*) malpighian tubule coloration for identifying *eyg* homozygotes, the development of synchronized first instar larvae from TM3 *Ser*/*eyg th st*; *ci* ey^2/*ci* ey^2 parents (Fig. 1, scheme ii) was closely followed. No evidence

for any differential larval survival was obtained whereas the viability of double homozygotes through pupation was significantly lower than that of *eyg* heterozygotes (Table 2).

Table 1. *Classification of eclosed adults and inviable pupae in the progeny from TM* 3 Ser/eyg th st; ci ey^2/ci ey^2 *parents*

	Genotype		Genotype		
	$\frac{TM3\,Ser}{eyg\ th\ st}\ \frac{ci\ ey^2}{ci\ ey^2}$		$\frac{eyg\ th\ st}{eyg\ th\ st}\ \frac{ci\ ey^2}{ci\ ey^2}$		
Total no. pupae	Adults	Inviable pupae	Adults	Inviable pupae	Undifferentiated pupae
314	168	70	0	67	9

The extreme reduction in head size and the corresponding reduction in the ptilinum must contribute to the lethality of double homozygotes but it is not clear whether death occurs before the onset of the muscular contractions that precede normal eclosion. In other words, is the lethality a direct consequence of the extreme effect on eye disk development, with associated brain damage preventing the co-ordinated movements necessary for eclosion as suggested by Baron (1935) and Shatoury (1963) for inviable *ey*^{2} pupae, or is there a separate lethal crisis prior to eclosion?

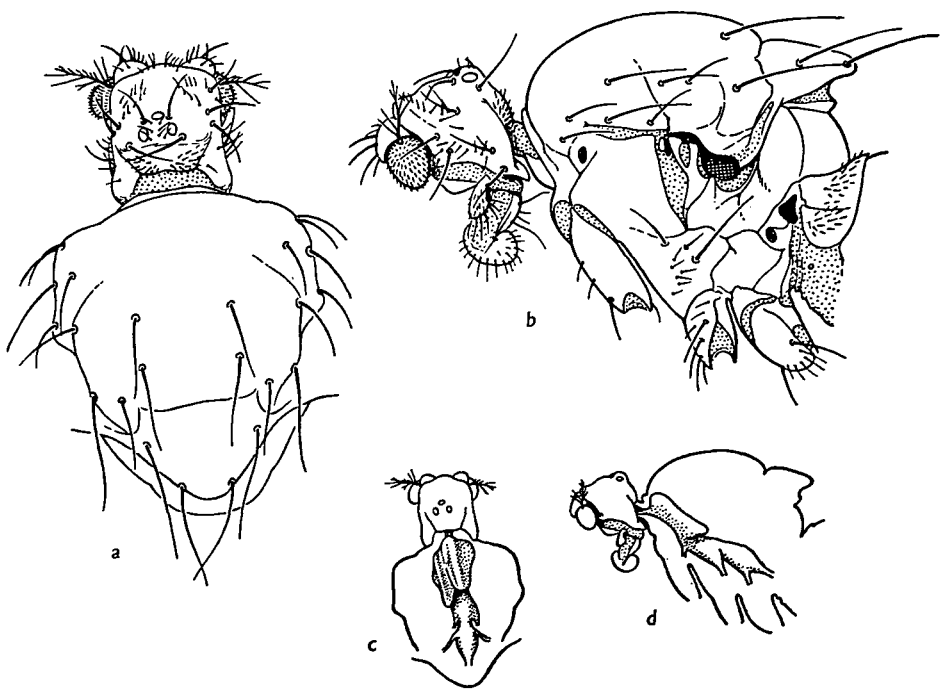

Fig. 2. Camera lucida drawings of the head and thorax of a viable double homozygote for *eye-gone* and *eyeless* after operculum removal. *a*, Dorsal view. Note the extreme reduction in head size. *b*, Lateral view. *c*, Dorsal view after dissection, showing location of brain in the anterior thorax. *d*, Lateral view after dissection.

In an attempt to distinguish these possibilities the operculi were removed from differentiated *eyg*/*eyg*; *ci ey*^{2}/*ci ey*^{2} and *eyg*/*eyg*; *ey*^{4}/*ey*^{4} pupae (Table 3). Non-operated control cultures yielded no eclosed double homozygotes whereas eight eclosed *eyg*; *ey*^{4} homozygotes and nine eclosed *eyg*; *ci ey*^{2} homozygotes were obtained after operculum removal. Of these eclosed double homozygotes, only six proved fully viable, the remainder dying almost immediately after emergence. Dissection of the viable adults revealed that the brain is located in the thorax (Fig. 2) and that

Table 2. *Viability at the larval-pupal ecdysis of* eyg th st/eyg th st; ci ey^2/ci ey^2 *and TM* 3 Ser/eyg th st; ci ey^2/ci ey^2 *larvae. P = probability level for the difference in viability of the two genotypes*

Genotype	No. 3rd instar larvae	No. pupae	% to pupate	*P*
eyg th st/*eyg th st*; *ci ey*^{2}/*ci ey*^{2}	47	33	70·2	
TM 3 Ser/*eyg th st*; *ci ey*^{2}/*ci ey*^{2}	91	86	93·4	< 0·001

the antennae, mouthparts, and ocelli are innervated by extended antennal and labial nerves and ocellar pedicel. These observations may be accounted for either by atrophy of the optic nerves and consequent failure of head eversion to pull the brain hemispheres forward (Shatoury, 1963) or by the small size of the head capsule preventing the entry of the brain.

Table 3. *Viability of differentiated* eye-gone; eyeless *homozygous pupae after operculum removal*

	No. operated	No. eclosed adults	No. fully viable eclosed adults
eyg; *ey*^{4} homozygotes	25	8	2
eyg; *ci ey*^{2} homozygotes	21	9	4

DISCUSSION

In contrast to the situation described by Edwards & Gardner (1966) for double homozygotes of the *eyes-reduced* (*eyr*) mutant and *ey*^{2} where a number of fully viable adults are obtained, albeit with reduced eye size, homozygous combination of *eyg* with either the *ey*^{2}, *ey*^{4}, or *ey*^{K} allele results in an almost complete curtailment of eye development and synthetic lethality, with the major lethal crisis towards the end of the pupal instar and a minor lethal phase at pupation. The observed malformation in head structure and the abnormal location of the brain in the anterior thorax may account for the pupal lethality although, even after the experimental removal of the pupal operculum, few double homozygotes manage to emerge and of those that do succeed many die almost immediately. This evident 'weakness' of double homozygotes, taken together with the semi-lethality at pupation, indicates a severe physiological disturbance in addition to the more obvious effects on

eye disk development and it must remain a possibility that this breakdown in homeostasis may contribute directly to the lethal syndrome. Death may occur, at least in some cases, before the onset of the muscular contractions necessary for eclosion, thus accounting for the poor viability of double homozygotes even after operculum removal.

Lucchesi (1968) discusses a number of synthetic lethal and semi-lethal systems in which pteridine biosynthesis is the primary site for gene action and it is not difficult to envisage that major disturbances in pteridine biosynthetic machinery may severely reduce viability. For a synthetic lethal situation to arise, a common biosynthetic target for the mutant action of both genes in question appears to be necessary and the evidence obtained from a consideration of the dietary interactions of the *eye-gone* and *eyeless* mutants in a standard genotype (Pacific wild), although complicated by the apparent heteroallelic behaviour of the four *eyeless* alleles examined, is in general agreement with such an interpretation (Hunt & Burnet, 1969; Hunt, 1969). The four Pacific *eyeless* strains are closely concordant in their sensitivity to the availabillty of RNA, cholesterol, and thiamine in the synthetic diet but differ with regard to casein, lecithin, biotin, pyridoxine, and riboflavin sensitivities. Clearly, the developmental system is sensitive not only to the major differences associated with changes in genetic background but also to the minor differences conferred by differing extents of mutational damage within the same structural gene. The Pacific *eye-gone* strain follows Pacific *eyeless* in sensitivity to RNA and cholesterol concentration while the incidence of antennal duplication is significantly increased by deficiency levels of dietary thiamine, but contrasts in sensitivity to deficiency levels of folic acid and riboflavin. Sensitive period determinations reveal that both mutants are sensitive to dietary change at similar developmental stages, with the action of the *eyeless* mutant always marginally preceeding that of *eye-gone* and it seems probable, especially in view of the extreme sensitivity in eye development as demonstrated by the heteroallelic behaviour of the *eyeless* alleles, that the differences in dietary sensitivities of the two mutants reflects this divergence in time course rather than dissimilar sites of gene action.

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