THE EFFECT OF THE LOZENGE PSEUDOALLELIC SERIES ON EYE PIGMENTATION IN *DROSOPHILA MELANOGASTER*. II. RED PIGMENT AND PIGMENTATION IN LOZENGE COMPOUNDS

FRANCES E. CLAYTON

Department of Zoology, University of Arkansas, Fayetteville, Arkansas

Received June 24, 1957

IN the investigation of pigmentation among the lozenge series of pseudoalleles in *Drosophila melanogaster*, the amount and distribution of red and brown pigments in the compound eyes of the lozenge mutants can be analyzed histologically by studying the genic effect on the two pigments separately. In an earlier report (CLAYTON 1957), the distribution of pigment in males of the lozenge mutants was described, as well as the distribution of brown pigment in lozenge males homozygous for the mutant brown. In continuing this study, an analysis has been made of lozenge females homozygous for vermilion (*v*) or scarlet (*st*) and eighteen compounds of females heterozygous for two different lozenge alleles but homozygous for scarlet.

OLIVER (1947) described the eye colors in ten of the lozenge alleles and the appearance of the eyes with scarlet and vermilion. GREEN (1948) analyzed quantitatively the amount of red pigment present in the eyes of seven types of lozenge females which were homozygous for a lozenge mutant and for vermilion. A number of investigations have been made on the lozenge effect in compounds; these studies are discussed in an earlier paper on the structural abnormalities of the ommatidia in lozenge compounds (CLAYTON 1954a). GREEN and GREEN (1949) observed that the phenotypic effects of the lozenge mutants, when classified on the basis of the quantity of red pigment present in the eyes, were distributed at random at the three loci assigned to these pseudoalleles. In the analysis of the ommatidial abnormalities in the compounds, no correlation between the loci involved and the severity of structural defects could be found.

In the present investigation, an analysis has been made to determine, by histological methods, the distribution of red pigment in ten of the lozenge mutants and in the compounds of these mutants in order to correlate the appearance of the eyes with abnormalities in eye structure and pigment distribution.

MATERIALS AND METHODS

The histological techniques used in this study are the same as those described in the first paper of this series; heads were fixed in Carnoy's solution, sectioned at ten microns and mounted on slides unstained. Due to the solubility of the red pigment in water, some loss of pigment occurred during the dehydration process in the preparation of stained slides. The loss of pigment during the preparation

of unstained slides was slight, but those individuals in which an observable amount of red pigment was lost were not considered in the analysis.

As controls, the mutant stocks scarlet (*st*) and vermilion (*v*) of *Drosophila melanogaster* were used. Balanced isogenic stocks were used (OLIVER 1947) in which each of the following lozenge alleles was homozygous for *st* or *v*: lozenge (*lz*), lozenge 37 (*lz*³⁷), lozenge Bar-Stone (*lz*^{BS}), lozenge glossy (*lz*^g), lozenge 34k (*lz*^{34k}), lozenge y4 (*lz*^{y4}), lozenge 3 (*lz*³), lozenge spectacle (*lz*^s), lozenge spectacle-Bishop (*lz*^{sB}), and lozenge 36c (*lz*^{36c}). The compounds examined were homozygous for the mutant scarlet and are listed in Table 1. The unstained slides of the compounds were compared with previously prepared stained slides.

TABLE 1

Comparison of eye structure and quantity of red pigment in lozenge compounds

Compound	Eye structure*	Amount of red pigment†
<i>lz</i> ³⁷ / <i>lz</i>	<i>lz</i> ³⁷	Normal
<i>lz</i> ³⁷ / <i>lz</i> ^{BS}	<i>lz</i> ³⁷	Normal
<i>lz</i> ³⁷ / <i>lz</i> ^g	<i>lz</i> ³⁷	Intermediate
<i>lz</i> ³⁷ / <i>lz</i> ³	<i>lz</i> ^g	Intermediate
<i>lz</i> ³⁷ / <i>lz</i> ^{sB}	<i>lz</i> ^{BS}	Normal
<i>lz</i> ³⁷ / <i>lz</i> ³⁶	<i>lz</i> ^g	Intermediate
<i>lz</i> / <i>lz</i> ^{BS}	<i>lz</i>	<i>lz</i>
<i>lz</i> / <i>lz</i> ^{y4}	<i>lz</i> ^g	<i>lz</i>
<i>lz</i> / <i>lz</i> ^s	<i>lz</i>	<i>lz</i>
<i>lz</i> ^{BS} / <i>lz</i> ^g	<i>lz</i>	Normal
<i>lz</i> ^{BS} / <i>lz</i> ^{y4}	<i>lz</i> ³⁴	Intermediate
<i>lz</i> ^{BS} / <i>lz</i> ³	<i>lz</i> ³⁴	Intermediate
<i>lz</i> ^{BS} / <i>lz</i> ^s	<i>lz</i> ^g	<i>lz</i> ^g
<i>lz</i> ^g / <i>lz</i> ³⁴	<i>lz</i> ^g	<i>lz</i> ^g
<i>lz</i> ^g / <i>lz</i> ^s	<i>lz</i> ^g	<i>lz</i> ^g
<i>lz</i> ^g / <i>lz</i> ³⁶	<i>lz</i> ³ or <i>lz</i> ^{y4}	Intermediate
<i>lz</i> ³ / <i>lz</i> ^s	<i>lz</i> ³ or <i>lz</i> ^{y4}	<i>lz</i> ³
<i>lz</i> ^{sB} / <i>lz</i> ³⁶	<i>lz</i> ^s or <i>lz</i> ^{sB}	None

* From CLAYTON 1954a.

† Each allele was homozygous for the eye color mutant, scarlet.

RESULTS

Effect of lozenge on red pigment

The effect of the lozenge type mutant on the quantity and distribution of red pigment was studied by adding the recessive genes scarlet or vermilion to the lozenge genotype. EPHRUSSI and HEROLD (1944) reported the suppression of brown pigment by scarlet with no interference of the red pigment. The recessive vermilion also suppresses brown and leaves only red pigment. OLIVER (1947) observed that all but one of the ten lozenge alleles reduce the red pigment when *st* is present, and some of the mutants alter the red pigment distribution. He found that more red pigment is produced with *v* than with *st* and that the greatest difference between scarlet and vermilion phenotypes is among the more severe al-

leles. GREEN (1948) reported marked differences in the amount of pigment produced in nine different lozenge mutants with vermilion present. Most of the mutants used in this study were tested with both *st* and *v*. The inversion associated with *Lz^{SB}* prevented the addition of vermilion to that genotype.

The eyes of scarlet are bright red in color, appearing in sections as light red. NOLTE (1950) noted that the postretinal pigment layer is slightly narrower in *st* than in the normal eye but that the density of the granules seems to be similar to wild type (Figure 1). The color throughout the eye is uniform, lacking the yellowish-red color found in the distal portions of the normal eye.

The eye of vermilion is also a bright red color. The appearance in sections is very similar to that of *st*, with a uniform light red color. JOHANNSEN (1924) described the color of *v* as being quite similar to wild type, with yellow granules sparsely distributed in the distal pigment regions and a heavy deposit of wine-red granules toward the basal regions. NOLTE (1954) described the colors of vermilion and scarlet as identical, in aggregate appearing light red. The distribution of the red granules is similar in these two mutants, and the only distinction that could be found in this study was that the over-all impression of red color seems to be slightly darker in the vermilion eye.

Lozenge 37

The least severe lozenge mutant, *Lz³⁷*, does not appear to affect the amount of red pigment present with either *st* or *v*. The only irregularities that could be observed were associated with the small regions of structural abnormalities. The irregularities in pigment distribution are like those previously described when both red and brown pigment are present. Pigmentation in the primary cells is normal except where fusion of facets occurs, but the distortion of the pigment cells is limited to the region of the basal membrane in most areas of the eye (Fig. 2). The pigment granules are bright red in color and the amount of pigment is normal. OLIVER (1947) reported that *Lz³⁷* was the only lozenge allele which did not reduce the amount of red pigment with either *v* or *st* in the genotype.

Lozenge

The structural abnormalities of *Lz* are similar to those described in earlier papers. OLIVER described the eye color with scarlet as a light scarlet color, sometimes giving a uniform orange appearance. The color with vermilion is bright red with shiny black spots and occasional light areas. Black facet-size spots are present on the eye surface; these correspond to the "eruption" facets described previously. The color of the pigment in unstained sections is similar to that described by OLIVER. The color with *st* is orange, lighter than the bright red of the *st* mutant. There appears to be a decrease in the amount of red pigment in all regions of pigment concentration. The eye with vermilion is darker than with scarlet but the distribution is not uniform. There are occasional lighter or darker areas of pigment accompanying the structural abnormalities. The irregularities of the primary pigment cells are more marked than in *Lz³⁷* as these cells are fre-

FIGURES 1-8.—Unstained sections of *scarlet*, lozenge pseudoalleles and lozenge compounds showing pigment patterns. Carnoy's fixative; original magnification $\times 200$. FIGURE 1.—*scarlet*. FIGURE 2.—*lz*³⁷ *st*. FIGURE 3.—*lz*^{BS} *st*. FIGURE 4.—*lz*^g *st*. FIGURE 5.—*lz*³ *v*. FIGURE 6.—*lz*³⁷/*lz*; *st*. FIGURE 7.—*lz*^{BS}/*lz*^g; *st*. FIGURE 8.—*lz*^g/*lz*³⁴; *st*.

quently present in a layer beneath fused facets. The secondary, basal and post-retinal pigment cells are more highly distorted, with accompanying abnormalities in pigment distribution.

Lozenge Bar-Stone

The eye color of lz^{BS} with st or v is not uniform in any region of pigment deposition. The irregular distribution is closely associated with the structural abnormalities, particularly where ommatidia are absent or severely distorted. These regions differ from the normal or slightly abnormal areas by having a heavy concentration of red pigment or lacking pigment completely. With st the color appears orange except in severely distorted regions where a greater density results in a bright red color (Fig. 3). The effect with vermilion is similar to that with st except that the over-all color is a brighter red.

Glossy

The eyes of lz^g with either v or st are bright red except for shiny areas where fusion of facets has occurred. The appearance in sections is a bright reddish-orange except where concentration is heavier in the primary pigment cells below the fused cornea. In these regions the color is bright red. The color appears to be slightly darker with vermilion than with scarlet. A heavy concentration of red pigment occurs at the marginal rim but no granules were found accompanying the postretinal layer of retinulae (Fig. 4). The quantity of red pigment in glossy is greater than in either lz^{BS} or lz .

Other alleles

Both lz^{34} and lz^{y4} with scarlet have reddish-orange eyes and an irregular distribution of the pigment. In sections the rim always has a dense concentration of orange granules but the arrangement of pigment in the remainder of the eye is very irregular. The scattered distribution of the granules, except in the marginal rim, gives the impression of a yellow color. The irregular patches of pigment are concentrated beneath the fused cornea in primary pigment cells and in secondary pigment cells surrounding the postretinal layer of retinulae. With vermilion, both lz^{y4} and lz^{34} have bright reddish-orange eyes similar to the color with scarlet.

The eyes of $lz^3 st$ are pink in color as the result of scattered spots of pigment throughout the eye and a narrow pigmented rim. In sections these spots appear to be the result of small concentrations of pigment just below the cornea. Only occasional streaks of pigment could be seen below the level of the primary pigment cell layer except in the margin where a dense concentration of pigment extends from the cornea to the basal region. This allele, when vermilion is present, has much more pigment than with scarlet. The rim is heavily pigmented and the patches of red granules below the cornea are deeper red in color (Fig. 5).

The "spectacle" alleles have no observable pigment with scarlet. The sections show no pigment present in any of the regions of pigment concentration. Two of the alleles, lz^8 and lz^{36} , were tested with vermilion and a small amount of pigment

could be observed in the marginal rim, with very small spots of pigment in other regions. The color of the pigment concentrated in the margin is a dull red, lacking the bright scarlet appearance of the less severe alleles. The small stippling of pigment below the cornea also appears to be dark red, although not as dark as the pigment of the rim.

Red pigmentation in lozenge compounds

An analysis was made of the red pigment in 18 compounds of the lozenge alleles with scarlet and the pigment irregularities were compared with the structural abnormalities described earlier (CLAYTON 1954a) and with the pigmentation in compounds described by OLIVER (1945) and OLIVER and GREEN (1947).

Compounds with lozenge 37

Six compounds of Lz^{37} with other members of the lozenge series were studied. The compounds of Lz^{37} with Lz or Lz^{BS} are similar in color to scarlet, thus normal in red pigmentation; the distal portions of the ommatidia are yellowish-orange in color and the basal regions are dark red. The structural abnormalities have been reported and the only irregularity in the red pigment distribution occurs among the disrupted basal and postretinal pigment cells in regions where abnormal postretinal elements occur. Occasionally the more distal pigment cells are disrupted by irregular retinulae penetrating the basal membrane. Such regions are usually limited to small regions of the eye (Fig. 6). OLIVER (1945) reported normal pigmentation in these compounds in a study based on the surface appearance of the eye color. The Lz^{37}/Lz^9 compound is similar to Lz^{37} males structurally but the red pigment appears more orange than the scarlet in structurally normal eyes. Some red pigment granules are present in irregular streaks surrounding the postretinal bundles. OLIVER (1945) reported a normal eye color in this compound. The eye structure of Lz^{37}/Lz^3 is intermediate between the two alleles, resembling the glossy phenotype. The eye color of the compound as seen in sections is a dark red. The distribution of pigment is irregular due to regions of fusion and disarrangement of the ommatidial elements. In fused areas where no normal facets are present the pigment of the primary pigment cells form a dense layer beneath the flattened cornea. Some pigment cells surrounding abnormally shaped pseudocones contain only a few granules or lack pigment completely. The eye color of this compound was described by OLIVER as resembling the homozygous Lz females.

The eye structure in the compound of Lz^{37} with Lz^{SB} is less irregular than the Lz^{37}/Lz^3 compound, resembling the Bar-Stone eye. The color is a bright scarlet, very similar to that of the st mutant. The compounding of Lz^{36} with Lz^{37} results in eyes which are intermediate in both color and structure. Abnormalities in structure of the ommatidia and the deposition of red pigment granules resemble those found in Lz^9 . The outer pigment cells are yellowish-red and the basal region is a deeper red. The postretinal pigment layer beneath normal ommatidia is thicker than in st and a dark red pigment deposit extending distally to the primary pigment cells is present in the marginal ommatidia. Except in the marginal region,

the pigmentation is irregular in the distal portions of the secondary pigment cells and in the primary pigment cells. The color varies from yellowish-red to orange in different regions of the eye. The "erupted" facets of the cornea are frequently accompanied by a layer of primary pigment cells containing reddish-brown granules.

Other compounds

The pigmentation in two compounds with *lozenge*, lz/lz^{BS} and lz/lz^s , is indistinguishable from the homozygous *lz*. The lz/lz^{y4} compound produces structural abnormalities and consequently, pigment distribution like that of glossy, but the eye color is not as dark as lz^g , having a bright orange color rather than the darker scarlet color of the glossy mutant.

With the exception of lz^{BS}/lz^g , the compounds of Bar-Stone with more severe alleles are intermediate phenotypically. The structural abnormalities and red pigment distribution of the compound with glossy are like the homozygous *lz*, a condition more nearly normal than either homozygote of the compound. The color is indistinguishable from *st*. The pigment distribution in lz^{BS}/lz^{y4} is like lz^{34} and the color varies from orange to bright red with the deeper pigmentation occurring in the rim and in the region of the basement membrane. The pigment in compounds of Bar-Stone with lz^3 and lz^s is bright red in the margin and bright orange or yellow in the flattened primary pigment cells below fused facets. In lz^{BS}/lz^3 the distribution is like lz^{34} while lz^{BS}/lz^s is less severe, with distribution like glossy.

Three compounds with lz^g were analyzed; two of these, lz^g/lz^{34} and lz^g/lz^s , are like glossy in the amount and distribution of the red pigment. The eyes are bright red with irregularities in distribution accompanying the structural irregularities, which are also similar to glossy. Very little pigment is present in the region of the postretinal retinulae. The heterozygous combination of lz^g with lz^{36} is lighter in color than the other two compounds, the over-all color appearing as a bright orange with a somewhat darker rim.

The two remaining compounds studied, lz^3/lz^s and lz^{sB}/lz^{36} , possess no normal ommatidia and the cornea is flattened, with a layer of pigment cells underneath. In lz^3/lz^s a rim of orange pigment is present, appearing as a dense solid mass in primary and secondary pigment cells in that region. Small scattered masses of yellow or orange pigment granules occur beneath the cornea in the flattened primary pigment cells but their distribution is very irregular. The compound of lz^{sB}/lz^{36} contains no red pigment that can be detected in unstained material.

DISCUSSION

With this analysis of red pigmentation in the *lozenge* mutants and in compounds of *lozenge* a more complete examination of the *lozenge* effect on pigmentation may be made. As described in an earlier paper (CLAYTON 1957), the series of *lozenge* pseudoalleles fall into two groups when both red and brown pigments are present, the first group consisting of those *lozenge* mutants with eyes of normal

color or darker than the wild type and the second group consisting of those alleles with an extreme reduction in red pigment. When the two pigments are considered separately by eliminating either red or brown, the mutants may then be placed in linear series on the basis of their effect on either red or brown pigment, but the seriation is not the same for the two pigments.

When the different lozenge mutants are homozygous for brown the pigment distribution and the quantity of brown pigment deposited in the pigment cells make possible a seriation of decreasing pigmentation which corresponds with increasing ommatidial abnormalities of structure. The only exception to this series is that *lz*, structurally more abnormal than *lz*³⁷, appears to have more brown pigment present than either wild type or *lz*³⁷. The detailed description of brown pigmentation was presented in the previous paper by the author (1957).

The sequence of the alleles when considered on the basis of their effect on red pigment as given by OLIVER (1947) and GREEN (1948) corresponds to the seriation based on this histological study. With the exception of *lz*³⁷, all of the members of the lozenge series reduce the amount of red pigment produced. Both OLIVER and GREEN found that *lz*³⁷ was normal in red pigmentation with scarlet or vermilion. Although there is a uniform reduction in the amount of red pigment in all regions of *lz* eyes, the distribution is normal except in fused regions. Green found that *lz* with vermilion had a red pigment value of only 29.2 percent relative to 100 percent value for the vermilion mutant. OLIVER reported that the eyes of *lz v* had more red pigment than with *st* and the same condition was found in this investigation. In structurally abnormal regions, however, *lz v* is more variable in the amounts of red pigment deposited than in *lz st* where the amount of red pigment even in fused areas appears to be uniform.

Both *lz*^{BS} and *lz*^g possess more red pigment than *lz*, a factor contributing to the darker eye color of these mutants. GREEN (1948) found the quantity of red pigment in Bar-Stone with vermilion to be 41.6 percent with *lz*^g *v* possessing 50.3 percent of the maximum. In both of these mutants the distribution is irregular and the color varies from bright orange to deep red in different regions of the eye. The eyes of *lz*³⁴ are similar to *lz* in the amount of red pigment produced; according to GREEN's analysis the values are 29.2 percent for *lz* and 27.3 percent for *lz*³⁴. These two mutants, which are quite easily distinguished on the basis of eye structure, are alike in their effect on red pigment, and the darker eye color of *lz*³⁴ may be partially explained on the basis of differences in the distribution of the pigment granules in relation to the structural abnormalities. Although the eyes of *lz*^{u4} appear darker than any of the previously mentioned alleles, the total amount of red pigment is considerably reduced. The localization of the pigment below the cornea would make the eye appear darker than in those mutants with elongated secondary pigment cells and definite basal and postretinal concentrations of red pigment.

In the remaining mutants, *lz*³, *lz*^s, *lz*^{sB}, and *lz*³⁶, the amount of red pigment in the pigment cells is greatly reduced. In this group, however, differences between the gene action with *st* and with *v* become quite apparent. With scarlet, *lz*³ has a

red marginal rim and small spots of red pigment beneath the cornea; with vermilion the pigment deposition is much greater. The rim is heavily pigmented and the remaining pigmented areas are a much deeper red. The lz^s , lz^{sB} and lz^{s6} alleles have no observable pigment with *st*; however, with vermilion both lz^s and lz^{s6} have dark red granules in the rim and very small widely scattered spots of dark red pigment in central regions of the eyes. GREEN (1948) found that the red pigment value for $lz^s v$ was about six percent and that red pigment could be extracted from $lz^s v$ and $lz^{s6} v$ but the quantity was so small that it could not be measured.

The differences in eye color of the various lozenge mutants are closely related to the amount of pigment deposited in the ommatidial cells and the degree of cellular abnormality. Whether the concentration of pigment in the cells is due to differences in the number of granules present or to the amount of pigment deposited in each granule could not be determined accurately. In those individuals where counts were attempted, the number of granules did not appear to vary significantly. Since the changes in eye color occur primarily in those mutants with rather severe structural irregularities of the ommatidia, pigmentation and eye structure must be considered together. A comparison of photographs of unstained sections (Figs. 9 through 16) indicates the extent of the irregularities in red pigment distribution. The figures are arranged in the order of increasing distortion of the pigment pattern, from the normal condition of scarlet (Fig. 9) to those intermediate in abnormalities (Figs. 10 through 13) and those individuals in which the ommatidia are highly distorted or absent (Figs. 14, 15, 16).

In lz^{s7} , where distribution of pigment is normal, the disruption of the pigment cells occurs only in the region of the basal and postretinal pigment concentrations and does not affect the eye color. This type of disturbance may be seen in Figure 11. Disruption of distal pigment cells is usually limited to small regions (Fig. 12). In the remaining mutants, however, irregular distribution of the pigment occurs in primary and secondary pigment cells as well as the more proximal pigmented areas, thus affecting the over-all eye color.

Fusion of facets and a changed orientation of the primary pigment cells around abnormal pseudocones alter the relationship between the eye surface and the pigment of these cells. This probably accounts for the variations in surface eye color when the amount of pigment remains almost normal. Severe corneal abnormalities accompany an extreme disruption of the primary pigment cells and the distal portions of the secondary pigment cells. In those regions where the cornea has large depressions and pseudocones are absent, the pigment may appear as a solid layer beneath the depression, or the pigment cells may be completely absent beneath the depressed area, forming masses of heavily pigmented cells around the unpigmented region (Figs. 13, 14). This results in a colorless spot surrounded by a heavy deposit of pigment. CASTEEL (1929) described the "pink" appearance of ommatidia from surface view, but he found that these ommatidia were without pigment and owed their color to light reflected from pigment of adjacent ommatidia. OLIVER (1947) suggested that the orange appearance of the eyes of lz^{s4}

FIGURES 9-16.—Unstained sections of *scarlet*, lozenge pseudoalleles and lozenge compounds selected to illustrate varying degrees of irregularities in pigment distribution. Carnoy's fixative; original magnification $\times 475$. FIGURE 9.—*st*. FIGURE 10.—*lz³⁷/lz*; *st*. FIGURE 11.—*lz^{BS}/lz^g*; *st*. FIGURE 12.—*lz³⁷ st*. FIGURE 13.—*lz^g/lz³⁴*; *st*. FIGURE 14.—*lz^g st*. FIGURE 15.—*lz^g/lz³⁶*; *st*. FIGURE 16.—*lz³ v*.

with scarlet is due to reflection of light through clear areas around which the red pigment is aggregated.

From the results of this study, it appears that this abnormal arrangement of the primary pigment cells together with the amounts of red and brown pigments deposited in the granules explains the appearance of irregular patches and streaks of color characteristic of the more severe lozenge mutants. In addition to depressions in the cornea, small facet-sized "erupted" facets occur in some of the mutants. These are the result of the elongation during pupal development of displaced cells above the pseudocone region (CLAYTON 1954b). Pigment distribution below such facets is similar to that beneath depressed regions; primary pigment cells may form a solid layer or may be aggregated around the eruptions.

The distortion of the secondary pigment cells which accompanies abnormalities of the retinulae also causes disruption of the normal pigment pattern. However, the most severely affected region is that of the basement membrane and postretina. The disruption of the basement membrane by abnormal retinulae in the postretina causes irregularities in the pigment distribution. The postretinal pigment regularly forms streaks of color about the postretinal retinulae in the least severe alleles, but with increasing severity of the structural abnormalities this pigment is sparse or absent, as may be seen by comparing Figures 12 and 13. Retinulae which penetrate the basement membrane produce small regions lacking pigment which are surrounded by heavy concentrations of basal and postretinal pigment.

The lz^3 mutant and the spectacle alleles, which show the severest structural abnormalities, also have the greatest irregularities in pigmentation. The heavy pigment deposit of the rim is present in elongated pigment cells at the margin of the eye, and the remainder of the pigment is deposited just below the cornea (Fig. 16). As observed in the development of lz^s (CLAYTON 1954b), many pigment cells outside the marginal layer appear to undergo degeneration during late pupal development. The small scattered spots of pigment seen in the adult eyes may be the result of this degeneration of pigment cells.

In the comparison of the effects of the mutants with st , v or bw , no differences in the severity of structural abnormalities were observed and differences in the distribution of pigment were not noticeable except in the postretinal concentration. With brown, the streaks of pigment accompany postretinal retinulae less frequently than with either scarlet or vermilion and, in several alleles, no pigment could be seen around these cells when only brown pigment was present.

The phenotypic effects on ommatidial structure and red pigment quantity of lozenge compounds are compared in Table 1. In the majority of the compounds the amount of red pigment corresponds to the severity of ommatidial abnormalities. However, in several compounds the dominance relationships are altered. In lz^{37}/lz^g the structure is like lz^{37} but the eye color is intermediate; lz^{37}/lz^{sB} is intermediate in eye structure but resembles lz^{37} in the quantity of red pigment present. Although the structure of lz/lz^{y4} resembles the glossy allele, the eyes lack the heavy deposit of red pigment characteristic of this mutant and it more closely resembles lz . The amount of red pigment in lz^{BS}/lz^g cannot be distinguished from

scarlet while the structural characteristics are like *Lz*. No correlation in interaction between the alleles and their loci (GREEN unpublished, cited in CLAYTON 1954a) could be found on the basis of the quantity or distribution of the eye pigments. GREEN and GREEN (1949) found that the mutants, when classified quantitatively with respect to the amount of red eye pigment formed, were distributed at random to the three lozenge loci; the histological study showed a similar random distribution.

OLIVER (1947) suggested that the eye color of the lozenge alleles was the result of both the quantities of red and brown pigments produced and the distribution of these pigments. The results of the present series of investigations indicate that both of these factors are involved in the final expression of eye color. The effect of the alleles on the production of red and brown pigment appears to be at least partially independent of the ommatidial abnormalities and the resulting irregularities in pigment distribution. Although the abnormalities in the arrangement and structure of the pigment cells, together with degeneration of cells in the severe alleles, can account for variations in the distribution of pigment and some quantitative changes, other factors seem to be involved. For example, *Lz^s* and *Lz^u* cannot be distinguished on the basis of structural abnormalities, ommatidial arrangement, or pigment distribution; yet the eye colors are quite different as a result of variations in the amount of pigment present. Both *Lz^s* and *Lz^B* have the same ommatidial structure and eye color but act quite differently in compounds with other lozenge alleles.

An investigation on the pleiotropic effects of specific mutants in mice, carried out by GRUNEBERG (1938, 1943), revealed that the multiple effects could be traced to a single biochemical change wrought by the mutant gene early in development. In the lozenge series of pseudoalleles, however, no single factor has been discovered which will explain all of the phenotypic effects of these genes. The effect of lozenge on the color of the compound eye can be explained in part on the primary effect on eye structure. The irregular cell differentiation during development of the eye results in abnormal ommatidia and secondarily in irregular pigment distribution. ANDERSON (1945) described a similar relationship in which reduced fertility in lozenge was a secondary effect of the abnormal development of the female genitalia. Although lack of normal cell differentiation may account for several of the pleiotropic effects of the lozenge pseudoalleles, some independence in the gene expression still seems to exist. Variations in the interaction of phenotypically similar lozenge mutants in compounds, semi-dominance of fertility as compared to recessiveness of other effects, as well as differences in the quantities of red and brown pigments indicate that a complex system of gene action is involved in the final expression of the lozenge phenotype.

SUMMARY

The quantity and distribution of pigment in ten lozenge pseudoalleles homozygous for scarlet or vermilion are described and the red pigmentation in eighteen compounds from lozenge alleles homozygous for scarlet are analyzed.

The differences in eye color are the result of variations in the amount of red pigment produced and the distribution of the pigment. The distribution appears to be directly related to the abnormalities in structure and arrangement of the ommatidial cells.

Abnormal cell differentiation during pupal development and degeneration of some abnormal pigment cells may account for several of the lozenge effects but does not satisfactorily explain differences in the amount of pigment deposited in the different alleles or variations in the expression of phenotypically similar mutants when in compounds.

LITERATURE CITED

- ANDERSON, R. C., 1945 A study of the factors affecting fertility of lozenge females of *Drosophila melanogaster*. *Genetics* **30**: 280-296.
- CASTEEL, D. B., 1929 Histology of the eyes of X-rayed *Drosophila*. *J. Exp. Zool.* **53**: 373-385.
- CLAYTON, F. E., 1952 Phenotypic abnormalities of the eyes of lozenge alleles in *Drosophila melanogaster*. *Univ. Texas Bull.* **5204**: 227-251.
- 1954a Phenotypic abnormalities of the eyes of lozenge compounds in *Drosophila melanogaster*. *Univ. Texas Bull.* **5422**: 189-209.
- 1954b The development of the compound eyes of lozenge alleles in *Drosophila melanogaster*. *Univ. Texas Bull.* **5422**: 210-243.
- 1957 The effect of the lozenge pseudoallelic series on eye pigmentation in *Drosophila melanogaster*. I. Brown pigment and pigmentation in lozenge males. *Genetics* **42**: 28-41.
- EPHRUSSI, B., and J. L. HEROLD, 1944 Studies of eye pigments on *Drosophila*. I. Methods of extraction and quantitative estimation of the pigment components. *Genetics* **29**: 148-175.
- GREEN, M. M., 1948 Red eye pigment determination in the lozenge allelic series of *Drosophila melanogaster*. *Am. Naturalist* **82**: 188-192.
- GREEN, M. M., and K. C. GREEN, 1949 Crossing-over between alleles at the lozenge locus in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U. S.* **35**: 586-591.
- GRUNEBERG, H., 1938 An analysis of the "pleiotropic" effects of a new lethal mutation in the rat (*Mus Norvegicus*). *Proc. Roy. Soc. London B* **125**: 123-144.
- 1943 Congenital hydrocephalus in the mouse, a case of spurious pleiotropism. *J. Genet.* **45**: 1-21.
- JOHANNSEN, O. A., 1924 Eye structure in normal and eye-mutant *Drosophilas*. *J. Morphol.* **39**: 337-349.
- NOLTE, D. J., 1950 The eye pigmentary system of *Drosophila*: The pigment cells. *J. Genet.* **50**: 79-99.
- 1954 The eye pigmentary system of *Drosophila*. IV. The pigments of the vermilion group of mutants. *J. Genet.* **52**: 111-126.
- OLIVER, C. P., 1945 Four lozenge alleles phenotypically alike which react differently with their other alleles. *Genetics* **30**: 16.
- 1947 Interrelationship between eye color and facet arrangement in lozenge alleles of *Drosophila melanogaster*. *Univ. Texas Bull.* **4720**: 167-184.
- OLIVER, C. P., and K. C. GREEN, 1947 Dominance and interaction effects of four isogenic lozenge alleles in *Drosophila melanogaster*. *Anat. Record* **99**: 629.