MODIFICATION OF GENE SUPPRESSION IN *DROSOPHILA MELANOGASTER* BY SEX CHROMOSOMES II. VARIEGATION IN EYES OF w^{m4} MALES

GREGOR KOLIAN TZ,* INGEBORG HARTMANN-GOLDSTEIN AND NICHOLAS FIELLER
*Genetics Department and Department of Probability and Statistics, Sheffield University,
Sheffield S10 2TN, UK*

SUMMARY

We studied position-effect variegation in left eyes of w^{m4} males with none, one or two Y chromosomes, reared at 25°C or 15°C. The results showed that an additional Y chromosome reduces both the number of variegated flies and the mean number of affected ommatidia, whereas loss of the Y chromosome markedly enhances both. Low culture temperature also enhances the variegation but is less effective than loss of the Y chromosome. Comparisons of variegation patterns between the different experimental groups were hampered by considerable variation between individuals within a group. "Average" patterns were therefore constructed for each group, which are presented in the form of computer-smoothed contour maps and three-dimensional perspectives. They revealed that the distribution of pigment differs in various respects from that observed in other white-variegated strains. Notably, the highest concentration of unpigmented ommatidia occurs in the middle of the eye, decreasing more or less radially though not all peripheral regions are equally affected. Pattern differences associated with differences in Y-chromosome constitution are ascribed to a combination of the factors which determine regions of sensitivity and those which enhance variegation.

1. INTRODUCTION

In *Drosophila*, genes lying next to a heterochromatic breakpoint of a chromosomal rearrangement may be fully expressed in some cells and not in others, which leads to position-effect variegation (for review see Spofford, 1976). The eyes of white-variegated strains of *D. melanogaster* contain pigmented and unpigmented ommatidia arranged in some defined pattern which is related to the chromosomal rearrangement responsible for the variegation. The distinction is usually made between large-spotted mosaicism in the form of sizeable patches with relatively sharp outlines, and the fine-spotted "pepper- and-salt" type where smaller groups and even single ommatidia appear, at least in some strains, to be superimposed on the large patches. Comparisons between position-effect patches and twin spots produced by mitotic crossing-over have shown that the patches represent clones of cells derived from a stem cell in which some developmental event has determined whether the white locus can subsequently become active or not. The size of the patches reflects the developmental stage at which this event occurs (Becker, 1978). A common characteristic of white-variegated eyes is a tendency for the affected, usually unpigmented, ommatidia to decrease in frequency towards the posterior edge of the eye, resulting

* Present address: Department of Biology, The University for Teacher Education, 49 Mobarezan Avenue, Tehran-15, Iran.

in a roughly anterior-posterior pigmentation gradient (e.g., van Breugel, 1972).

Some preliminary observations on the eyes of w^{m4} flies from a strain used for studies on Malpighian tubules (Hartmann-Goldstein and Koliantz, 1981) confirmed the fine-spot variegation initially reported in w^{m4} (Muller, 1930) but suggested that neither as regards pattern nor gradient could the variegation be allocated unequivocally to either of the types cited above—a conclusion also reached by Becker (1978). Furthermore, the disparate descriptions of w^{m4} eyes to be found in the literature seem to imply an unusual variability in the expression of the phenotype, although some of the discrepancies may be due to differences between different w^{m4} lines (Schultz, 1943). Observations on variegation patterns over a range of w^{m4} expression would, it was hoped, lead to a clearer understanding of eye variegation in this strain. Accordingly, the eyes of w^{m4}/YY , w^{m4}/Y and $w^{m4}/0$ males reared at 25°C and 15°C were studied.

2. MATERIALS AND METHODS

(i) Stocks

The w^m/Y males used for these studies were taken from a w^{m4} strain kindly provided by the University of Umeå. To produce $w^{m4}/0$ flies we crossed the w^{m4}/Y males with females of the attached-X strain $O/C(1)RM y^2 su(w^a) w^a/XYL.YS v f B$. Males with an additional Y chromosome were obtained by crossing the w^{m4}/Y males with $In(1)w^{m4}-N^{264-84R}/In(1)w^{m4}/Y$ females. The F1 $In(1)w^4/Y$ females were then crossed with w males (our criterion for the selection of flies with the desired genomic constitution is given in Hartmann-Goldstein and Koliantz, 1981). F2 males showing white-variegated or predominantly wild-type eyes were selected and presumed to have either one or two Y chromosomes; investigation showed that those with the additional Y chromosome had, in general, markedly fewer colourless ommatidia than the males with only one. The putative w^{m4}/YY males in which variegation was scored were subsequently individually progeny tested. All data were taken from 3-day old flies. For descriptions of strains and mutants, see Lindsley and Grell (1968).

(ii) Measurement of variegation

Preliminary examination of the eyes of a large number of w^{m4} males with none, one or two Y chromosomes showed that variegation invariably occurs either in both eyes or not at all. No marked differences between the two eyes could be observed and in accordance with other workers we used left eyes only. For each fly investigated, an image of the eye was projected onto a separate sheet of graph paper at a magnification such that the total area of the eye corresponded to about 800 subdivisions. The colourless regions found in $w^{m4}/0$ flies were outlined; since the eye contains about 800 ommatidia (Shoup, 1966), the number of subdivisions within an outlined region was an approximation of the number of ommatidia it contained. Any pigmented ommatidia within such a region were recorded individually, as were the unpigmented ommatidia found in the eyes of w^{m4}/Y and w^{m4}/YY flies. To simplify graphic presentation, each tracing was overlaid

by a grid in which each square corresponded to 9 ommatidia. The average number of unpigmented ommatidia in each of the 108 sites (complete and incomplete squares) thus obtained was determined for each of the six experimental groups.

(iii) *Statistical methods*

The pooled relative frequencies of unpigmented ommatidia at the affected sites in each of the experimental groups, displayed in plates 2 and 3, have been "smoothed" to remove local fluctuations attributable to sampling errors by fitting a series of regression surfaces, *i.e.*, the three-dimensional analogue of regression lines, to the pooled frequencies. The 108 sites of the eye were divided into 16 regions and separate regression surfaces were fitted in each region subject to the constraint that each surface joined up smoothly with its neighbour. This is technically described as a bicubic spline surface (Hayes and Halliday, 1974). This method is liable to produce spurious numerical anomalies at the edges of the region of estimation, so the numerical calculations were performed by adding "dummy frequencies" at artificial sites around the edges of the eye, extrapolated from those at the perimeter. These dummy frequencies are included in the diagrams.

The numerical calculations were performed on an ICL 1906S computer, and use the "Numerical Algorithms Group" library routines E02DAF, E02DBF and E02ZAF (NAG, 1976). The diagrams in plates 2 and 3 were drawn with the aid of a CALCOMP 1036 incremental plotter and utilise isometric projection routines, with removal of hidden lines, provided by the Computing Services Department of the University of Bradford.

3. RESULTS

(i) *Phenotype*

The number and distribution of unpigmented ommatidia vary markedly between individuals with the same chromosome constitution and cultured at the same temperature. In w^{m4}/Y and w^{m4}/YY flies they are scattered singly or in small groups in the more central regions of the eye, frequently in a limited area, and their distribution—as far as one can tell from the small numbers involved—appears to be non-random, with some tendency towards a more or less radial arrangement. The eye colour in these flies is predominantly wild-type and the unpigmented or lightly pigmented ommatidia (Nolte, 1950) appear somewhat darker than the background (plate 1(a)), probably because, unless viewed vertically, the pigment from the neighbouring ommatidia is visible through their transparent cell walls (Muller, 1930). In $w^{m4}/0$ flies most of the unpigmented ommatidia occur in larger groups and appear white. Some individuals have predominantly pigmented eyes with small white patches in the middle and at the anterior, ventral and dorsal periphery; at the other extreme, pigmented ommatidia occur in radial streaks, small groups, or even singly against a white background. Plate 1(b) illustrates an intermediate pattern. The pigmented ommatidia of $w^{m4}/0$ flies are brownish and paler than wild-type; they darken with age but never achieve the red of the wild-type.

FIG. 1. Eye areas affected by variegation. The symbols indicate which of the experimental samples (each consisting of 40 flies) contained at least one individual with unpigmented ommatidia in the particular area of the eye.

	w^{m4}/YY		w^{m4}/Y		$w^{m4}/0$		No. of flies
	25	15	25	15	25	15
+	+	+	+	+	+	37
-	+	+	+	+	+	12
+	-	+	+	+	+	3
+	+	-	+	+	+	3
-	-	+	+	+	+	3
-	+	-	+	+	+	5
+	-	-	+	+	+	1
+	+	-	-	+	+	1
-	-	-	+	+	+	10
-	-	+	-	+	+	1
-	+	-	-	+	+	3
+	-	-	-	+	+	2
blank	-	-	-	-	+	+	27
No. of flies	47	61	58	74	108	108	

Note that every part of the eye contained unpigmented ommatidia in at least one $w^{m4}/0$ animal in each temperature group.

(ii) *Variegation frequency*

From table 1 it can be seen that most w^{m4} males with the normal sex-chromosome constitution had variegated eyes, although in general only a very small proportion of the ommatidia in a given eye were unpigmented. Upon loss of the Y chromosome all males were variegated at both temperatures, and there was a more than 10-fold increase in the number of unpigmented ommatidia. Conversely, an additional Y chromosome significantly reduced the occurrence of variegated flies and caused a 3- and 4-fold reduction in unpigmented ommatidia; at least in regard to the occurrence of affected ommatidia, loss of the Y chromosome is evidently far more effective than gain. In spite of the marked variation between individuals in a given group, the differences between the mean number of unpigmented ommatidia were statistically highly significant, in the series w^{m4}/YY 25°C, w^{m4}/YY 15°C, w^{m4}/Y 25°C, w^{m4}/Y 15°C, w^{m4}/O 25°C, w^{m4}/O 15°C. At the low culture temperature the proportion of unpigmented ommatidia were roughly doubled in the w^{m4}/Y and w^{m4}/YY groups and increased by about 50 per cent in the w^{m4}/O flies. Temperature had however an insignificant effect on the number of variegated individuals in any of the chromosomal groups, suggesting that culture temperature is a less effective modifier of variegation than Y-chromosome constitution.

(iii) *Variegation patterns*

In attempting a quantitative analysis of variegation patterns in different experimental groups, cognisance must be taken of the considerable variation within each group. For example, in one w^{m4}/Y fly cultured at 25°C there were only 13 unpigmented ommatidia distributed between 11 of the 108 sites into which the eye was arbitrarily divided, while another fly in the same group had 45 unpigmented ommatidia in 27 sites; only 8 of the affected sites corresponded in the two individuals. Such variation is obscured by the pooling of data but sampling errors may persist if relatively small numbers of flies are studied. It is not to be expected, therefore, that every site in the eye which is affected by variegation in a given experimental group will necessarily also be affected in a group with more extensive variegation, even if the pattern is not fundamentally different.

Fig. 1 illustrates, for each of the experimental groups, the sites which contained unpigmented ommatidia in at least one fly. The 25°C-reared w^{m4}/YY group had, as already shown, the fewest variegated flies and the smallest mean number of unpigmented ommatidia *per* eye. These ommatidia were distributed over a smaller proportion of the eye area than in any other group, namely in only 47 of the 108 possible sites. The affected area covered the centre of the eye and extended towards the postero-ventral rim. In the sample of 15°C-reared w^{m4}/YY flies, with about twice as many unpigmented ommatidia *per* eye, a total of 61 different sites exhibited variegation, *i.e.*, there was a net increase of 14 sites. Five sites affected in the w^{m4}/YY 25°C group were however not variegated in any w^{m4}/YY 15°C fly, probably due to random sampling error. At the lower temperature the area of eye affected by variegation was enlarged fairly symmetrically but perhaps with a tendency to extend ventro-anteriorly and dorso-posteriorly. The proportion of unpigmented ommatidia in 25°C-reared w^{m4}/Y animals was about twice that in the w^{m4}/YY 15°C group but the areas involved scarcely

TABLE 1
Effect of Y chromosome constitution on frequency of w^{m4} variation at two culture temperatures

Culture temperature Sex chromosome constitution	25°C			15°C		
	w^{m4}/YY	w^{m4}/Y	$w^{m4}/0$	w^{m4}/YY	w^{m4}/Y	$w^{m4}/0$
Flies examined	200	200	200	200	200	200
Variegated flies	158	192	200	163	192	200
* <i>P</i>	<0.001	<0.02		<0.05		
Variegated flies	40	40	20	40	40	20
Total no. of unpigmented ommatidia [†]	316	1003	6686	553	1951	10 512
Per cent unpigmented ommatidia/eye [‡]	0.99	3.13	41.79	1.73	6.10	65.70
† <i>P</i>	<0.001	<0.001		<0.001	<0.001	
Unpigmented ommatidia/eye (<i>M</i> ± <i>SE</i>)	7.90 ±0.625	25.075 ±1.515	334.3 ±21.91	13.825 ±1.379	48.775 ±2.930	525.6 ±18.02

* Two by two contingency test.

! Counts from left eyes only.

‡ Assuming an average of 800 ommatidia/eye.

† Wilcoxon's rank test.

PLATE 1. Photomicrographs of the left eyes of adult flies, mounted in oil. Magn. $\times 162$. (a) w^{m4}/Y fly reared at 25°C . Unpigmented ommatidia, isolated or arranged in patches of various sizes, appear darker than the surrounding wild type ommatidia (see text). (b) w^{m4}/O fly reared at 25°C . Most ommatidia in this specimen are unpigmented and appear colourless; the darker, pigmented ommatidia are arranged in isolated patches or approximately radial streaks.

XYY

PLATES 2-4. Mean number of colourless ommatidia in each of the 108 arbitrary areas into which the eye was divided. In (a) and (c) the lines represent contours of equal variation, fitted to computer-smoothed data; (b) and (d) show perspective views of three-dimensional surfaces fitted to the same data (for details see Materials and Methods). X refers to the chromosome carrying $ln(l)w^{m4}$.

XY

PLATE 3

XO

PLATE 4

differed in size or shape, and in fact slightly fewer (58) sites were affected in the more variegated group. In 15°C-reared w^{m4}/Y flies, with a further and approximately two-fold increase in variegation, the area affected was increased to a total of 74 sites, and essentially only the dorsal and ventral edges of the eye remained unaffected. All 108 sites were affected in w^{m4}/O flies reared at either temperature.

Plates 2 and 3 show, respectively, computer-smoothed contour maps and three-dimensional perspective views of variegated eyes drawn using pooled data for each of the experimental groups. These figures include the area information inherent in fig. 1, with the additional information on the relative frequency of unpigmented ommatidia at each of the affected sites.

In all experimental groups there was a marked tendency for unpigmented ommatidia to occur preferentially near the centre of the eye. In w^{m4}/YY animals reared at either temperature (plate 2) the probability of finding unpigmented ommatidia decreased rapidly and approximately symmetrically from the centre, but with a somewhat increased incidence just at the posterior edge of the eye. The pattern in 25°C-reared w^{m4}/Y animals (plates 3(a and b)) was very similar, except that the posterior "hump" was absent; this apparent difference in pattern may have been due merely to sampling error. With increased variegation in the 15°C-reared w^{m4}/Y group the overall pattern remained approximately radially symmetrical (plates 3(c and d)) with perhaps a slight tendency for elongation along the posterior-anterior axis, *i.e.*, somewhat less variegation dorsally and ventrally. In the highly variegated w^{m4}/O animals (plate 4) unpigmented ommatidia were found in all parts of the eye, but still with the highest proportion near the centre; a number of other smaller regions with a relatively high proportion of unpigmented ommatidia were present around the periphery of the eye.

4. DISCUSSION

(i) *Types of variegation patterns*

The extensive literature on position-effect variegation includes descriptions which are apparently inconsistent or even frankly contradictory, largely due to the wide variety of criteria applied over the years and to the varied manifestation of the phenomenon in different strains and in the same strain under different genetic and environmental conditions. The initial description of w^{m4} (Muller, 1930) corresponds closely to the phenotype shown by our w^{m4}/Y and w^{m4}/YY flies, whereas a subsequent description (Schultz, 1943) tallies with patterns we found in the w^{m4}/O groups; there are presumably different dark and light lines of this strain such as those described by Muller (1930) for other strains with the fine-spotted type of pattern. The distinction between fine-spotted and large-spotted patterns is itself not always agreed on (*e.g.*, compare Spofford, 1976, and Becker, 1978, in relation to $w^{m258-18}$) and to distinguish sharply between these two types may be an over-simplification.

In eyes regarded as showing large-spotted variegation the unpigmented ommatidia are typically restricted to one or more of about 20 regions, each of which represents a clone of cells derived from a precursor cell present in the first larval instar. Where unpigmented ommatidia are mostly distributed singly or in small groups, on the other hand, the pattern is thought

to result from a developmental event occurring at or about the time of the final cell division in late third larval instar or the early pupal stage (Becker, 1978; Spofford, 1976). Although none of the w^{m4} eyes we studied were typically large-spotted, in some cases the unpigmented ommatidia, mingled with pigmented ones, did appear to be confined to areas corresponding to clonal regions. Spofford (1976) has suggested that such a distribution is due to stem cells in the early eye *Anlage* being determined in such a way that all the descendants of a given cell have an equal probability of subsequently undergoing inactivation of the white locus. Most of the patterns we observed in w^{m4} are consistent with a relatively late developmental event, the precise time of which is not however identical in all individuals. For example, in some eyes the unpigmented ommatidia were distributed in diffuse, elongated groups oriented more or less vertically in the eye, and lying across several of the supposed clonal boundaries. Such groups might be interpreted as resulting from an event affecting all cells, which happened at that moment to be in a particular stage of the cell cycle, since it is known (Ready *et al.*, 1976) that waves of mitosis start at the back of the eye and travel forwards. In other cases, particularly in the largely unpigmented eyes of $w^{m4}/0$ animals, relatively small, compact and elongated groups of pigmented ommatidia lay with the long axis of the groups pointing towards the centre of the eye. Studies of X-ray-induced twin spots (Becker, 1978) indicate that in the earliest cell divisions in the eye the mitotic spindles are oriented parallel to the long axes of the clonal areas seen subsequently, but that in later mitoses the spindle is more commonly at right angles to these axes. Some of the radially-arranged streaks of ommatidia may be due to an event occurring just before the end of the mitotic period; it is not however clear whether all such groups can be explained in this way, since the orientation of the last few cell divisions in different parts of the eye is not known.

(ii) *Pigmentation gradient*

Becker's observations on the ventral half of the eye of $w^{m258-18}$ (1961) showed clearly that the posterior region is less frequently affected than the anterior region, resulting in an anterior-posterior gradient. This agrees with the observations of Sutton-Gersh (1952) that the mid-dorsal region is least susceptible and in some cases the only region which does not show variegation; in $Dp(1; 3)N^{264-58}$ and *mot-28* Sutton-Gersh found a similar pigment distribution, the dorso-posterior region being unaffected in all *mot-28* flies examined. Our cursory observations on $w^{m158-21}$ (unpublished) also showed pigment preferentially located in the ventro-posterior region. A more detailed investigation of $T(1; 4)w^{mKrist}$ in *D. hydei* (van Breugel, 1972) revealed an anterior-posterior gradient and a less marked oblique dorso-ventral gradient, pigment being preferentially located in the upper posterior part of the eye. In w^{m4} the pigment appears to be distributed in a very different manner: the unpigmented ommatidia are concentrated towards the middle of the eye in all groups, and their frequency decreases towards the rim. The flies without a Y chromosome *i.e.*, those with very marked variegation are more susceptible to position effect in anterior than posterior regions and are in this respect similar to the other variegated strains of *D. melanogaster*. There is also a trend towards the oblique in the sense that

susceptibility appears to be greater in the upper anterior than lower posterior region on the one hand and in the lower anterior than upper posterior region on the other. The most obvious feature of all w^{m4} eyes we looked at, however, namely that the region of greatest susceptibility is in the middle, sets the pattern aside from those cited above.

The marked tendency for unpigmented ommatidia to occur preferentially near the centre of the eye in w^{m4} is difficult to interpret in developmental terms. To account for her observations in other strains, Sutton-Gersh (1952) postulated a diffusion gradient starting at the back of the eye and suggested that in regions where the concentration of the hypothetical diffusing substance is high at some critical time most or all cells subsequently become pigmented, while in regions with a lower concentration, such as the front of the eye, the pigmentation is more variable. Such a posterior-to-anterior gradient explains neither the patterns we found nor those of Judd (1975), who observed "halo" patterns, with unpigmented cells in the middle of the eye "... almost as if the w^+ gene were not functioning in the eye itself but is forming its product in surrounding tissues with diffusion of the product into the eye". Variations of the basic pattern occurred and could be inherited; in one group of variants Judd found the pigment was distributed in a variegated pattern closely resembling the pepper-and-salt type. It is possible that some form of radial diffusion gradient could explain our observations along the lines suggested by Judd. As an alternative, one might postulate the formation in the eye itself of a substance which inhibits pigmentation. Diffusion of such a substance out of the eye into the neighbouring tissues could result in the formation of a gradient which might formally explain the observations, but there is no evidence to suggest what such a material might be.

(iii) *Effect of temperature and Y chromosome constitution on pattern*

The results show that in each of the chromosomal groups the variegation pattern is similar at both culture temperatures. One must conclude that the lower temperature, in increasing sensitivity to the position effect, emphasises the pattern shown at the higher temperature but does not change its nature. Although most evident in the middle of the eye, this is shown in a general way at the periphery, where one might expect the small number of flies involved, the low frequency of unpigmented ommatidia at least in the w^{m4}/YY and w^{m4}/Y groups, and the difficulty in reliably counting or estimating the proportion of unpigmented ommatidia in sites around the rim of the eye, to lead to greater experimental error than in the more central area.

In considering whether the Y chromosome has an effect on pattern, several factors must be taken into account. It has already been pointed out in the previous section and elsewhere (Hartmann-Goldstein and Koliantz, 1981) that Y chromosome constitution is a more potent modifier of w^{m4} position effect than culture temperature. Also of relevance are the asymmetric shape of the eye itself and the observations that sensitivity varies round the edge of the eye and that the most sensitive region is more or less in the middle. Within these constraints, the apparent differences in the overall pattern associated with differences in Y chromosome constitution are most readily interpreted as a consequence of the spread of the sensitive

area and increase in intensity of the effect, with successive enhancement of variegation brought about by both lowered temperature and reduction in Y chromosome material. In other words, the different patterns seemingly associated with different numbers of Y chromosomes are due to the combination of the factors determining the degree of the effect, and reflect the spread of, and increase in, sensitivity within the limits dictated by the constraints referred to above; they do not reflect an effect of Y chromosome constitution *per se*. According to this view, the changes in pattern follow the same sequence shown by variegation frequency, *i.e.*, w^{m4}/YY 25°C, w^{m4}/YY 15°C, w^{m4}/Y 25°C, w^{m4}/Y 15°C, $w^{m4}/0$ 25°C, $w^{m4}/0$ 15°C. Consistent with this view is the fact that the two most alike patterns are found in the 15°C-reared w^{m4}/YY group and the 25°C-reared w^{m4}/Y group.

(iv) Eye colour

Each ommatidium in *Drosophila* contains primary, secondary and tertiary pigment cells (Ready *et al.*, 1976). Two main types of pigment are normally present: a red pigment believed to be made up of three closely related compounds, the drosopterins (Ziegler and Harmsen, 1969) and a brown pigment, which is an ommochrome belonging to the ommatin class (Dickinson and Sullivan, 1975). According to Shoup (1966), the granules bearing the ommochromes are present in primary and secondary pigment cells whereas the drosopterin-bearing granules are restricted to the secondary pigment cells. In a histological study of eye-pigmentation, Nolte (1950) observed that the pigment granules in w^{m4} males with the standard sex-chromosome constitution are morphologically the same as those in the wild type but can be absent in some of the normally pigmented cells within a given ommatidium. Such partially pigmented ommatidia would not have been readily detected in the present work but if there were a tendency for pigmentation to be absent from secondary pigment cells rather than randomly from both types, it might be responsible for the brownish colour we observed in $w^{m4}/0$ males, where the inversion affects many more ommatidia than in either w^{m4}/YY or w^{m4}/Y flies. Ommochrome is already visible in the eyes of a two-day-old pupa whereas drosopterin synthesis does not start until the third day of pupation (Shoup, 1966); an alternative interpretation for the brownish colour is that the white locus becomes inhibited after the formation of the brown but before the synthesis of the red pigment. The latter speculation makes certain predictions as to the timing of the control of pigment formation which should be testable.

5. REFERENCES

- BECKER, J. H. 1961. Untersuchungen zur Wirkung des Heterochromatins auf die Genmanifestierung bei *Drosophila melanogaster*. *Zool. Anz. Suppl.*, 24, 283–291.
- BECKER, H. J. 1981. Mitotic Recombination and position effect variegation. In Gehring, W. J. (ed.) *Results and Problems in Cell Differentiation*, vol. 9, Springer-Verlag, Berlin, Heidelberg, pp. 29–49.
- BREUGEL, F. M. A. VAN 1972. The effect of eye reduction on white position-effect variegation in *Drosophila melanogaster*. *Genetica*, 43, 25–42.
- DICKINSON, W. J. AND SULLIVAN, D. T. 1975. Gene-enzyme systems in *Drosophila*. In Beermann, W., Reinbert, J. and Ursprung, H. (eds.) *Results and Problems in Cell Differentiation*, vol. 6, Springer-Verlag, Berlin, pp. 1–163.

- HARTMANN-GOLDSTEIN, I. AND KOLIANITZ, G. 1981. Modifications of gene suppression in *Drosophila melanogaster* by sex chromosomes. 1. Effect of the Y chromosome on variegation in Malpighian tubules of w^{m4} larvae. *Heredity*, 47, 337-345.
- HAYES, J. G. AND HALLIDAY, J. 1974. The least squares fitting of cubic spline surfaces to general data sets. *J. Inst. Math. Applics.*, 14, 89-103.
- JUDD, B. H. 1975. Genes and chromosomes of *Drosophila*. In Peacock, W. J. and Brock, R. D. *The Eukaryote Chromosome*, Aust. Nat. Univ. Press, Canberra, pp. 169-184.
- LINDSLEY, D. L. AND GRELL, E. H. 1968. Genetic variations of *Drosophila melanogaster*. *Car. Inst. Wash. Publ. No. 627*.
- MULLER, H. J. 1930. Types of visible variations induced by X-rays in *Drosophila*. *J. Genet.*, 22, 299-334.
- NAG, 1976. NAG Mini Manual for the ICL 1900-series. Numerical Algorithms Group Limited, 7 Banbury Road, Oxford.
- NOLTE, D. 1950. The eye-pigmentary system of *Drosophila*: the pigment cells. *J. Genet.*, 50, 79-99.
- READY, D. F., HANSON, T. E. AND BENZER, S. 1976. Development of the *Drosophila* retina, a neuro-crystalline lattice. *Developml. Biol.*, 53, 217-240.
- SCHULTZ, J. 1943. A derivative of $In(1)w^{m4}$ showing a different reaction to heterochromatin. *Dros. Inf. Ser.*, 17, 64-65.
- SHOUP, J. R. 1966. The development of pigment granules in the eyes of wild type and mutant *Drosophila melanogaster*. *J. Cell Biol.*, 29, 223-249.
- SPOFFORD, J. B. 1976. Position-effect variegation in *Drosophila*. In Ashburner, M. and Novitski, E. (eds.) *The Genetics and Biology of Drosophila*, vol. 1c, Academic Press, London, pp. 955-1018.
- SUTTON-GERSH, E. 1952. Pigmentation in a mottled white eye due to position effect in *Drosophila melanogaster*. *Genetics*, 37, 322-338.
- ZIEGLER, I. AND HARMSSEN, R. 1969. The biology of pteridines in insects. *Adv. Insect Phys.*, 6, 139-203.