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MODIFICATION OF GENE SUPPRESSION IN *DROSOPHILA MELANOGASTER* BY SEX CHROMOSOMES 3. HETEROCHROMATISATION ASSOCIATED WITH THE $w^{m}4$ PHENOTYPE

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SUMMARY

The position effect of $In(1)w^{m4}$ on the inversion-bearing X chromosome was studied in salivary glands of w^{m4}/YY, w^{m4}/Y and $w^{m4}/0$ larvae cultured at 25°C and 15°C. Heterochromatisation extended along not more than 6 bands, usually fewer. It occurred adjacent to the proximal inversion breakpoint but not the distal one, suggesting that the whole or part of the white locus lies within the inverted region. Several lines of evidence are consistent with the hypothesis that the distal breakpoint is within the locus, separating a regulatory from a structural subunit.

The *Y* chromosome constitution markedly affected the proportion of cells exhibiting heterochromatisation, which ranged from 0 per cent in w^{m4}/YY larvae reared at 25°C to 22·4 per cent in $w^{m4}/0$ larvae reared at 15°C. Comparison of the data with our previous findings on white-variegation in larval Malpighian tubules and adult eyes showed good correlation between the proportion of affected cells in salivary glands and Malpighian tubules but the eyes were relatively more affected. These differences between larval and adult tissues might point to a relationship between the developmental stage at which an organ is determined and the degree to which it is affected by the inversion.

1. INTRODUCTION

A series of fundamental studies on variegated position effect in *Drosophila* led Schultz (*e.g.*, 1965) to the conclusion that cell phenotype is causally related to the state of compaction of the chromosome region containing genes which govern that phenotype. The conclusion was based on the observations that (1) many of the variegated phenotypes occur in flies which have chromosomal rearrangements with a breakpoint in heterochromatin, (2) the genes with mosaic expression are near the heterochromatic breakpoint, and (3) in salivary gland chromosomes, the regions containing the affected genes are compacted in some cells. The observation that variegation and heterochromatisation of the relevant loci are well-correlated (Hartmann-Goldstein, 1967; see also Henikoff, 1981, for analogous findings on a translocated heat shock puff locus) is consistent with Schultz' conclusion. It is now widely accepted that the compaction is a position effect exerted by the neighbouring heterochromatin, and that it impairs gene function.

In variegated tissues the distribution of affected cells is non-random and, particularly in eyes, certain fundamental variegation patterns can often be identified which differ from strain to strain (reviewed by Spofford, 1976).

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However, it has been observed repeatedly that the degree to which variegation for a particular character is expressed may differ from one tissue to another; in a given tissue differences can also occur between individuals from the same strain. Some of the factors which influence the phenotype, such as distance of the affected locus from the breakpoint and the time during development at which the cell potential is determined, have been identified. Others, for instance the direction in which the position effect passes along the chromosome, are speculative. Regrettably there are many observations for which explanations cannot as yet even be guessed at.

One way forward is to determine which of the factors influencing the phenotype do so by altering chromosome conformation. So far it has not been possible to study gene expression and chromosome morphology in the same cells. An alternative, albeit less direct, approach is to analyse the phenotype of affected organs and heterochromatisation in the same strain. We recently obtained quantitative information on white-variegation in Malpighian tubules and eyes of males carrying *In(1)w*^{m4} (Hartmann-Goldstein and Koliantz, 1981; Koliantz *et al.*, 1984). The present paper deals with observations on the salivary gland chromosomes of *w*^{m4}*/YY*, *w*^{m4}*/Y* and *w*^{m4}*/0* larvae in relation to some of the questions arising from phenotypic studies.

2. Materials and Methods

The stocks and breeding programmes used for our phenotypic studies of *w*^{m4} variegation, more fully described in Hartmann–Goldstein and Koliantz (1981), were again employed. Male larvae with the normal sex-chromosome constitution were taken from strain *In(1)w*^{m4}. The *w*^{m4}*/O* larvae were derived from the cross *O/C(1)RMy*^{2} *su(w*^{a}*)w*^{a}*/XYL.YS v f B* × *In(1)w*^{m4}*/Y*. To obtain *w*^{m4}*/YY* larvae, *In(1)w*^{m4}*N*$^{264\text{-}84R}$, *y sn/In(1)w*^{m4}*/Y* females were crossed with *w* males and from the male progeny with predominantly wild-type Malpighian tubules larvae with two Y chromosomes were selected by ganglial metaphase analysis.

Salivary gland analyses were done on lacto-aceto-orcein and aceto-orcein squash preparations of glands from late third instar larvae which had been kept throughout embryonic and larval life at 25°C or 15°C. To distinguish between heterochromatised and non-heterochromatised regions we used the criteria set out by Hartmann-Goldstein (1967).

3. Results and Discussion

In(1)w^{m4}, diagrammatically represented in fig. 1, is a long inversion in the X chromosome with the distal breakpoint between polytene chromosome bands 3C1 and 3C2 and the proximal breakpoint in region 20 (plate 2a). The 3C2 region is thus located adjacent to basal heterochromatin, and becomes heterochromatised in some cells (plate 2b). The proportions of affected cells in our material are shown in table 1. An earlier investigation of *w*^{m4} by Prokofieva-Belgovskaya (1947) revealed heterochromatisation in 26 per cent of cells, which seems to be much greater than in the strain we examined, but since the genetic and chromosomal constitution of the larvae she studied is not recorded the reason for the apparent discrepancy between the two studies is unclear.

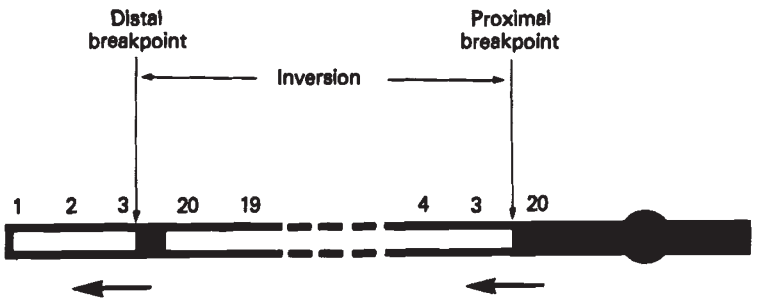

FIG. 1. Diagrammatic representation of *In(1)w*^{m4}. Shaded regions indicate basal heterochromatin, position of centromere is expanded. Heavy arrows denote direction in which position effect could theoretically spread; in practice, heterochromatisation was observed adjacent to the proximal breakpoint only. For details of breakpoints and position of white locus, see text.

TABLE 1

Proportion of salivary gland cells showing heterochromatisation adjacent to the proximal breakpoint of In(1)w^{m4}

Culture temperature	Sex-chromosome constitution	Larvae	Cells analysed	Cells heterochromatised	%
25°C	w^{m4}/YY	23	545	0	0
	w^{m4}/Y	31	368	5	1·36
	w^{m4}/O	39	483	78	16·15
15°C	w^{m4}/YY	25	588	3	0·51
	w^{m4}/Y	29	402	7	1·74
	w^{m4}/O	36	410	92	22·44

The white locus has been variously assigned to sites between bands 3C1 and 3C2 (Lefevre and Green, 1972; Judd, 1975), in 3C2 (Lefevre and Wilkins, 1966) and in distal 3C (Sorsa *et al.*, 1973; Bingham, 1981). An inversion with a break in centric heterochromatin can lead to variegation either if the affected locus is included in the inversion and transposed to the vicinity of centric heterochromatin, or if it is distal to the euchromatic break and thus adjacent to the inverted heterochromatin. For *In(1)w*^{m4} there is strong genetic evidence (*e.g.*, Lefevre and Wilkins, 1966) that the white locus lies within the inversion, and Bingham *et al.*, (1981) have mapped the white locus distal to the euchromatic break by molecular methods. Our cytological observations lead to the same conclusion. The data on heterochromatisation of the chromosomal region transposed to the vicinity of the proximal breakpoint (table 1) correspond closely to the data on colourless cells in Malpighian tubules (Hartmann-Goldstein and Koliantz, 1981; see also fig. 3) and observations on other white-variegated strains (Hartmann-Goldstein, 1967 and unpublished data) showed that there is good agreement between the frequency of heterochromatisation of the *white* region and of colourless Malpighian tubule cells. None of the almost 3000 cells we examined showed heterochromatisation of the non-inverted region distal to the euchromatic break. According to Prokofieva-Belgovskaya (1947), the inverted heterochromatin has a tendency to become morphologically

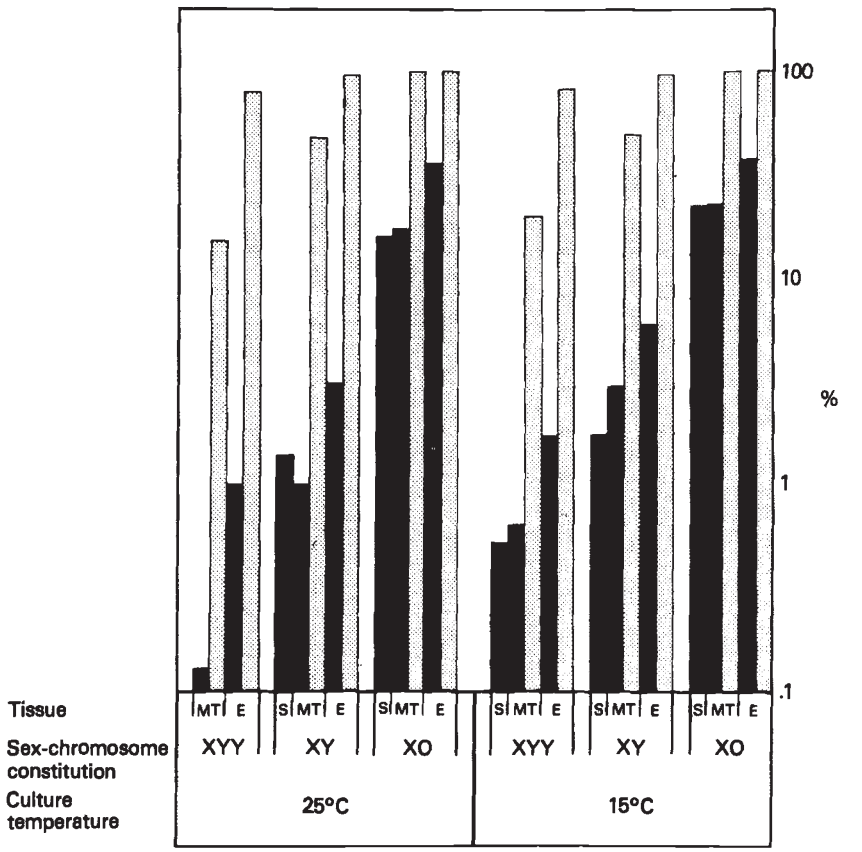

FIG. 3. Frequency of heterochromatisation at 3C2-3 in salivary glands (S) and of w^{m4} variegation in Malpighian tubules (MT)* and eyes (E)†, presented as proportion of affected individuals (shaded columns)‡, and mean proportion of affected Malpighian tubule cells and ommatidia in variegated individuals (solid columns). Log scale.

* data from Hartmann-Goldstein and Koliantz (1981)
† data from Koliantz *et al.*, (1984)
‡ not available for salivary glands: see text
'X' refers to the chromosome bearing $In(1)w^{m4}$

euchromatic, which argues against a potential for a heterochromatic position effect and supports our conclusion that, since the position effect is visible only adjacent to the centric heterochromatin, the white locus has been inverted. It should be noted in this connection that there are inherent problems in scoring heterochromatisation (Hartmann-Goldstein, 1967), especially when inversions are being analysed (Hartmann-Goldstein and Wargent, 1975), which are exacerbated when the breakpoint is in or near a region prone to distortion, such as 3C2-7. We cannot exclude the possibility that some of the regions we classified as heterochromatised were merely distorted, nor can we be sure that in attempting to analyse only regions which could confidently be assigned to either the euchromatic or heterochromatic state we non-randomly excluded heterochromatised regions whose appearance was intermediate between the two states. However, since the

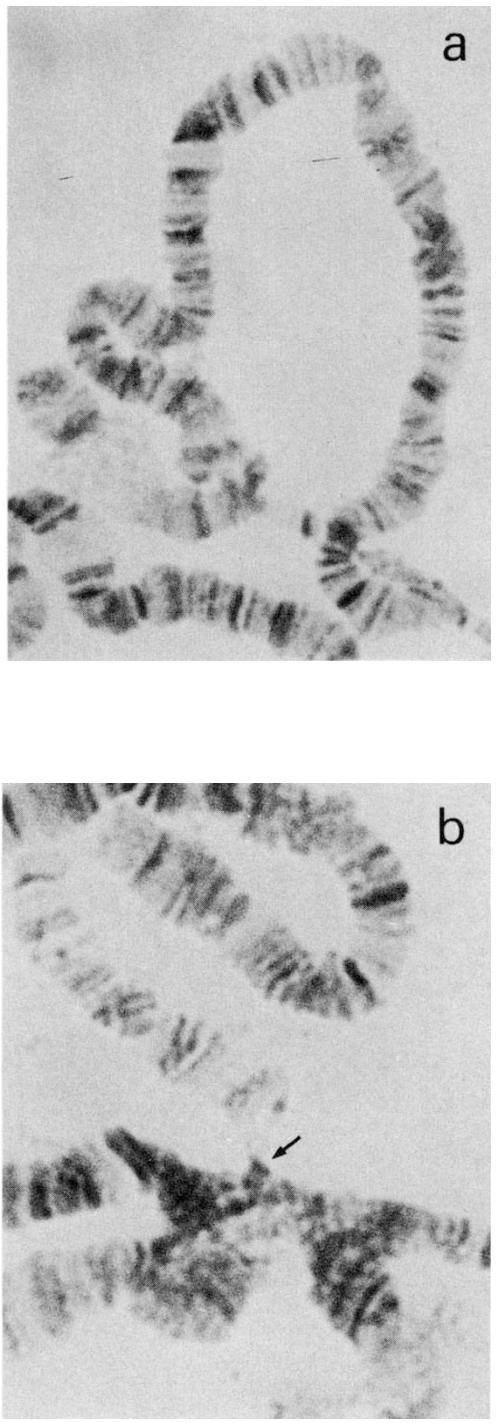

PLATE. 2. a. Heterozygous w^{m4} inversion. b. Heterochromatisation (arrow) in w^{m4} hemizygote.

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former type of misclassification would lead to an over-estimate of heterochromatisation frequency and the latter to an underestimate, errors would have tended to counteract each other.

In view of the early recognition (Gowen and Gay, 1934) that the Y chromosome modifies position-effect variegation, there has been surprisingly little information about its effect on heterochromatisation. Prokofieva–Belgovskaya (1947) reported that region 1AB1–20ABC of the sc^8 chromosome is more frequently heterochromatised in XX than in XXY females; according to Schultz (1965) an extra Y chromosome reduces heterochromatisation in the 3D region of $w^{m258\text{-}21}$ heterozygotes; lastly, the studies of Ananiev and Gvozdev (1974) on *Dp(1;f)R* suggested that loss of the Y chromosome is accompanied by heterochromatisation of bands 1A3-4 in a large proportion of cells. The general conclusion from these findings, that heterochromatisation-frequency is reduced on addition of a Y chromosome and increased when the Y chromosome is lost, is confirmed by our data on w^{m4} males set out in the table. The table also shows that suppression of the position effect was relatively greater when a Y chromosome was added to the w^{m4}/O than to the w^{m4}/Y complement. The same applied to w^{m4} variegation in Malpighian tubules (Hartmann-Goldstein and Koliantz, 1981) and eyes (Koliantz *et al.*, 1984). A summary of the data for the three tissues is illustrated in fig. 3. It can be seen that the frequencies of salivary gland and Malpighian tubule cells affected by the rearrangement were almost identical in w^{m4}/O larvae and very similar both in w^{m4}/Y and w^{m4}/YY larvae. The values for affected ommatidia were consistently higher. Since ommatidia consist of groups of cells and since in w^{m4} flies some ommatidia contain both pigmented and unpigmented cells (Nolte, 1950), it is not strictly valid to compare variegation in eyes with that in Malpighian tubules or salivary glands, but in our view this is unlikely to account for the two- to-three-fold differences observed. Similar differences between eyes and testis sheath have been ascribed to the fact that eyes are of ectodermal and testis sheath of mesodermal origin (Hessler, 1961). We could analogously invoke ectodermal origin of eyes and endodermal origin of Malpighian tubules, but since both salivary glands and eyes are ectodermally derived, embryonic origin does not provide an adequate explanation either. When salivary glands are squashed the relative positions of the cells are disrupted, which precludes an analysis of the patterns of affected and unaffected cells in the intact glands. The fine-spotted variegation pattern in Malpighian tubules and eyes of w^{m4} males suggests that in these organs inactivation of the white locus is specified shortly before mitotic divisions cease (Spofford, 1976). Salivary glands and Malpighian tubules develop at roughly the same time and very much earlier than the eye discs (Fullilove and Jacobson, 1978). A relationship between developmental stage at which a tissue is determined and the degree to which it is affected by a variegation-inducing re-arrangement would thus account for the differences we observed between larval and adult tissues and would be consistent with the observation by Schultz (1965) that gonads, separated off very early in embryonic development, are not affected at all.

In quantifying position-effect variegation and its modification, it is useful to distinguish between the average frequency of affected cells within individuals and the frequency of affected individuals in the population. When heterochromatisation is being scored this distinction is not possible.

In our experience, maximally 10 per cent of salivary gland nuclei are adequately analysable, even in cytologically favourable material, so that one cannot determine whether *all* cells in the glands from a particular larva are unaffected. We did however note the number of larvae in which at least 10 nuclei were analysed all of which were unaffected; by this criterion, 15 per cent of 15°C-reared w^{m4}/YY larvae, 10 per cent and 20 per cent respectively of 25°C- and 15°C-reared w^{m4}/Y larvae, and all w^{m4}/O larvae, were variegated, which is consistent with the proportions of individuals which have variegated Malpighian tubules and variegated eyes (fig. 3). The data do not reveal whether the relative difference between loss and gain of a Y chromosome, which is so marked when cells are being considered, also applies to individuals. Investigation of a strain in which the proportion of variegated flies is lower than in w^{m4} should provide such information.

In *D. hydei* van Breugel (1970) noticed that in white-mottled inversion strains with a euchromatic breakpoint proximal to the white locus the variegation pattern is fine-spotted, whereas if the breakpoint is distal to the locus the eyes show large-spotted variegation. He postulated that the locus contains two functional regions operating at different developmental stages, and proposed that the pattern is large- or fine-spotted depending on which of the two regions is closer to the breakpoint. In *D. melanogaster* several lines of evidence (Judd, 1975) suggest that in the rather complex white locus the proximal portion has regulatory function and the distal portion contains the structural gene sequence. One example quoted by Judd is strikingly reminiscent of the w^{m4} phenotype: a group of recessive white-spotted mutations involving the proximal region causes pigment to be deposited in small groups of facets scattered across the eyes, decreases the quantity of pigment formed, and results in a yellowish-brown pigment instead of the wild-type red. Judd suggested that these characteristics are best explained by some change in the control mechanism of the locus. To explain the generally fine-spotted nature of w^{m4} variegation in *D. melanogaster* on van Breugel's model, we must suppose that the proximal part of the locus is closer to the breakpoint than the distal part. This could theoretically be due to a position of the locus distal to the euchromatic breakpoint and thus affected by the inverted heterochromatin, but as already stated above, such an interpretation is not borne out by genetic, molecular or cytological evidence. There is however another possible explanation, consistent both with the model and the existing evidence: that the distal inversion breakpoint lies within the locus such that it separates the proximal from the distal portion. Heterochromatisation would then affect only the controlling portion. It is not clear what effect a wide separation of the putative structural and regulatory portions would have on the function of the locus in the absence of heterochromatisation. It is however interesting to note that there are regulatory mutants of *white* which act in both the *cis* and *trans* configurations (Bingham, 1980).

Position effect spreads from the heterochromatic breakpoint along the transposed chromosome region. Lefevre and Wilkins (1966) pointed out that in the few inversions known which are associated with white variegation, the breakpoint is very close to the white locus, whereas in many of the relatively numerous translocations affecting *white* the distance between the breakpoint and the locus is much greater. They concluded that position effect extends further in translocations than inversions. This is consistent

with our observation that in the w^{m4} chromosome heterochromatisation usually terminated at 3C2-3 and never spread beyond 3C7; the maximum extent was thus 6 bands as compared for example with 65 bands in the $w^{m258\text{-}21}$ translocation (Hartmann-Goldstein, 1967). In no case did we observe variegation for *rst*, *vt* or *N* all of which are located in the 3C2-7 region; Reuter *et al.* (1983) similarly found no phenotypic spreading in w^{m4} flies of normal chromosome constitution. During their extensive and valuable investigation of the effects of enhancer and suppressor mutants on w^{m4} variegation, Reuter and his collagues did however succeed in inducing spreading and involvement of adjacent genes with combinations of heterochromatin deficiencies, low culture temperatures and genetic modifiers. Thus there appear to be no lethal genes between *w* and *rst*. The reason for the relatively very limited spread of the position effect in the absence of such manipulations remains obscure.

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