PIGMENTATION IN A MOTTLED WHITE EYE DUE TO POSITION EFFECT IN DROSOPHILA MELANOGASTER

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THE phenomenon of phenotypic variegation in Drosophila, due to the translocation of a gene in a euchromatic region of the chromosomes to the neighborhood of a heterochromatic one, has been studied widely and interpreted in various ways (see BELGOVSKY 1946; EPHRUSSI and SUTTON 1946). After an analysis of one example of this variegation, the mottled white eye of the stock T(1;4)$w^{258\text{-}18}$ of *D. melanogaster,* DEMEREC and SLIZYNSKA (1937) suggested that proximity to heterochromatin may render the *w* locus unstable, so that it mutates from time to time to an allelic form, which may be reproduced and maintained for several cell generations, but which may in its turn mutate to another allele. The data indicate that, if this is so, the progression from one allele to another is not completely at random, but is restricted to a small number of the possible alleles of the *w* locus.

In T(1;4)$w^{258\text{-}18}$, the X chromosome has been broken just proximal to the *w* locus (following 3C4, salivary gland chromosome map) and the fourth chromosome in 101F. The distal end of the X, including the *w* locus, has been reattached to the stump of the fourth (chromocentral region) and the distal part of the fourth to the proximal part of the X.

According to DEMEREC and SLIZYNSKA, "on the eyes of homozygous 258-18 flies, cream, cherry and red colors . . . appear in definite patterns," and in their discussion the authors relate these colors to three alleles of the *w* locus, w^{cr} (cream), w^{ch} (cherry) and w^{+} (wild) respectively. In this paper, the cream color will be denoted as w^{cr} though no self-colored mutant at the *w* locus has been thus designated. It should not be confused with cream (*cr*), a series of modifiers of the eosin (w^{e}) allele of the *w* locus (BRIDGES and BREHME 1944).

If the mechanism of variegation is such as they postulate, one would expect, in a histological study of the eyes of these flies, to find three distinct types of ommatidia, whose pigmentation would correspond to that of the self-colored eyes of the mutants cream and cherry and of the wild type.

When I started to examine the eyes of T(1;4)$w^{258\text{-}18}$ flies histologically, I knew of only one published statement on the histology of white-mottled eyes. In one of his papers PANSHIN (1938) wrote to the following effect (translated from Russian): "The difference in pigmentation of facets depends on the fact that within one facet some cells have a normal quantity of pigment while the others have none." If this statement were found to hold true for the eyes of T(1;4)$w^{258\text{-}18}$ the hypothesis of DEMEREC and SLIZYNSKA would be

invalidated. It would be possible, moreover, on this basis to explain a considerable range of variation in pigmentation as seen from the surface of the eye; for one might expect the color to vary in intensity according to whether one or more layers of pigment cells were pigmented, and to appear opaque or transparent according to whether or not the upper layer of pigment cells was pigmented (MAINX 1938). In addition one might expect intermediate effects due to refraction, like that found by CASTEEL (1929) in a small area of an eye which, histologically, showed only normal pigmentation or complete lack of pigment.

PANSHIN, however, did not publish evidence for his statement, nor did he work with T(1;4)$w^{258\text{-}18}$, and it seemed of interest therefore to record in the present paper the results of a histological examination of the eyes of this particular stock. Subsequently, NOLTE (1950) has published some observations on the histology of the eye in white-mottled-4, referred to below.

In the course of work with T(1;4)$w^{258\text{-}18}$, an observation of a different sort was made. In the paper of DEMEREC and SLIZYNSKA it is affirmed that cherry or red spots on a cream-colored background are distributed at random over the eye. I found, on the contrary, that the major dark-pigmented areas are localized rather constantly in one part of the eye. This observation was confirmed in other stocks with mottled eyes. It has also been noticed by SCHULTZ (1941) who says: " . . . at low temperatures there is a small dark patch at the back of the eye . . . . " Some data relative to this phenomenon are presented here, in addition to the histological observations.

MATERIALS AND METHODS

All stocks of flies for this work were raised at about 18°C. A stock of T(1;4)$w^{258\text{-}18}$ was obtained from DR. DEMEREC at Cold Spring Harbor. The stock was carried by mating heterozygous females (T(1;4)$w^{258\text{-}18}/w$) with w males, but viable homozygous $w^{258\text{-}18}/w^{258\text{-}18}$ females and hemizygous $w^{258\text{-}18}$ males were obtained from this stock. The two latter types show patches of darker pigmentation on a cream-colored background, and also mottling for roughest (*rst*).

Eyes of T(1;4)$w^{258\text{-}18}/w$ females and T(1;4)$w^{258\text{-}18}$ males (which occur with a low frequency in the stock) were prepared in one of the following ways: (1) Whole flies or half heads fixed in absolute alcohol, passed through toluene series, imbedded in paraffin, serially sectioned, mounted on slides, cleared with xylol and mounted unstained in clarite or balsam. (2) Whole flies fixed by the freezing-drying method (GERSH 1948) infiltrated with paraffin and treated subsequently as in (1) with this difference—the sections were mounted on the slide by the dry method.

Eyes of males and females of six other stocks, namely Ore-R (Oregon-R, wild type), *bw* (brown), *v* (vermilion), w^{bf} (white-buff), w^{ch} (white-cherry) and w^{e} (white-eosin), were prepared in the same manner. In addition, some sections of Ore-R and w (white) eyes were examined after staining with Delafield's haematoxylin and eosin. All flies were aged at least two days after

emergence. Sections were made at 6 μ or 10 μ, and a few of the Ore-R eyes at 2 μ.

The study of the localization of the pigment in T(1;4)$w^{258\text{-}18}$ was supplemented by an examination of two other stocks—Dp(1;3)$N^{264\text{-}58}$ and mottled-28. The former is a stock homozygous for *w* which has in addition a small section of the X chromosome including the *w* locus inserted in the chromocentral region of the third chromosome. Flies of this stock have mottled eyes owing to the proximity of the *w* locus to heterochromatin, as in T(1;4)$w^{258\text{-}18}$. Mottled-28 (*mot-28*) is a recessive mutation at a locus near the centromere in the left arm of the third chromosome, with no detectable chromosomal aberration. The eyes are mottled with dark brown pigment and the stock is peculiar in that this pigment is formed even when flies are homozygous for white (*w/w; mot-28/mot-28*). It was in flies of this genotype that the eyes were studied. For further details of these stocks, see BRIDGES and BREHME (1944).

RESULTS AND DISCUSSION

The pigment cells in sectioned material

1. *General.* The histology of the adult wild type eye has been described by previous workers (*e.g.*, JOHANNSEN 1924; HERTWECK 1931). Figure 1 shows the structure of the individual ommatidium. In this diagram (A) are shown three layers of pigment cells: a distal layer of *primary pigment cells* (two in each ommatidium), a middle layer of *secondary pigment cells* (nine surrounding each ommatidium) and a proximal *post-retinal pigment cell* (probably one underlying each ommatidium).

The number of secondaries sheathing each ommatidium has been variously cited as from six to twelve. I agree with NOLTE (1950) that there are generally nine of these cells, although there often seem to be eight or ten owing to confusion with the cells of contiguous ommatidia.

There has been some doubt also as to whether the post-retinal pigment, lying below the basement membrane, is contained in a separate series of cells, or in processes extending through this membrane from the pigment cells above it (for discussion see NOLTE 1950). I am again in agreement with NOLTE that there is a layer of post-retinal pigment cells; in sections stained with haematoxylin and eosin, one can see their large nuclei with heavily staining nucleoli, and these nuclei correspond roughly with the overlying ommatidia (figures 13, 14). These cells contain granules similar to those in the primary and secondary pigment cells.

JOHANNSEN (1924) states that there is a fourth layer of *basal pigment cells,* and this was reaffirmed by PILKINGTON (1941) and NOLTE (1950), but HERTWECK (1931) was unable to convince himself that there were any such cells as distinct from the rudimentary eighth retinula cell which occupies a basal position in the ommatidium above the basement membrane. I have not found any nuclei between those of the eighth retinula cells and those of the post-retinal pigment cells.

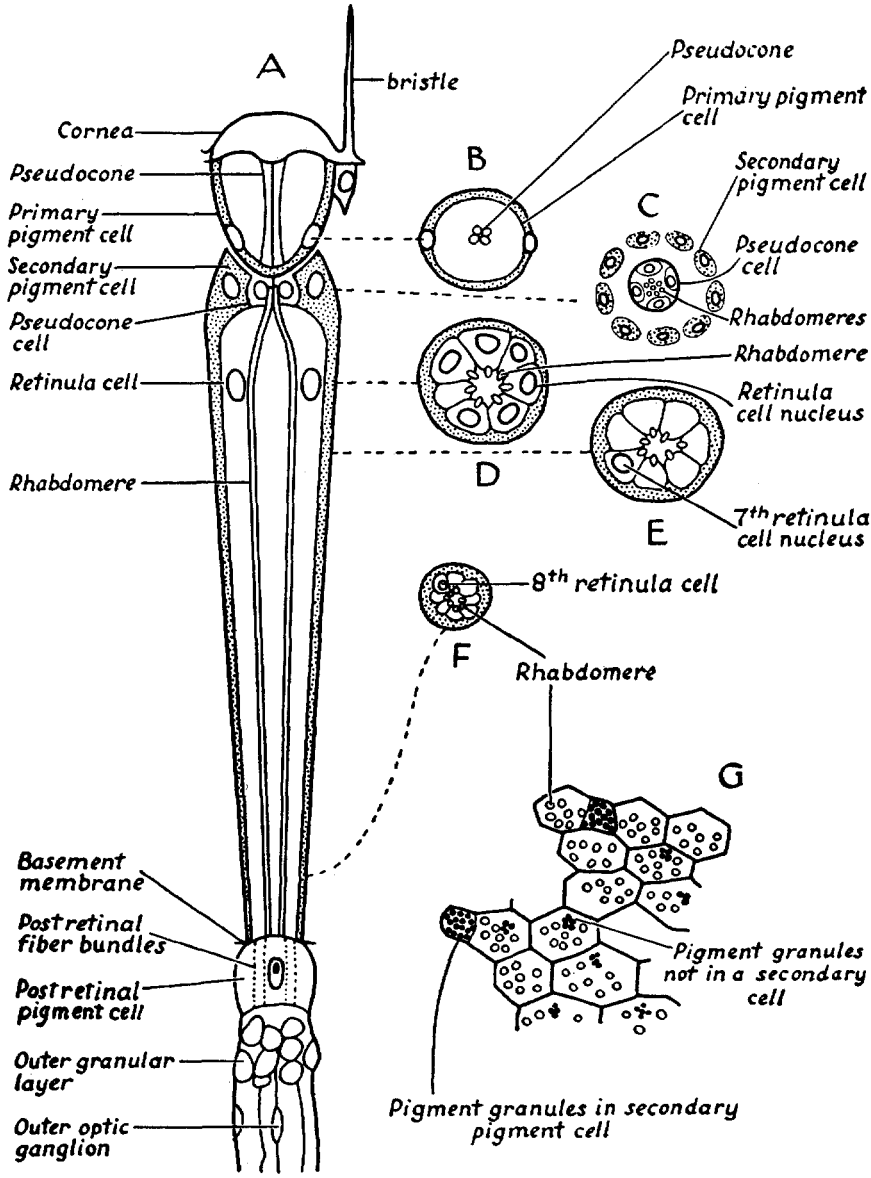
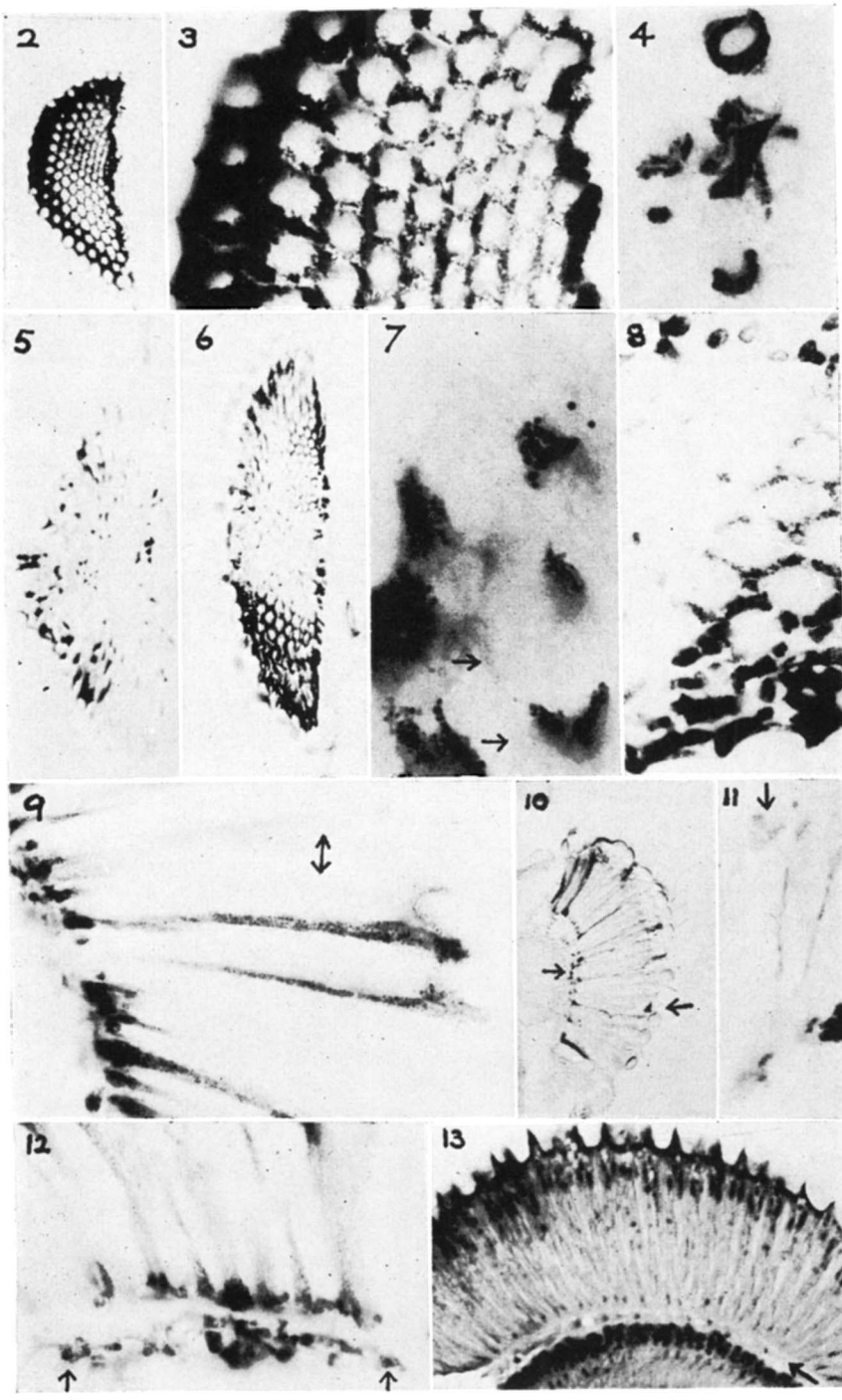


FIGURE 1.—A–F, diagrams of a single ommatidium to show location of pigment cells, for orientation purposes. A, longitudinal section; B–F, transverse sections at different levels. G, drawing of part of the eye in figure 5 to show small groups of pigment granules oriented with respect to the rhabdomeres. (A–F after HERTWECK 1931 with modifications; G, camera lucida, *ca.* × 680, drawn by MRS. E. B. PATTERSON.)



FIGURES 2–13.—Microphotographs of unstained sections through eyes of wild type Oregon-R (2, 3) and mottled white $T(1;4)w^{258\text{-}18}$ (4–12), and a stained section of a white eye (13).

There are two distinct pigments, the red and the brown (EPHRUSSI and HEROLD 1944). Both are present in both primary and secondary pigment cells, but MAINX (1938) found that the red predominates in the primaries and the brown in the secondaries. The pigments are confined to granules, and (again according to MAINX) the individual granules containing mainly the red pigment (as in *st* flies) appear in sections as " yellow," " ochre yellow," or " salmon red," while those containing mainly the brown pigment appear (as in eyes of *bw* flies) as " lilac red " (OSTWALD's Normenatlas, 1923). I consider those terms adequately descriptive of the colors of individual granules in the eyes of vermilion and brown flies respectively, whether fixed in alcohol or by freezing and drying. It is therefore necessary to bear in mind that in a histological description the colors of individual granules are not the same as those seen in a gross view of the surface of the eye; the reddish granules give a gross effect of brown, while yellowish granules give a gross effect of red.

In the wild-type eye the granules have been described as " yellow " or " golden-brown," and " wine-red," the former being concentrated in the distal region, especially in the primaries.

2. *Pigment cells in* $T(1;4)w^{258\text{-}18}$. In the eyes of flies of the $T(1;4)w^{258\text{-}18}$ stock, two types of primary pigment cells were observed. One type was pigmented with golden brown granules similar to those of the wild type eye but often more sharply demarked from, and contrasting more with, the underlying wine-red pigment of the secondaries. The other type was not visibly pigmented. A mosaic of the two types of cell is shown in figure 4, where the two primaries of one ommatidium (in transverse section) form an entire circle of

FIGURE 2.—Wild type, transverse section through ommatidia at level of secondaries, showing regular pattern formed by pigment cells. *ca.* × 140.

FIGURE 3.—Same as figure 2, × 680.

FIGURE 4.—Mottled white, transverse section through ommatidia at level of primaries; above, complete circle formed by two pigmented primaries; below, half-circle formed by one pigmented primary, the other being unpigmented. × 680.

FIGURES 5, 6.—Mottled white, transverse section through ommatidia of two different flies at level of secondaries. In these two eyes different proportions of pigment cells are fully pigmented, the remainder being either unpigmented or having pigment granules of an intermediate color. The two latter types are indistinguishable here. Compare with figure 2. *ca.* × 140.

FIGURE 7.—Same as figure 5, × 1300; arrows indicate position of cells with intermediate pigmentation.

FIGURE 8.—Same as figure 6, × 680. Compare with figure 3.

FIGURE 9.—Longitudinal section through ommatidia from same eye as figure 5; arrows indicate position of secondaries with intermediate pigmentation. × 680.

FIGURE 10.—Mottled white, longitudinal section through ommatidia; upper arrow shows pigmented post-retinal area underlying unpigmented secondaries; lower arrow, pigmented primary overlying unpigmented secondaries, and immediately below this, a pigmented secondary with corresponding primaries unpigmented. *ca.* × 140.

FIGURE 11.—Mottled white, longitudinal section through ommatidia, arrow indicates pigmented primary with corresponding secondaries unpigmented. Adjacent secondary pigmented. × 680.

FIGURE 12.—Mottled white, longitudinal section through bases of ommatidia; arrows show pigmented areas below basement membrane underlying unpigmented secondaries. × 680.

FIGURE 13.—White, longitudinal section through ommatidia stained with haematoxylin and eosin; arrow indicates post-retinal layer of pigment cells. × 415.

pigment, while in another ommatidium the pigment in a single crescent-shaped primary is contrasted with the lack of pigment in the cell which completes the circle.

The secondary pigment cells are of three types: (1) fully pigmented and comparable to those of the wild type fly; (2) with no visible pigment; and (3) very slightly pigmented, densely packed with granules of a pale yellowish pink color. The third type was identified in eyes fixed by freezing and drying, but not after alcohol fixation. As in the case of the primaries, the individual ommatidium may be a mosaic, with secondaries of two or even three different kinds. The cells with intermediate pigmentation are not confined to the immediate neighborhood of those with wild type pigmentation, but are found, mingled with colorless cells, at a distance of several ommatidia away from any wild-type secondary.

Figures 5 to 9 show various aspects of the pigmentation of the secondaries. Figures 5 and 6 are of contrasted eyes with, respectively, very few and rather many darkly-pigmented cells. Figures 6 and 8 should be compared with figures 2 and 3, which are photographs of a wild type eye at the same magnifications. The intermediate type of secondary does not show well in photographs, but the location of such cells is indicated in figure 7, and in the longitudinal section of the ommatidia in figure 9.

In the unpigmented cells no granules were visible, but a slide stained in iron haematoxylin showed that granules are present in all pigment cells. There is, however, variation in the stainability of the granules which corresponds roughly with the initial degree of pigmentation. This was determined by making camera lucida drawings to show the pigmented areas before the slide was stained with haematoxylin. After staining it was found that cells in the most strongly pigmented region (dorsal part of the eye) contained very dark brown to black granules. In isolated small patches of fully pigmented cells in the more ventral region the granules varied from pale brown to black, while in all other pigment cells they were very pale. There was no noticeable difference in granule size or density in different cells.

In some eyes there were regions where pigment was present below the basement membrane (see figure 1 A) although there was no pigment surrounding the ommatidia immediately above this membrane (figures 10 and 12). A study of sections stained with haematoxylin and eosin confirmed NOLTE'S (1950) view that this pigment is contained in separate post-retinal cells (figures 13, 14). The pigment in these cells is usually very dense, like that of wild type, but there are a few intermediate and colorless cells like those in the secondaries.

Transverse sections through the proximal parts of one eye showed a small patch of pigment in many ommatidia which were otherwise unpigmented. This patch was always located in the same position within the ommatidium, close to the rhabdomeres and between corresponding rhabdomeres (which form a pattern repeated from one ommatidium to the next); and it was much smaller in cross section than a secondary pigment cell at the same level (figure 1 G). It seems possible that this pigment is contained in the rudimentary eighth retinula cell (figure 1 F), which was first described by DIETRICH (1909).

In sections of many of the eyes examined, the pigment cells of ommatidia in the dorsal region were all strongly pigmented. Ventrally, isolated ommatidia or groups of ommatidia contained some fully pigmented cells (figure 10). In this region it is possible to find ommatidia in which the primaries are pigmented and the secondaries are not (figures 10, 11) and *vice versa* (figure 10). Pigmentation of primaries and secondaries thus seems to be independent (in contrast, apparently, to white-mottled-4, where NOLTE states that unpigmented sectors extend from the post-retinals to the primaries), but in areas where the secondaries are uniformly pigmented throughout many ommatidia, the primaries tend likewise to be uniformly pigmented. This will be referred to again in the following section.

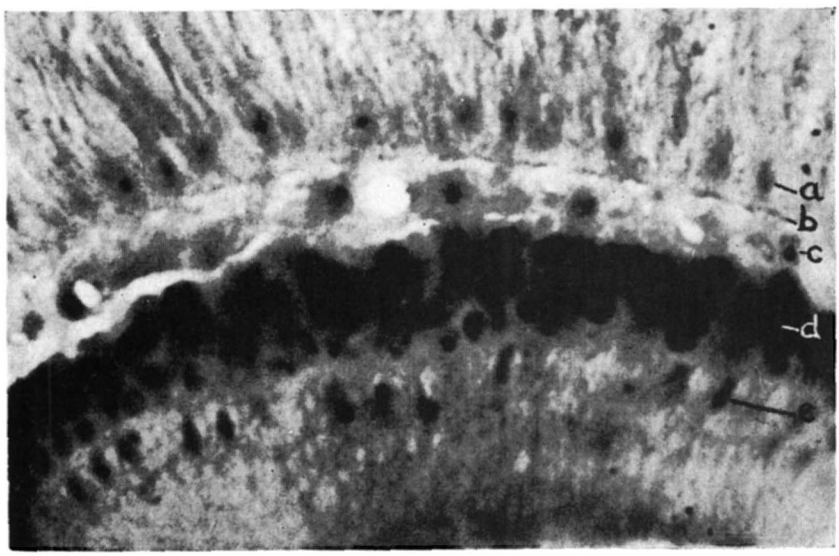


FIGURE 14.—Longitudinal section through bases of ommatidia of a white eye stained with haematoxylin and eosin (same section as figure 13). a, layer of eighth retinula nuclei; b, basement membrane; c, fenestrated zone, with tracheae (lacunae) and nuclei of post-retinal pigment cells; d, layer of monopolar neurons; e, nuclei of external optic glomerulus. × 1700.

To sum up, pigmentation can vary from cell to cell, and is not necessarily uniform within a whole ommatidium; but it is not, for the secondary and post-retinal pigment cells at least, a simple all-or-none effect, for a third, intermediate type occurs. This again contrasts with NOLTE's finding of an all-or-none effect in white-mottled-4.

3. *Pigment cells in some intermediate alleles of the w locus.* White-buff (w^{bf}). This mutant was chosen because it is one of the palest in the series of white alleles and may be considered as comparable in that respect with the cream background of the white-mottled eyes described above. In gross view the eyes of males are slightly lighter than those of females; in sections they are indistinguishable. The basal pigment cells are packed with granules, but they are colorless or almost so. Numerous colorless granules are also present in the

secondaries, but at the apex of these cells a few bright yellow granules are clearly distinguishable. There are also a few such granules in the primaries.

White-cherry (w^{ch}). This mutant was studied by way of comparison with the intermediate (cherry) pigmentation of the white-mottled eye in T(1;4)$w^{258\text{-}18}$. In males there is a very dense concentration of orange-pink granules in the post-retinals and at the bases of the secondaries. The granules are less numerous in the distal parts of the secondaries and in the primaries, where they are mostly golden-brown. In females (whose eyes appear darker than those of males in gross view) the granules of the post-retinals are pinker and less orange than those in the males. In other respects males and females are similar. All granules in cherry eyes are more intensely pigmented than the "intermediate" of the white-mottled eye.

White-eosin (w^e). In males there are many pale pink granules in the post-retinals. They are more intensely pigmented than the "intermediates" of the white-mottled eye. The secondaries contain a very sparse sprinkling of browner granules, and the primaries are thinly pigmented in a similar manner. In females (whose eyes are darker in gross view) the only difference is that the pigment granules in the post-retinals are an intense orange-red.

White (w). It may be mentioned here that in sections of w eyes stained with haematoxylin and eosin, the pigment cells are seen to be as densely packed with granules as in wild type eyes similarly treated.

4. *Discussion.* A comparison of the intermediate secondary pigment cells found in T(1;4)$w^{258\text{-}18}$ with the secondaries of the intermediate mutants at the w locus described above reveals two marked differences. First, the individual granules in the intermediate cells of the white-mottled eye are much paler than those in any of the mutants studied (except for the post-retinal granules in white-buff). Second, the number of pigmented granules in the secondaries of the white-mottled eye does not appear to be reduced, while in the mutants examined there is an obvious reduction in the number of pigmented granules in the secondaries. Thus, the pigmentation of the cherry eye and the similar coloring of the cherry patches in the white-mottled eye are apparently produced in different ways: the former, largely by reduction in the number of pigmented granules as compared with wild type; and the latter, by reduction in the amount of pigment per granule as compared with wild type.

While the histological picture in T(1;4)$w^{258\text{-}18}$ seems at first sight to be consonant with the hypothesis of mutability as a result of change in position of the w locus, the comparison with mutant alleles at the w locus gives the impression that the changes that occur within the white-mottled eye are of a different kind from the mutational change from w^+ to a lower allele, or *vice versa.*

5. *Advantages of fixation by freezing and drying.* Some of the results obtained in the course of this work show clearly the superiority of the freezing-drying method for this type of study.

For instance, it has been noted that the intermediate cells in the white-

mottled eye were not detectable after fixation with absolute alcohol, though they were after freezing and drying. This is undoubtedly because the small amount of pigment in the granules of these cells is dissolved out during even a short period in alcohol. It is conceivable that intermediate cells could also be demonstrated in white-mottled-4 with the freezing-drying technique (see NOLTE 1950).

Again, MAINX (1938) did not detect any colored granules at the distal ends of the ommatidia in white-buff, but these are very clear in the frozen-dried white-buff eyes. This discrepancy may be due in part to a genetic reduction in the amount of pigment in the w^{bf}; *bw* as well as in the w^{bf}; *st* eyes which MAINX examined. The distal pigment is much more striking than the proximal, though, and it seems unlikely that it would be reduced to a greater extent, except by solution during alcohol fixation (MAINX avoided the use of water in mounting sections). NOLTE's (1950) observations on pigmentation of the wild-type eye indicate that pigment is more readily lost by solution from the distal parts of the eye than from the proximal.

Finally, although some shrinkage and consequent distortion occurs during freezing and drying, it is far less than during alcohol fixation. The superiority of freezing-drying in this respect can be seen by comparing figure 14 of this paper with NOLTE's (1950) figure 4, from material fixed in modified Carnoy. In the latter, distortion is particularly noticeable in the fenestrated zone immediately below the basement membrane and in the region below the nuclei of the external optic glomerulus. The basement membrane stains with haematoxylin (see figure 14 in which it shows some slight discontinuities), and I believe that shrinkage in NOLTE's material has disrupted the membrane into discrete parts which can be mistaken for nuclei. The " basal nuclei " seen in cross or oblique sections (NOLTE's figure 3) cannot, of course, be accounted for in this way, but may conceivably be the bases of the rhabdomeres, which also stain strongly with haematoxylin.

The gross pigmentation pattern of the eye

In order to study the localization of pigment, the surface of the eye was arbitrarily divided into six regions, as shown in figure 15 A. In the flies examined, both eyes were scored for presence or absence of a major dark-pigmented area (one-quarter of a region or more) in each of these regions. The results, expressed as percentage of eyes with dark pigmentation in a given region, are shown in figure 15 B–E.

At 18°C, the T(1;4)$w^{258\text{-}18}$ males show a strong localization of pigment in middle-caudal (4) and upper caudal (2) regions. Sixteen out of seventy flies had dark pigment restricted to region 4 in both eyes; thirty others had one eye in which the pigment was so restricted. Eight exceptional eyes lacked pigment in region 4, but it was then present in region 2 or (in one eye) 6.

In heterozygous females, T(1;4)$w^{258\text{-}18}$/*w*, the pigment is more generally distributed, chiefly in the caudal regions 2, 4 and 6. Only five flies out of sixty

had the pigment restricted to region 4 in both eyes, seventeen in one eye. Six eyes lacked pigment in region 4, though it was present in region 2 or in 2 and 6.

In the Dp(1;3)$N^{264\text{-}58}$ stock, homozygous for white, the darkly-pigmented area is usually larger. Males and females, both of which may be either homozygous or heterozygous for the w^+ duplication, appear to have similar eyes, but only females were scored. Again region 4 is most frequently pigmented. Only one fly out of twenty-five had this area lacking in pigment in both eyes, and five in one eye. Here again, 2 or 6 was pigmented if 4 was not, except in two eyes where no large dark spot occurred.

Mottled-28, a stock in which the mottling is apparently of an entirely different type, showed a dorsal shift in the location of the pigment, which was chiefly in region 2, but also frequently in region 1 or 4. One fly out of ten

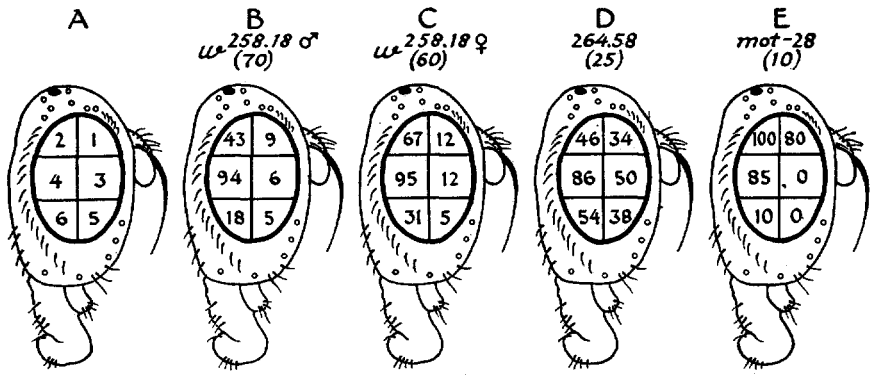


FIGURE 15.—Diagram of right eye of Drosophila, showing A, regions 1–6 used in mapping distribution of pigment; B–E, distribution of pigment in eyes of four different groups of flies, as shown by percentage of eyes having major pigmented areas in each of the regions 1–6. Total number of eyes scored given in parenthesis under name of stock. Drawn by MRS. E. B. PATTERSON.

lacked pigment in region 2 in one of its eyes, which was pigmented only in region 4. Eyes of males and females are similar.

A cross was made between T(1;4)$w^{258\text{-}18}$ males and females from an inbred stock of brown scarlet (*bw; st,* 18 generations of brother-sister matings). F_1 females, heterozygous for the translocation and for *bw* and *st,* were backcrossed to *bw; st* males and the male progeny of this backcross was examined, and some individuals mated again with *bw; st* females to test their genotype with regard to *bw* and *st*. In this male progeny, T(1;4)$w^{258\text{-}18}$ males of the classes $bw^+; st^+$, *bw/bw* and *st/st* all showed localization of pigment, while T(1;4)$w^{258\text{-}18}$*; bw/bw; st/st* males had uniform and almost colorless eyes. This shows that both the red and brown pigments are affected, and confirms the observation in sectioned material that where one type of pigment cell is mainly pigmented, so too is the other type. The same conclusion was reached by CHEN (1948): "Dans les deux cas ($w^{258\text{-}18}$*; bw* and $w^{258\text{-}18}$*; st*) le mode de panachure est le même que chez le mutant $w^{258\text{-}18}$."

Now, we can assume that mutation is the cause of differences in pigmentation in different regions of the mottled white eye, and explain the observed pattern in this way. In the upper caudal region, mutation from w^{cr} to w^{+} can occur at an earlier or later stage in the development of the eye, and in the former event the w^{+} allele remains stable throughout several cell generations giving rise to a large wild type spot (although in a related stock of $T(1;4)w^{258\text{-}18}$, apparently differing only in certain genetic modifiers, DEMEREC and SLIZYNSKA concluded that the w^{+} allele was unstable, mutating to w^{ch} and w^{cr}). In the rostral and ventral regions, on the other hand, mutations of the w^{cr} allele occur only at the later stages, giving rise to the small patches of ommatidia in which some or all of the pigment cells show more intense pigmentation. In other words, some property of the upper caudal region favors mutation of the w^{cr} allele especially at early stages in the development of the eye.

There are some objections to this explanation. It assumes that the size of darker spots on the cream background depends on cell lineage and on the stage of development at which a mutation occurs. CHEN (1948) has shown that the number of dark-pigmented facets can be modified by temperature changes during the pupal stage, and it is also known (DEMEREC 1950) that all, or nearly all of the ommatidial precursors are laid down by the last day of larval life. If at higher temperatures the size of the dark spots is increased (and the extent of the dorso-caudal pigmented area certainly is increased) the greater size of a spot cannot be correlated with a greater number of cell generations in which its ommatidia were derived from a single initial ommatidium or cell, because new ommatidia are not being formed during this pupal temperature sensitive period. The addition of new wild type or cherry facets under the influence of high temperature during the pupal stage must then be assumed to be due to independent mutations from w^{cr} within individual ommatidia during their development from the four-cell precursor stage to complete differentiation. Moreover, as the temperature effective period seems to extend to the last day of pupal life, when pigment formation has already started, "mutations" which alter pigmentation at this stage would presumably occur independently of cell division, which is unlikely to be taking place at this time.

According to CHEN, temperature changes are ineffective during the larval period, so that changes from w^{cr} toward wild-type apparently do not occur except during pupal, and possibly during embryonic, stages. There are some data (J. W. GOWEN, personal communication) indicating that there is a temperature sensitive period, for some white-mottled stocks at least, very early in development. Very early changes may, therefore, produce a pattern determined by cell lineage; but when the changes at the pupal stage are superimposed on the former, a final pattern of pigmentation which is not entirely dependent on cell lineage will be the result.

Another difficulty arises in connection with the direction of change. In accordance with DEMEREC and SLIZYNSKA, this discussion has assumed that in the light background stock of $T(1;4)w^{258\text{-}18}$ used in this work w^{cr} changes

to w^+, with or without w^{ch} as an intermediate stage. The work of DUBININ and SIDOROV (1935) and PANSHIN (1935) provides some evidence that in position effects associated with a break in heterochromatin the affected gene retains, in all essentials, the form it had before rearrangement; in other words, a wild type allele remains wild type and can, theoretically, be re-extracted in the normal form by crossing over into a normal chromosome. $T(1;4)w^{258\text{-}18}$ was obtained by irradiating a w^+ male. If it is assumed that the darker spots in the eye are due to changes from w^{cr} to w^{ch} or w^+, it seems necessary also to assume an initial change very early in ontogeny from the original w^+ allele to w^{cr}. It is hardly possible to suppose that this change occurred as a mutation at the time of irradiation, because DEMEREC and SLIZYNSKA report that light and dark background stocks were derived from the original one by selection, and in the dark background stock they postulate that the direction of change is from w^+ towards w^{cr}. In the light stock, then, the direction of mutation (if such it is) must be reversible, so we might expect, at least occasionally, to find one or more cream facets within a darker spot. The absence of such observations can only be explained by assuming that change from w^+ to w^{cr} is confined to an early embryonic stage, while changes from w^{cr} to w^+ can occur subsequently. In the dark stock, on the other hand, changes from w^+ to w^{cr} can presumably occur during pupal life, and changes from w^{cr} to w^+ not at all.

These arguments are not conclusive, but they indicate the difficulty of presenting a consistent hypothesis in terms of mutability, *sensu stricto,* as an explanation of the variegated phenotypes found in a large group of position effects.

As has been seen, the histological picture suggests that the type of pigmentation found in a white-mottled eye is different from that of the mutant alleles of the *w* locus.

The gross pattern of variegation in the eye may be determined by some developmental factor other than mutation. The preferential localization of pigment in regions 2 and 4 sems to be a general phenomenon which is manifested independently of the existence of a position effect; for it occurs also in the *mot-28* stock, in which there is no demonstrable position effect. It may also be relevant that in another position effect, associated with In(2LR)40d, the abnormality of a deeply pigmented crust forming on the surface of the eye occurs preferentially in the ventral part of the eye (HINTON 1949); while in several mutants that affect the structure of the eye, the ventral half is more severely affected than the dorsal (*e.g.,* in almondex, Deformed, Kidney, Lobe). It is not probable that somatic mutation is involved in all these cases, but a common factor might affect gene mutation in the position effects, and some other stage between gene and end-result in the mutant stocks; or a common effect on the *action* rather than the essential structure of the gene seems a possible explanation of the facts.

It is rather striking, in connection with the localization of pigment, to find the following passage in UMBACH'S (1934) description of the development

of pigment in the eye of the mealmoth, *Ephestia Kühniella* Z.: " Bei der Pigmentbildung in den Retinulazellen ist ebenso wie für die Formwandlungsprozesse der dorsocaudale Bezirk Differenzierungsmittelpunkt. Tägliche Beobachtung unter dem Binokular ziegt, wie in den ersten 10 Tagen der Puppenzeit ein ' Pigmentstrom ' über die Augenanlage hinzieht. Die Pigmentierung schreitet ventralwärts schneller als in rostraler Richtung fort. . . . Am 10 Tage nach der Verpuppung erscheint die gasamte Fläche der Augenanlage durch Pigment verdunkelt." UMBACH adds that pigmentation of the ommatidia is independent of their stage of development, some becoming pigmented at an early and others at a later stage of differentiation, according to their position within the eye.

Is it merely a coincidence that in some Drosophila eyes the pigment is confined to approximately the same region where it first begins to develop in the eyes of Ephestia?

In Drosophila, those who have studied the development of the eye (*e.g.*, KRAFKA 1924; BODENSTEIN 1938; STEINBERG 1943) have observed no regional differences in the time of onset of ommatidial differentiation; nor is there evidence that pigmentation of the ommatidia is other than simultaneous. In fact, the retinula cells, which alone show the " Pigmentstrom " in Ephestia, remain unpigmented in Drosophila.

In order to determine more certainly whether pigmentation in Drosophila occurs simultaneously over the whole eye or not, flies were raised at a low temperature (about 15°C) to slow down development. In the conditions of the experiment, puparium formation started about fifteen days after the culture was started (with non-virgin parents), and adult flies emerged about thirteen days from puparium formation. Some time after the sixth day (not accurately timed) from puparium formation, the earliest stage of pigmentation that could be seen was in the form of a very pale yellow color distributed uniformly over the whole surface of the eye.

Thus there seems to be no obvious relationship between the localization of pigment in Drosophila and the site of its initial formation, and some other factor must be sought for.

It might be postulated that the localization of pigment is related to a gradient which is established within the eye before the beginning of pigment formation, and which is effective in the white-mottled eye (though not in the wild-type eye nor those of most other mutants) by virtue of a change in the sensitivity of the pigment cells to the factor which determines the gradient. Suppose, for instance, that a diffusible substance were at the highest concentration in the dorsocaudal center, diminishing to a lower concentration peripherally; and that pigment formation could proceed wherever the concentration reached a threshold inversely related to the sensitivity of the individual pigment cell. In the wild type eye it could be assumed that both concentration and sensitivity are high enough in all parts of the eye to ensure uniform pigmentation. If in the white-mottled eye the pigment cells tended to be less

sensitive, owing to modification of the w^+ locus, then we might expect pigment formation to be normal in the area of highest concentration and elsewhere to be limited to those cells whose sensitivity was normal or nearly so. This would result in the pattern of a dorsocaudal pigmented area with smaller peripheral spots. Sensitivity could conceivably vary discontinuously, depending on discontinuous states (temporary modifications, not strictly mutant states) of the w^+ locus, and thus account for the intermediate type of pigmentation. The effect of high temperature could presumably be either to increase the sensitivity of the pigment cells or to reduce the steepness of the gradient (for instance, by increasing the rate of diffusion). Where temperature changes are effective in very early stages of the life cycle, the former mechanism would operate, and during the pupal temperature sensitive period the latter might also come into play.

A diffusion gradient is not, of course, the only form which can be invoked to explain the pattern of pigmentation. Many other factors could play a similar role. One such factor which may influence pigmentation has been mentioned by BODENSTEIN (1943) who relates that in the development of wild type eye-discs transplanted (together with ring-glands) into adult hosts, "we observe most frequently that only certain eye regions are pigmented while others are still white," though he does not give further details as to which regions are preferentially pigmented. He suggests that this phenomenon is due to the position of the graft in the host, and more directly to local differences in oxygen supply. It is possible that such differences occur also in normal development of the eye, and are significant only for certain genotypes.

SUMMARY

The structure of the ommatidium in *D. melanogaster* is discussed.

In the mottled eye of T(1;4)$w^{258\text{-}18}$ the individual secondary pigment cells appear to be fully pigmented, unpigmented or intermediate. The post-retinal pigment cells are mostly fully pigmented, but may be unpigmented or intermediate. Only two types of primary pigment cells, fully pigmented and unpigmented, were distinguished.

The intermediate pigmentation in T(1:4)$w^{258\text{-}18}$ differs histologically from that of three intermediate alleles of the white locus. It is argued that the position effect is not due to mutation, in the strict sense, at a locus that has become unstable.

Further histological details of the pigmentation in T(1;4)$w^{258\text{-}18}$ are described.

The gross pattern of pigmentation, as seen at the surface of the eye, is not entirely random, pigment being most frequently concentrated in the dorsal caudal region of the eye. The size of this pigmented area does not seem to be a function of its cell lineage. It is suggested that the pattern may be due in part to a developmental factor influencing the manifestation of the position effect, rather than being fundamental to the mechanism of position effect.

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