Genet. Res., Camb. (1967), **10**, *pp.* 143–159
With 7 text-figures and 1 plate
Printed in Great Britain

On the relationship between heterochromatization and variegation in *Drosophila*, with special reference to temperature-sensitive periods

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(*Received* 21 *March* 1967)

1. INTRODUCTION

Certain chromosomal rearrangements in *Drosophila*, involving transposition of euchromatic chromosomal segments to the vicinity of heterochromatin, result in variegated phenotypes (for summary see Lewis, 1950). The variegation is attributed to suppression by heterochromatin of the activity of adjacent genes in some, but not all cells of the affected tissues. Morphologically, in larval salivary gland chromosomes the transposed euchromatin may take on a heterochromatic appearance (Caspersson & Schultz, 1938; Prokofieva-Belgovskaya, 1941). A causal relationship between gene inactivation and heterochromatization is supported by the fact that temperature can affect both phenomena. In w^{258-21} flies, which have variegated eyes and malpighian tubules, heterochromatization is enhanced by low temperature during development (Schultz, 1941). In strain sc^8, where variegation affects bristles, heterochromatization is depressed by temperatures either lower or higher than normal (Prokofieva-Belgovskaya, 1947). Gowen & Gay (1933) demonstrated that low temperatures enhance mottling of the eyes. Chen (1948) found that in mottled-eye strains temperature is effective during the pupal stage, and these results were confirmed by Becker (1960, 1961). On the other hand, variegation in adult malpighian tubules (Schultz, 1956) and bristles (Noujdin, 1945) is affected by temperature only during the embryonic stage.

The facts are consistent with the view that heterochromatin can, subject to environmental conditions, cause near-by euchromatin to become heterochromatic, and that this heterochromatization is causally related to gene inactivation and phenotypic variegation.

In the hope that further information would be obtained on this relationship and its connexion with developmental processes, a systematic and quantitative study of the effect of temperature on heterochromatization and variegation was undertaken.

2. MATERIALS AND METHOD

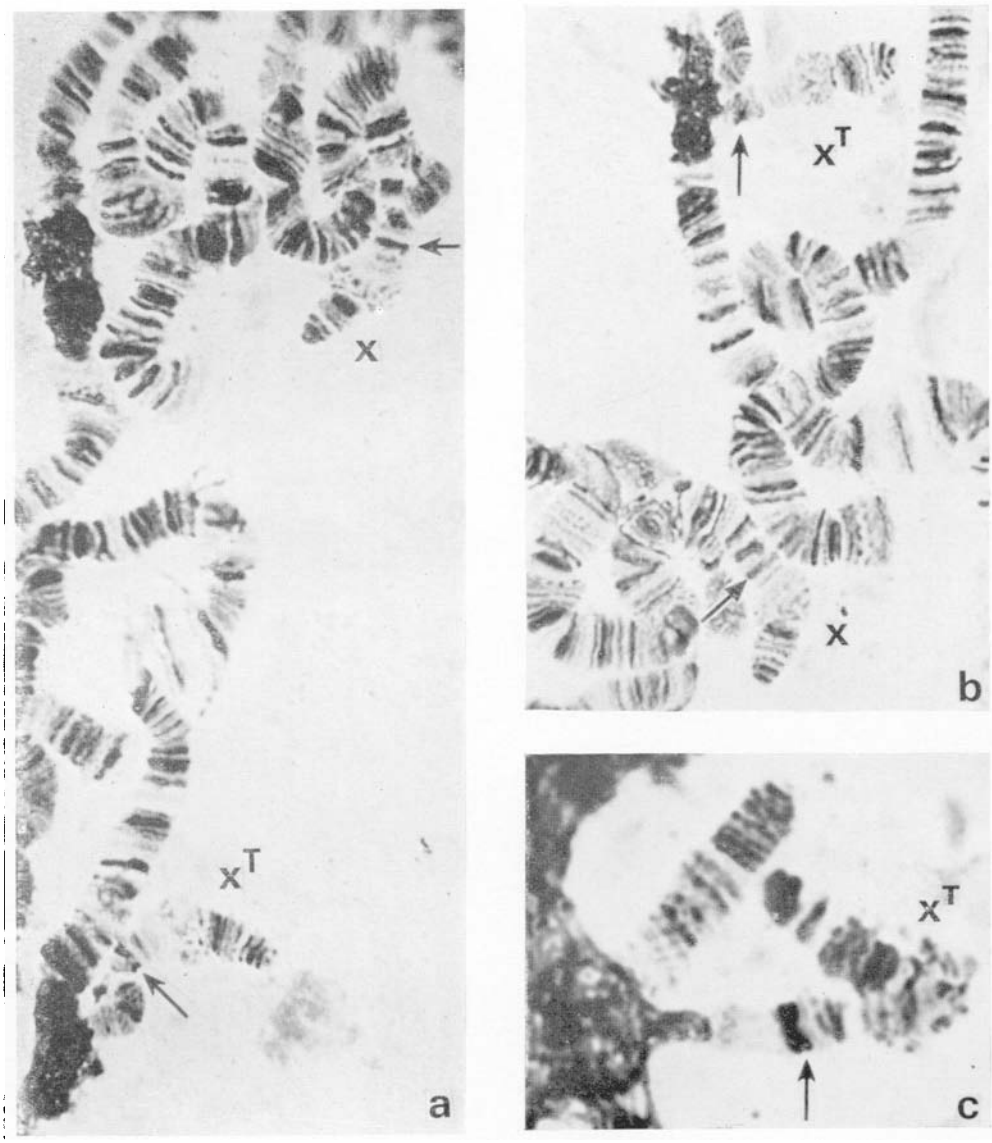
In *D. melanogaster*, heterochromatic position effect on band 3C7 of the X chromosome results in the notched phenotype, a wing abnormality with variable expression (for an analysis of this locus, see Welshons, 1965). Variegation in malpighian

tubules is expressed as patches of colourless cells in an otherwise yellow tissue, and results from a position effect of heterochromatin on the white locus situated on the X chromosome between bands 3C1 and 3C3, probably 3C2 (Lefevre & Wilkins, 1966).

The strain used for the present investigation, $\frac{T(1:4)\,w^{258-21}}{IN(1)\,\Delta 49\,w\,lz^{+}}$, carries an X-4 translocation having breaks between bands 3E5 and 3E6 and following 101 (Bridges & Brehme, 1944). The notched and white region is thus translocated to the vicinity of the chromocentre. Females heterozygous for the translocation and for the mutant gene *white* (*w*), which exhibit variegation of malpighian tubules and *Notch* (*N*), were used for these observations.

Three temperature groups were set up as follows: Twenty fertilized females, laying well, were introduced into fresh culture bottles and allowed to lay for 24 hours at 25°, 19°, and 14°C. respectively. They were then removed and the eggs left to complete development. Preliminary observations had indicated that *N* and variegation in malpighian tubules is affected by temperature during early embryonic life. To confirm this, two groups of cultures kept at 19°C. for the initial 24 hours were transferred to 25° and 14°C. respectively on removal of the adults. Since development from deposition till eclosion from the egg takes roughly 55 hours at 19°C., most of these individuals would have spent approximately half their embryonic life at 19°C. For observations on the effect of temperature at different times within the embryonic period, eggs were collected over one-hour periods on agar at 25°C. Half the cultures so obtained were transferred to 14°C., the rest left at 25°C. At 14°C. the embryonic stage lasts for roughly 100 hours, whereas at 25°C. the mean hatching time was found to be 22 ± 2 hours. Accordingly, cultures were transferred from 25° to 14°C. at intervals from 3 to 24 hours after the midpoint of the egg-laying period, and cultures at 14°C. were transferred to 25°C. after 4·5 to 120 hours. Some cultures were left at each of the original temperatures and served as controls.

Lactic-acetic-orcein squashes of salivary gland chromosomes were made from third instar larvae. The best preparations were selected and the extent of heterochromatization shown by the translocated segment was recorded for ten nuclei from each individual, according to the cytological map of Bridges (1938). Using these data, it was possible to calculate for each temperature group the percentage of nuclei showing heterochromatization up to and including band 3C1, 3C7 or not reaching 3C7. Larvae from which salivary glands had been used were dissected and the malpighian tubules exposed. The approximate number of colourless cells was noted for each individual, and larvae classified as having either no colourless cells (class 0), 1–9 % (class 1), 10–24 % (class 2), 25–49 % (class 3), 50–74 % (class 4) or 75–85 %—the highest percentage to be observed (class 5). To get a specific percentage for each larva, the mean of the class to which it had been assigned was ascribed to it, e.g. an individual belonging to class 3 was recorded as having 37·5 % variegation. Using these percentages, the mean variegation percentage for each temperature group was calculated. Finally, adult heterozygous females were collected, the



Heterochromatization in the translocated segment. The heterochromatized region is stretched and distorted (*a*), incorporated in the chromocentre (*b*), resembles the chromocentre (*c*). In (*a*) and (*b*) translocated and non-translocated homologues in the same nucleus are seen. Magnification (*a*) and (*b*) × 2,200, (*c*) × 3,000.

X = non-translocated homologue.
X^T = translocated homologue.
Arrows point to region 3A1-4.

https://doi.org/10.1017/S0016672300010880 Published online by Cambridge University Press

number showing any evidence of N recorded, and the percentage of N was calculated for each temperature group. Initially N was also calculated on the same basis as variegation in malpighian tubules, i.e. individuals were assigned to classes representing the approximate notched wing area, and the mean percentage for each temperature group was calculated. Comparison of figures from both types of calculations showed that although values from the latter were lower, correlation within each temperature group was good. For subsequent estimations of N only calculations based on the total number of flies showing N were employed, and no attempt was made to assess the degree of N.

To investigate the effect of temperature on the expression of N during the pupal period, eggs were collected over 1-hour periods on agar at 25°C. Each agar slab was then cut in half, one half transferred to 19°C. and the other left at 25°C. Late third instar larvae were picked off the sides of the culture bottles, transferred to petri dishes and kept under observation. White prepupae were collected in fresh culture bottles and transferred to the reciprocal temperature. Controls were treated similarly but kept at the original temperature.

To avoid subjective bias during observation, on setting up the experiments all test groups were assigned code numbers, and the results decoded only after the observations for each experiment had been completed.

3. RESULTS

(i) *Variation in appearance of heterochromatin*

In salivary gland chromosomes there is often considerable variation in the position of the border-line between heterochromatin and euchromatin (Dobzhansky, 1944). In the present material the heterochromatization, spreading from the translocation break distally along the X chromosome, varied in extent and appearance. The part nearest to the break was sometimes frankly heterochromatic, but varied in both intensity of staining and compactness of the chromatin, being densest when it extended furthest along the translocated segment. It often appeared shorter than the homologous euchromatic section of the non-translocated chromosome. Such heterochromatin was frequently separated from the euchromatin by a region intermediate in appearance, subject to similar variability. In addition, although bands were generally visible in the transitional regions, those nearest to the heterochromatin occasionally stained more deeply and appeared larger (due to increased nucleic acid content, as shown by Caspersson & Schultz, 1938). In a few instances, and exclusively in nuclei having relatively short heterochromatized regions, such bands were unusually pale.

(ii) *Effect of developmental temperature on heterochromatization*

To employ a consistent measure for the extent of heterochromatization, all clearly identifiable bands were classified as being euchromatic. Thus the most proximal distinct band visible was noted, and the heterochromatization taken to

Table 1. *Mean values for variegation in larval malpighian tubules*, Notch *and Heterochromatization at bands* 3C1 *and* 3C7 *from different temperature groups after conversion of percentages to degrees (Fisher & Yates*, 1963)

Temperature group	Variegation in malpighian tubules		Heterochromatization at 3C1		*Notch*		Heterochromatization at 3C7	
	No. of larvae	Mean	No. of cells	Mean	No. of flies	Mean	No. of cells	Mean
25	14	0·98 ± 1·48	122	0	629	6·19 ± 4·47	122	12·38 ± 6·02
19	22	25·56 ± 4·88	215	24·40 ± 8·15	638	50·67 ± 3·24	215	40·77 ± 8·88
14	15	50·27 ± 6·03	145	52·01 ± 8·65	223	73·33 ± 7·63	145	62·98 ± 7·59
19–25	19	21·0 ± 6·94	190	25·04 ± 3·84	326	38·58 ± 4·26	190	43·52 ± 7·68
19–14	20	24·25 ± 7·47	200	35·36 ± 6·46	59	61·00 ± 5·90	200	52·93 ± 5·47

reach as far as the adjacent, no longer distinguishable, band. The most proximal region of the translocated segment was frequently obscured by the chromocentre, and bands between 3D1 and the break were particularly difficult to see. For the data presented in Table 1 and Figs. 1–4, analysis was therefore carried out only distal to this region.

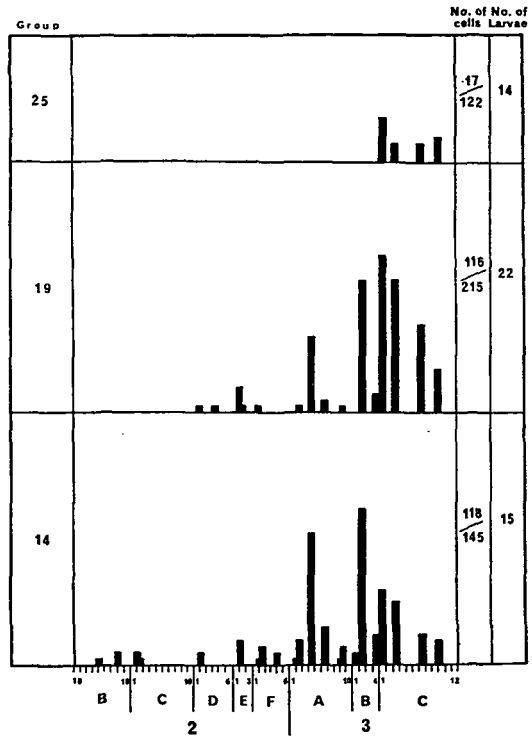


Fig. 1. Relation of heterochromatization to temperature during development. Each block of the histogram represents the number of nuclei exhibiting heterochromatization up to and including the chromosomal position indicated on the abscissa. The nuclei are divided into groups representing the temperature during development (25°, 19° or 14°C.). The second-last column gives the number of nuclei showing heterochromatization, over the total number examined in each group. The last column gives the number of individuals from which the nuclei were taken. A clear-cut correlation is seen between temperature and heterochromatization.

The results obtained in the various temperature groups are presented in the figures and Table 1. In all chromosomes studied the heterochromatized region was continuous with the chromocentre. In other words, cells showing heterochromatization at 3C1 were invariably heterochromatized also at 3C7, but not conversely. This is the cytological equivalent of the spreading effect associated with V-type position effects. An apparent tendency was noted for heterochromatization to progress preferentially to certain bands, e.g. 3C7, 3C2 and 3B2, all of which are relatively large.

Table 1 and Fig. 1 show the extent of heterochromatization in individuals kept

at three different temperatures throughout development. There was in general a good correlation between heterochromatization and temperature, both the number of nuclei showing heterochromatization, and the extent of heterochromatization, being greater at a lower temperature. The most distal band at which heterochromatization was seen to occur was 2B13 in an individual from the 14°C. group. This is a distance of 65 bands from the break. While comparison between the different groups gave clear-cut results, there was considerable variability inside a given group. Heterochromatization also varied between different nuclei from a

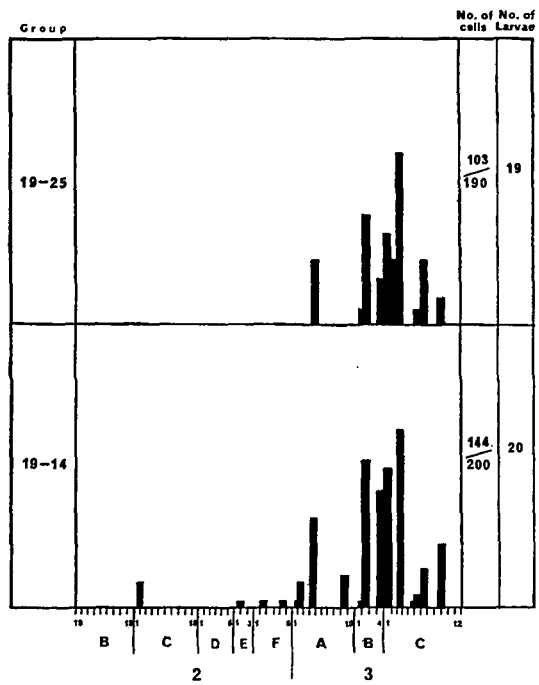


Fig. 2. Relation of heterochromatization to temperature during development, in individuals transferred from 19° to 25°C. or to 14°C. after 24 hours. Symbols as in Fig. 1. Animals left at 19°C. throughout development (see Fig. 1) show slightly more heterochromatization than those transferred from 19° to 25°C. after 24 hours, and slightly less than animals transferred from 19° to 14°C.

single individual, none being observable in some and other nuclei showing it to a varying extent. A tendency was, however, noticed for cells lying near each other in the squash preparation to have a similar degree of heterochromatization. Figure 2 shows the extent of heterochromatization in individuals of groups 19–25 and 19–14 (those transferred from one temperature to another after 24 hours). Since at 19°C. embryonic development takes roughly 55 hours, any effect of the earlier temperature must have been operative during the early embryonic period. The data show that heterochromatization after transfer to 25°C. resembled more closely that found in individuals kept throughout at 19°C. than in those kept throughout at 25°C. Comparison of Fig. 2 with Table 1 shows that individuals

transferred from 19° to 14°C. show more heterochromatization than those kept at 19°C., but less than those kept at 14°C. throughout.

It appears, therefore, that exposure to 19°C. during the first part of embryonic development is sufficient to induce the formation of more heterochromatin than development at 25°C., and that subsequent development at 19°C. causes only a moderate further increase. Development at 14°C. during the early period similarly

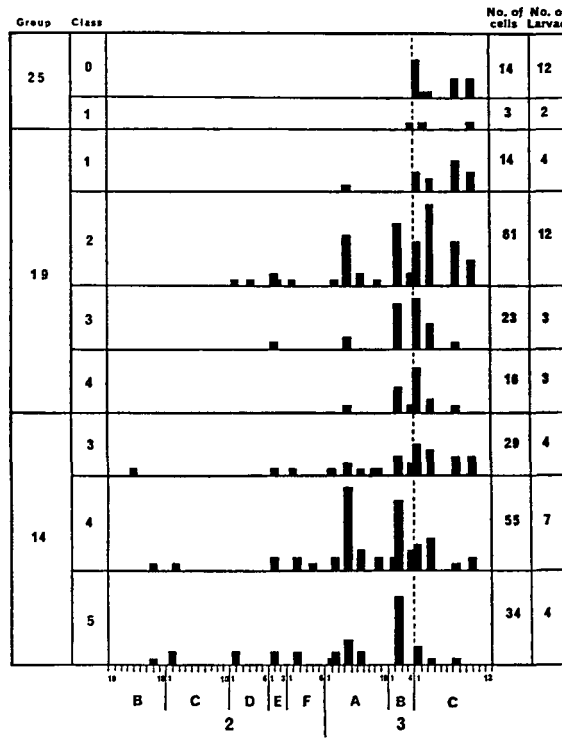


Fig. 3. Relation of heterochromatization to variegation in individuals reared at 25°, 19° or 14°C. Symbols as in Fig. 1, except that (1) the groups of nuclei are further divided according to the class of malpighian tubule variegation shown by the individual (see text), and (2) the second-last column gives the number of nuclei showing heterochromatization in each group. Close agreement is seen between variegation and developmental temperature, but there is no apparent correlation between heterochromatization and variegation inside a given temperature group.

causes more heterochromatization than does development at 19°C., and some further increase results from continued exposure to the lower temperature. It might be argued that the tendency towards increased heterochromatization in the 19–14 group and decreased heterochromatization in the 19–25 group, as compared with the 19 group, could be due to those individuals laid only shortly before transfer and which therefore underwent most of their embryonic development at 19°C. However, only the earliest larvae to crawl on the sides of the culture bottles were selected for study. This should have excluded the youngest individuals and largely eliminated this source of error.

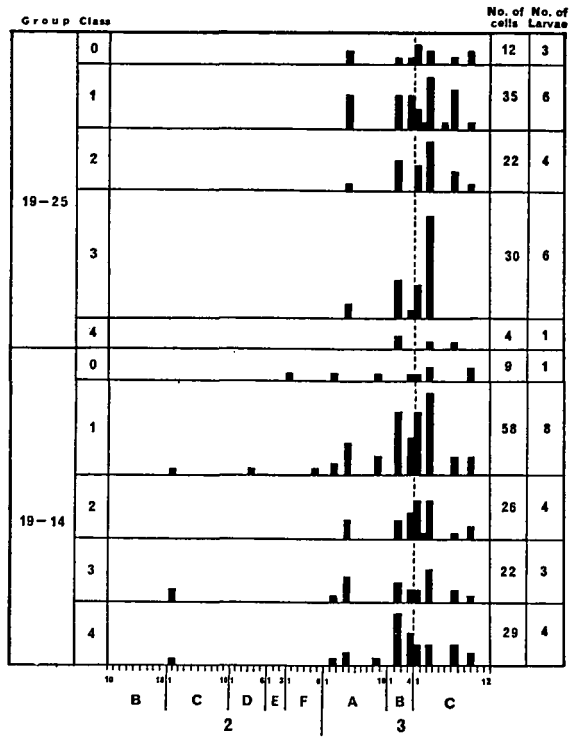


Fig. 4. Relation of heterochromatization to variegation in individuals transferred from 19° to 25°C. or to 14°C. after 24 hours. Symbols as in Fig. 3. There is no correlation between heterochromatization and variegation inside a given temperature group.

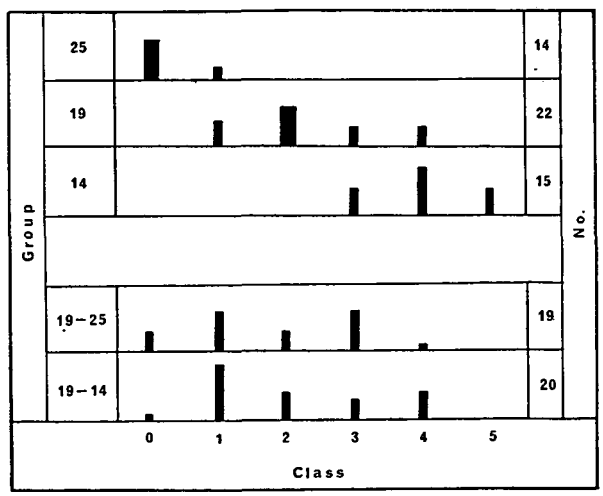


Fig. 5. Relation of malpighian tubule variegation to temperature during development. The histogram represents the number of individuals in each temperature group exhibiting the degree of variegation indicated on the abscissa (see text). The last column gives the total number of individuals in each group.

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In order to analyse more closely the events during early development cultures were transferred from one temperature to another at various stages during the embryonic period. The results are presented in Figs. 6 and 7. In addition to the incidence of heterochromatization at 3C1 and 3C7, the graphs show the percentage of nuclei with *any* heterochromatization. Since this involved analysis of the region between 3D1 and the break these data may be subject to more error than the others presented.

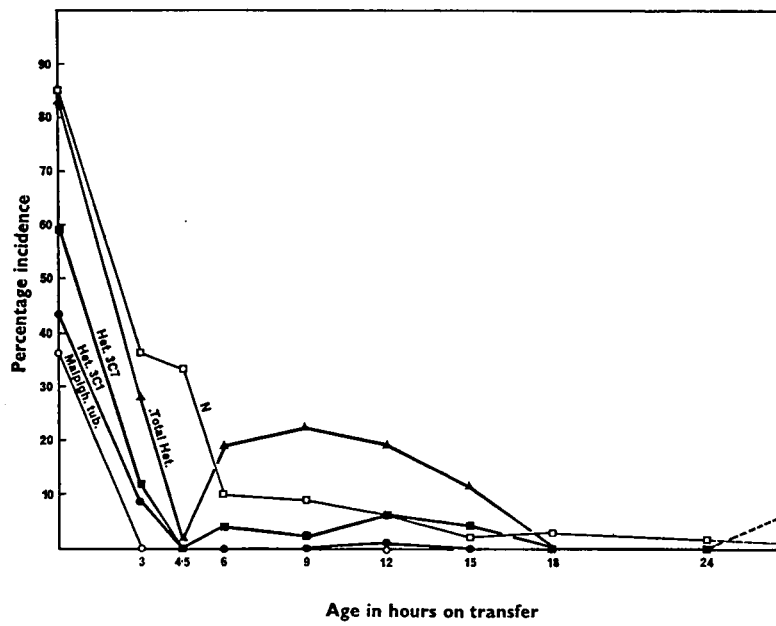

Fig. 6. Variegation and heterochromatization in individuals transferred from 25° to 14°C. at various times during the embryonic period. The abscissa represents the age in hours on transfer. The plotted lines indicate the percentage incidence of *Notch*, malpighian tubule variegation and heterochromatization at 3C7 and 3C1. The percentage incidence of *any* heterochromatization ('Total Het.') is also given. Each point on the graph represents data from approx. 300 adults, 20 larvae or 100 nuclei.

The data support the previous finding that heterochromatization is temperature-sensitive during early embryonic life. The greatest sensitivity to cold occurs during the first 4·5 hours, and the effect lessens up to about 18 hours. After eclosion from the egg some sentitivity to low temperature is still shown, however. Exposure to 14°C. following embryonic development at 25°C. affects only the most proximal region of the translocated chromosome, not involving 3C1 or 3C7. In individuals exposed to 14°C. after embryonic development at 19°C. (cf. Table 1) these two loci are affected. In other words, the enhancing effect on heterochromatization of 14°C. after eclosion is relatively slight. Where there is initially no heterochromatization (after embryonic development at 25°C.) development at 14°C. will not result in

heterochromatization as far as 3C7, but where there is already considerable heterochromatization (after early development at 19°C.) a further slight increase at 14°C. will involve both bands.

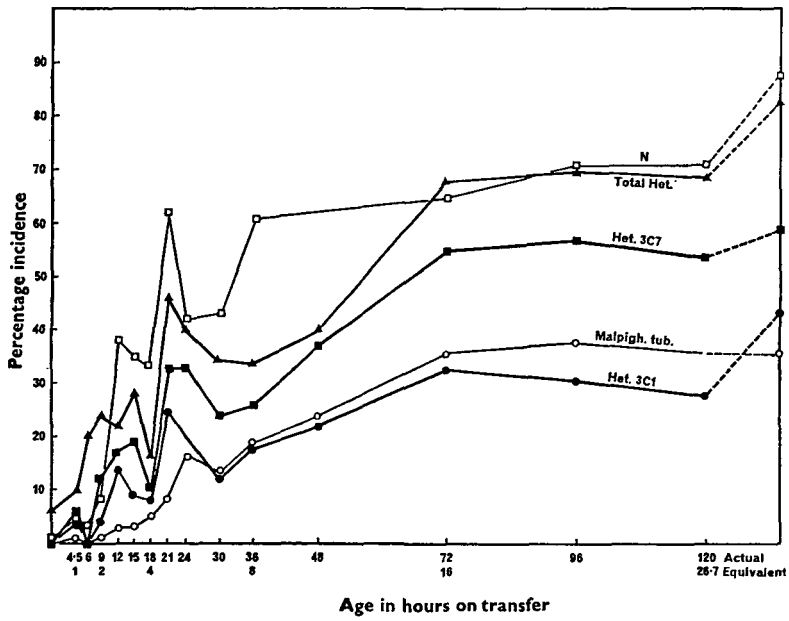

Fig. 7. Variegation and heterochromatization in individuals transferred from 14° to 25°C. at various times during the embryonic period. Description as in Fig. 6.

The marked fluctuations in the amount of heterochromatization after temperature transfer at varying stages of development, revealed in the graphs, is discussed below.

(iii) *Effect of developmental temperature on variegation*

It will be seen from Table 1 and Fig. 5 that variegation of malpighian tubules and the incidence of *N* was negligible in individuals kept at 25°C. throughout development, was appreciable at 19°C. and marked at 14°C. Malpighian tubule variegation in individuals transferred after 24 hours at 19°C. to either 14° or 25°C. was similar to that in individuals kept at 19°C. throughout. This suggests that temperature-sensitivity is limited to the early embryo. On the other hand, the incidence of *N* in flies kept throughout at 19°C. was lower than in those transferred from 19° to 14°C., and higher than in those of the 19–25 group. This indicates that *N* is affected by temperature both during and after the early embryonic period. It is supported by the data in Figs. 6 and 7 which show that, as with heterochromatization, malpighian tubule variegation and *N* are most sensitive to cold during the first 4·5 hours of embryonic life. After eclosion from the egg some sensitivity to cold is still shown by *N* but not by the malpighian tubules. To determine whether the sensitivity of *N* to cold extends to the pupal stage, individuals were transferred from one

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temperature to another during the prepupal phase. Table 2 shows the percentage of *N* in the adults from test groups and controls. There appears to be no temperature effect during the pupal stage.

Table 2. *Percentage* Notch *in adults transferred from* 25°C. *to* 19°C. *and vice versa during the prepupal stage*

	Change from 25 to 19°C.	Controls left at 25°C.	Change from 19 to 25°C.	Controls left at 19°C.
No. adults scored	301	461	288	368
No. adults showing *N*	2	3	191	239
%*N*	0·66	0·65	66·3	64·9

(iv) *Relationship between heterochromatization and variegation*

Table 1 and Figs. 3 and 4 show good agreement between heterochromatization at 3C1 in larval salivary glands and variegation in larval malpighian tubules, comparing groups of animals reared at 25°, 19° and 14°C. There is also a close correlation between *N* in adults and heterochromatization at 3C7 in larvae from the same experimental group. As shown in Figs. 3 and 4, however, there appears to be no correlation between the amount of variegation in malpighian tubules and the extent of heterochromatization in the same individual, comparing animals within a given experimental group. In some cases individuals showing heterochromatization beyond the white locus in salivary glands showed no variegation in malpighian tubules.

From Figs. 6 and 7 it can be seen that although the results fluctuated, depending on the time during the embryonic period at which animals were transferred from one temperature to another, there was a striking correlation between the extent of heterochromatization on the one hand, and the incidence of *N* and malpighian tubule variegation on the other. In animals transferred from 19°C to either 14° or 25°C. at the end of the embryonic period, however (Table 1 and Fig. 5), the change in temperature affected heterochromatization at the 3C1 locus without influencing variegation of the malpighian tubules.

4. DISCUSSION

(i) *Relationship between heterochromatization and variegation*

In studying the relationship of heterochromatization to variegation, it must be remembered that the incidence of heterochromatization in the malpighian tubules (which presumably controls variegation in that organ) may not be identical with that observed in the salivary glands. In the case of *N* the salivary gland chromosomes and the phenotype cannot even be examined in the same individual. The apparent absence of correlation between heterochromatin and phenotype revealed

by the present work when animals within a single experimental group are compared could therefore be due to random variation. Only if heterochromatization and variegation were observed simultaneously in a single cell could this be excluded. Bearing this in mind, the bulk of the present evidence is consistent with evidence from other organisms that low temperature can induce or enhance heterochromatization (e.g. Darlington & La Cour, 1940; La Cour *et al.*, 1956; Dyer, 1964) and that heterochromatization induces gene inactivity (e.g. Russel, 1961; Grumbach *et al.*, 1963; Ohno, 1965; Ohno & Lyon, 1965).

The present investigation showed that transfer of animals from 19° to 14°C. at the end of the embryonic period increases the amount of heterochromatization at 3C1, but does not materially affect variegation in malpighian tubules. In attempting to explain this apparent anomaly, it must be remembered that in development determination must occur before expression. An environmental stimulus may thus affect determination at an early stage of development, or may later modulate expression, perhaps by acting on cytoplasmic biochemical systems. One may suppose that during the early embryonic period low temperature induces heterochromatization in *Drosophila*, and blocks the action of genes situated in the heterochromatized region (having, in the case of *N*, the same effect as a deletion). Pycnotic regions are, in fact, already visible in eggs approximately 3 hours old (Kaufmann, 1934). Pigment in malpighian tubules is normally already present by the 16th to 18th hour of embryonic development (Poulson, 1950), so that the relevant cellular determination is presumably completed by this stage and can no longer be affected by subsequent heterochromatization. Since pigment formation continues in the larval stage, and since exposure to cold at this time, and the resultant heterochromatization, has no observable effect on the amount of pigment per cell, it appears that the white locus is not directly involved in this part of the process. It is tempting to speculate that either messenger RNA produced by the locus is long-lived, or that the cytoplasmic systems immediately responsible for pigment formation are stable or capable of autonomous replication. On the other hand, systems responsible for the formation of adult characters develop later. For example, pigmentation of the eye starts only at the end of the second day after puparium formation (Bodenstein, 1950). Chen's results (1948) indicate that a sensitive period for inducing mottling of the eye is found in the early pupa, varying somewhat from one strain to another. Environmental alterations may here be acting at the cytoplasmic and not at the gene level (see also Becker, 1961). Similarly, the wing begins to differentiate during the third larval instar and development is complete in the 28-hour-old pupa. The present observations that *N* may be affected by temperature during both the embryonic and larval stages may be similarly interpreted.

(ii) *Analysis of events during the embryonic period*

Figures 6 and 7 show the relationship of heterochromatization, malpighian tubule variegation and *N* to the time of transfer from one temperature to another. In both figures a general trend is discernible which indicates that the period of maximum

sensitivity to cold lies early in embryonic development. The similarity in shape of the curves is further confirmation of the correlation between phenotype on the one hand and heterochromatization of the relevant locus on the other. The marked and unexpected fluctuations of the curves are, however, striking and appear to show that in some cases a short application of cold has more effect than a longer one. Some of these observations were repeated, and although results varied slightly, the shape of the graphs remained essentially unchanged. This makes it unlikely that some random factor, such as inconstancy of the temperature in the incubators, or unexplained changes in the experimental population, could be responsible for the fluctuations observed.

There are several possible explanations for the fluctuations. Firstly, it is conceivable that a temperature shock, i.e. the temperature change itself, may affect the phenomena under discussion. Certain stages of development might be particularly susceptible to such shocks, and a change in temperature at such a stage might either enhance or inhibit heterochromatization and variegation. Thus the unexpectedly low points in Fig. 7 might be interpreted as being due to periods when heterochromatization is inhibited by a change in temperature, or alternatively the high points as times when a temperature shock facilitates the process. The postulated sensitive periods might be related to early developmental processes. For example, the two relatively prominent phases occurring between 1 and 6 hours of development might be connected with blastoderm formation and gastrulation respectively. Another possibility is that cold may have different effects depending on the time at which it acts. In different strains of flies cold may either increase or decrease heterochromatization (Prokofieva-Belgovskaya, 1947) and the effect of cold may not be constant during the development of an individual. Presumably temperature qualitatively affects development by differentially affecting the rates of linked metabolic processes, and as the metabolism changes during development the effect of cold may also change. Thus cold may tend to enhance heterochromatization during one stage of development and inhibit it during another.

If the above hypothesis is correct, it raises the interesting further possibility that heterochromatization may be reversible. Although it seems to be generally accepted that heterochromatization is a permanent condition of the chromosome, Hsu (1964) has pointed out that heterochromatin must uncoil at least during that period of the cell cycle when it replicates. The usually heterochromatic Y chromosome of several *Drosophila* species has been shown to be capable of uncoiling and gene activity during a short period in development (Hess & Meyer, e.g. 1963), and it seems extremely doubtful that the heterochromatic state implies either permanent condensation or permanent gene inactivity. The relationship between heterochromatin and heterochromatized euchromatin is far from clear, and it may be that the euchromatin of polytene chromosomes, once having become heterochromatic, must remain so. There is, however, no direct evidence for this, and the regular appearance of puffs demonstrates that the longitudinal constituents of these chromosomes are capable of reversible uncoiling. It is tempting to regard puffing

and heterochromatization as the two extremes of chromosomal coiling behaviour. If this were the case, there would be no *a priori* reason for assuming heterochromatization to be irreversible. Schultz (1956) concludes that, since transfer from one temperature to another at the end of the embryonic period has no discernible effect on the malpighian tubules of adults, '... an irreversible differentiation, with respect to the activity of the white locus, occurs in the variegation phenomenon.' His results and those reported here do not, however, exclude the possibility that reversal of heterochromatization occurs during the embryonic stage, nor that under some conditions it might occur at a later stage without affecting the expression of the white locus, which would already have failed to act.

(iii) *Variability in extent and distribution of heterochromatization*

The fact that heterochromatization proceeds progressively along the chromosome suggests strongly that the factor or factors responsible are genetically and biochemically unspecific and of short range. Diffusion of some substance of high molecular weight, or a physical factor such as a mechanical interference with the state of coiling of the chromosome, are obvious possibilities, but further speculation appears pointless at present.

It seems as if heterochromatization progresses preferentially to certain bands, e.g. 3C7, 3C3 and 3B2. Since most of the bands concerned are relatively large, the observation may be merely due to the fact that large bands are more readily distinguished than smaller ones. But it is possible that chromosome regions may genuinely behave as units with respect to heterochromatization. The labelling experiments of Plaut & Nash (1964) and Plaut, Nash & Fanning (1966) indicate that salivary chromosomes of *D. melanogaster* are differentiated into longitudinal series of DNA replicative units. These units were recorded on the basis of Bridges' cytological map (1938), but label could sometimes be localized more precisely than can be described by the lettered subdivisions of the map, and it seemed likely that label was often incorporated into a single cytological band. Since the rate of replication is probably different for heterochromatin and euchromatin, it appears to be not unreasonable to suggest that heterochromatization of a 'replicon' should be an 'all-or-none' phenomenon, and thus always progress to the border between two such units.

The phenomenon of variegation depends on the fact that cells which are (presumably) genetically identical may react differently to a given stimulus. The present work has confirmed earlier observations that the extent of heterochromatization may differ widely in cells from the same salivary gland, and this variability is one of the outstanding unsolved problems connected with heterochromatin. All the cells examined here were among the largest in the preparation, coming from the distal part of the gland. The fact that neighbouring cells in a preparation tended to exhibit a similar degree of heterochromatization suggests that heterochromatization may be affected by some strictly local tissue factor, which only influences a small

group of cells. Alternatively, near-by cells may belong to a clone derived from one parent cell, and heterochromatization may be inherited on the cellular level. In this context, Becker (1957, 1961) found that pale eye sectors, caused by heterochromatization, were identical with sectors induced by X-irradiation at the end of first larval instar. He presents evidence and arguments suggesting that at this stage of development presumptive eye-cells undergo stable differentiation, such that all progeny of a given cell behave similarly i.e. either all produce pigment, or all do not.

The data presented here appear consistent with the view that the genetic effects of heterochromatin can be profound, being at the same time largely non-specific and random. Further study of heterochromatization in relation to ontogenetic processes might throw some light on this anomaly.

SUMMARY

Using a strain of *D. melanogaster* carrying an X-4 translocation, comparison between groups of individuals cultured at 14°, 19° and 25°C. showed good correlation between heterochromatization, variegation in malpighian tubules and *Notch*. All these phenomena are enhanced by low temperatures. Correlation was less good within each temperature group, and considerable variability was observed between individuals within the group, and between nuclei from one individual.

Experiments involving a temperature change during the embryonic period indicate that (1) heterochromatization is especially temperature-sensitive during the early embryonic period but may be increased by low temperature later, (2) larval malpighian tubules are sensitive to temperature only during the early embryonic stage, and (3) *N* is influenced by temperature during early embryonic life and also during the larval period.

Our observations, in conjunction with those of other workers, could be explained as follows: exposure of individuals to low temperature at a time when a specific system is beginning to differentiate will cause the processes concerned to be blocked in some cells. At least during the early embryonic stages this appears to involve heterochromatization of the relevant locus. Once the processes are established which will lead to the formation of a character, further heterochromatization has no effect on the phenotype. Temperature may affect pupal or adult phenotype in this way or by a direct action on the metabolic processes of cells.

Further experiments showed: (1) the greatest temperature-sensitivity of all three phenomena within the first 6 hours of embryonic life; (2) striking fluctuations of the effect of temperature, especially within the early embryonic period; (3) close correspondence between all three phenomena in time of response to temperature. Some alternative interpretations are considered.

The stimulus for this work was provided by a period spent at the Genetics Foundation of the University of Texas, and I am deeply grateful to Professor Wilson S. Stone, who made this possible, and to Professor Burke Judd for providing the strain.

The work was carried out under Medical Research Council Grant 964/318/B.

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