THE EYE-PIGMENTARY SYSTEM OF *DROSOPHILA*

V. THE PIGMENTS OF THE LIGHT AND DARK GROUPS OF MUTANTS

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(With One Text-figure)

In continuation of the investigation of eye-colour mutants in *Drosophila melanogaster* the second and third groups of eye phenotypes were selected for study. These groups are the most contrasting, being the lightest and the darkest. The former consists of the mutants light (*lt*), lightoid (*ltd*) and persimmon (*pers*), while in the dark class sepia (*se*), clot (*cl*), Henna-recessive (Hn^r) and Plum^2 (Pm^2) are included. These phenotypes result from point mutations excepting persimmon, which is inseparable from an inversion, *In*(3*L*)*pers*, and Plum^2, which is inseparable from *In*(2*LR*)Pm^2 with the left break near the spindle attachment and the right break near the locus of brown (*bw*).

The methods applied in this investigation were similar to those of previous studies, viz. histological examination of the pigment cells, spectrophotometric analysis of the pigments and a quantitative estimation of the relative amounts of red and brown pigments by means of photometric determination of light absorption by AEA and AMA extracts.

Observations

The data will be described under three headings.

(1) *Phenotype.* In the light group the eye colour of light on emergence is a yellow orange, darkening through yellowish pink to a dull, but translucent, ruby on ageing; that of lightoid is a reddish orange on emergence, darkening through a deep red orange to a dull orange ruby; the eyes of persimmon are a deep orange on emergence, darkening to a dull orange and ultimately to a dull orange ruby. The eye colour of light varies slightly with temperature, the description being for cultures raised at 25° C., at which temperature all comparisons are made.

In the dark group the eye colours of newly emerged flies of all four mutants are a translucent brownish red (but rather more reddish brown in the case of Plum^2), rapidly darkening to a maroon in clot, less rapidly to a dull maroon or chocolate in Henna-recessive, to a dull sepia in the mutant sepia and to a dull reddish brown in Plum^2. After about 10 days of ageing the colour has darkened to a deep sepia in the mutant sepia, to a dark maroon in the case of clot and Henna-recessive (perhaps duller in the latter), while Plum^2 appears to be a deep dull garnet, thus lighter than the previous two mutants. Two additional facts on the colour phenotypes should be noted: first, Plum^2 is homozygous lethal and is thus maintained in the heterozygous state, and in the strain examined was not found to be variegated as described by Bridges & Brehme (1944); secondly, the body colour of the mutants sepia and clot appears to be darker than that of Henna-recessive or of the wild-type.

(2) *Histology*. The histological picture of the eyes of the two groups, with special reference to the pigment granules, was found to be as follows. In the light group eye-sections of persimmon appear to be light brown due to brown-pigmented granules; in the primary pigment cells the granules in aggregate show up as more yellowish in comparison with those of the other pigment regions; in the secondary pigment cells, with a normal density of granules, a small percentage of these granules is somewhat larger than the normal type; the post-retinal granules seem to be more densely pigmented or packed. In the mutant light the brown granules give an impression, in aggregate, of a lighter brown than in persimmon; some of the granules, especially in the distal parts of the secondary cells, are larger than the normal type. In the mutant lightoid the general impression of colour is a reddish brown. The post-retinal pigment region is very narrow, with granules of more or less normal size, while the primary and basal pigment cells are not clearly delineated by their pigmented granules, though in stained sections the basal cell nuclei are regularly present; in some sections the primary cell granules appear as very irregular distal clumps. Through clumping of granules the secondary pigment cells appear as very uneven columns of granules, clumping occurring especially in the inner or proximal third to a half of these cells; the number of these granules is greatly below the normal, and they show a great range in size, most granules having three to six or more times the normal diameter (all granules seem larger than normal). In sections of pupal eyes of lightoid, at about 84 hr. after puparium formation, with an eye colour only slightly lighter than the adult, the general histological picture shows much greater regularity, the middle and basal parts of the secondary pigment cells have a regular distribution of granules, with most of these granules already large but with a scattering of normal granules; clumping of granules now appears distally, i.e. in the primary pigment cells and/or the distal parts of the secondary cells.

In the dark group of mutants the dark phenotypes result from greatly different histological bases. In the mutant sepia the general impression is the type of coloration of the mutant brown (Nolte, 1950), with the primary pigment cells brown and the other pigment regions a type of purple; it differs from the brown mutant, however, in that the primary pigment cells are more yellowish or yellow-brown and, in addition, the distal parts of the secondary pigment cells have a similar colour, while the remaining pigment regions are a darker purple than in the brown mutant—the colour may be described as a wine purple as compared with the purplish brown of the latter. Brown granules occur in the primary cells, while in the other pigment regions purplish brown granules are found, all granules being apparently normal in size. Immediately after emergence of the flies the brown granules, and thus the yellowish colour tone, are limited to the primary cells but are not as distinct as in older flies. After extraction in AEA the general histological picture of the eyes of sepia remains unchanged excepting that the brown granules and the more yellowish tone seem restricted to the primary pigment cells. The eyes of the mutant clot histologically more nearly resemble those of the wild-type (Nolte, 1950), with the primary cells and the distal parts of the secondary cells a yellowish or salmon red and thus containing a lighter reddish type of granule, while the remaining pigment regions are a more purplish red though darker than in the wild-type. After extraction in AEA the outer lighter red has disappeared with much less colour remaining in the granules of the primary cells and distal parts of secondary cells, the overall appearance of all pigment regions now being more purplish like that of sepia. The mutant Henna-recessive is very

similar to the wild-type in the general histological appearance of the eyes with the outer regions of the ommatidia a yellowish red, in this case, however, due to the granules being more brownish than in the wild-type and of a more permanent nature (in the wild-type this region becomes more like the rest after ageing); the inner pigment regions are purplish red but with a darker tone than in the wild-type. The histological picture of the eyes of Plum[2] gives the general impression of the brown type of coloration but the tone is darker. In the primary pigment cells brown granules give a more yellowish general colour tone, while in the other pigment regions the darker granules give a more purplish brown tone; in the basal cells the granules occur as small compact masses as was described for the mutants brown and carnation (Nolte, 1950). No variegation in eye pigmentation could be ascertained in the strain examined.

(3) *Photometric analysis.* The light-absorption curves of the two pigments of the light group show no differences from those normally obtained for these pigments, while the curves of the brown pigment of the mutants of the dark group likewise are normal for that pigment; for example, the spectrophotometric curve of the brown pigment of sepia is identical with that of the wild-type, only the amounts of absorption being somewhat higher. In the case of the AEA extracts of the eyes of this group, however, great differences occur. On first observing the colours of these extracts it was noticed that whereas for Plum[2] and Henna-recessive the extracts were orange, which is normal for the red pigment dissolved in AEA, in the case of sepia the extract was a green-yellow colour, as was also observed by Mainx (1938) for an extract in water; for clot the colour was found to be yellow rather than orange. It was further observed that on standing the green-yellow extract from sepia becomes pale yellowish and ultimately nearly colourless, while the yellow extract from clot becomes more and more orange. The spectrophotometric curves of these two extracts, for 10 heads per 1 c.c. AEA, are compared in Fig. 1. In the ultra-violet absorption it will be noticed that the curves are very similar to those of the wild-type and the mutant vermilion as shown in a previous study (Nolte, 1953), but in the visible light region the two curves differ greatly from each other and from the bell-shaped curve of the normal red pigment. In the case of sepia the drop in absorption from the ultra-violet continues with only a slight peak with maximum at 415 mμ, while that of clot shows a rise in absorption with a peak at 430 mμ and a tendency to a peak at 480 mμ. In the inquiry into the properties of the modified AEA-extracted pigments of these mutants various tests were made. In one experiment with clot the yellow extract was measured photometrically 24 hr. after the beginning of extraction, i.e. immediately after extraction was terminated, and the extinction (E) was found to be 0·370 at 480 mμ and 0·460 at 430 mμ; when allowed to stand for 144 hr. the extract became orange in colour and gave an E of 0·335 at 480 mμ and of 0·193 at 430 mμ. An absorption analysis of this extract in the visible light range now gave a bell-shaped curve as for the normal red pigment.

In the case of the mutant sepia several experiments were set up to determine the duration of loss of colour and the possible causes of this loss. In all cases change in colour proceeds from the green-yellow of the fresh extract to an ultimate very pale yellow or cream solution, the spectrophotometric curve of which is given in Fig. 1. The times taken to effect this loss of colour proved very variable, e.g. in one case it took 72 hr., while in another a very pale colour was reached in 5 hr. after the first measurement of absorption when the extract was still a bright green-yellow. When, however, this process was followed photometrically the duration for the fullest loss of colour was found to be longer. In one

experiment the following values were obtained for E at 480 and 415 mμ respectively: 0·058 and 0·380 at 24 hr. after the beginning of extraction; 0·045 and 0·325 after another 48 hr.; 0·043 and 0·295 after a further 72 hr.; after about 1 month the values remained constant at about 0·027 and 0·005. In all other tests it was found that the longer the period of time elapsed after extraction the relatively greater became the loss of absorption at 415 mμ than at 480 mμ. It should also be noted that shaking the AEA extract seemed to accelerate the process of decolorization. The effects of oxidation

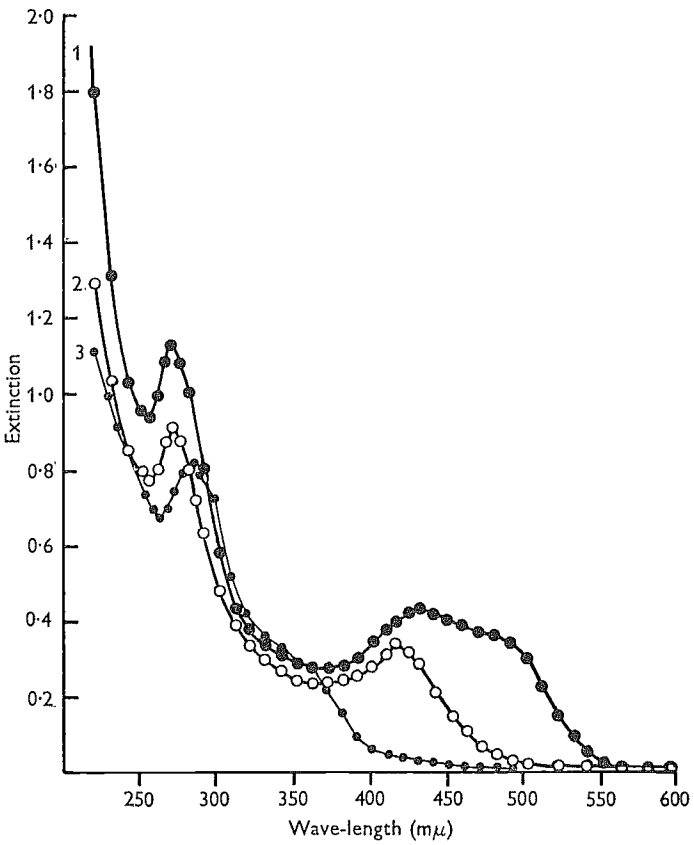


Fig. 1. Light-absorption curves of AEA extracts of the heads of the mutants clot and sepia, for 10 heads per 1 c.c. 1, for the mutant clot; 2, for the mutant sepia 24 hr. after the beginning of extraction; 3, the same extract of sepia after 5 days.

and reduction on the loss of colour were then tested. In one experiment three extracts of the same concentration were prepared: to the first was added one drop of a 5 % solution of sodium hydrosulphite, to the second one drop of a 5 % solution of hydrogen peroxide, and the third served as control. In the first extract the green-yellow was immediately replaced by a very light yellowish green, while in the second no visible change in colour took place. After 3 days the peroxide-treated and control extracts were still greenish, but after being decanted into new tubes they became more yellowish within 1 day, and after another 6 days had acquired a light yellowish tinge though still darker than the first extract; only after about 2 weeks were the three extracts similar in colour, with a very light yellowish tinge.

The quantitative determination of the red and brown pigments of the light group of mutants is given in Table 1, the measurements being of E at 480 mμ for the red pigment and at 444 mμ for the brown pigment, for concentrations of 10 heads per 1 c.c. solvent. For the dark group of mutants the comparison of relative amounts of brown pigment was as for other mutants, at the peak of 444 mμ for AMA extracts as given in Table 2, but in the case of the red pigment a problem was raised by the fact that in the mutant sepia a modified pigment was extracted by AEA with a peak at 415 mμ, and that in clot there apparently exists a mixture of the normal red pigment and the modified pigment of sepia, with a peak at 430 mμ and a near-peak at 480 mμ. In Table 2 the quantitative data are therefore given as E at 480 mμ for Henna-recessive and Plum^2, but at 415 mμ for sepia and 430 mμ for clot, though the relative amounts at 480 mμ were also obtained for the latter two mutants; all these measurements were taken immediately after an extraction period of 24 hr.

A test was made of the effect of temperature on the eye pigmentation of Plum^2; a number of cultures were divided into three lots of which the first was maintained at 25° C., the second removed to 20° C. at about 40 hr. after puparium formation, while the third was removed to 20° C. at about 40 hr. after hatching. The quantitative results obtained for the pigment content were as follows, being given as E for extracts from equal numbers of heads in AEA and AMA: control AEA extract $= 0{\cdot}111 \pm 0{\cdot}0089$, AMA extract $= 0{\cdot}0760 \pm 0{\cdot}0017$; second lot AEA extract $= 0{\cdot}1134 \pm 0{\cdot}0026$, AMA extract $= 0{\cdot}0757 \pm 0{\cdot}0002$; third lot AEA extract $= 0{\cdot}1400 \pm 0{\cdot}0082$, AMA extract $= 0{\cdot}0827 \pm 0{\cdot}0003$. It will be noted that no significant differences occur when the temperature is lowered to 20° C. during the critical period for pigment formation in the pupa, but that relatively greater amounts of both pigments are produced when the greater part of larval and pupal life is spent under conditions of lower temperature—it must be added that in the latter case an increase in the duration of these life stages resulted in larger-sized flies emerging, thus with larger eyes.

DISCUSSION

This study of the two series of eye-colour mutants of *Drosophila* has yielded some new data on the interrelationships of genes in eye pigmentation, and also facts relevant to the study of genic action in general.

(1) In these two groups of contrasting eye colours the fact is again established that the equivalent visible or gross phenotypes are not always the result of similar histological bases (Nolte, 1952*a*). In the light group the mutants lightoid and persimmon have very similar eye colours, especially after ageing, yet whereas the pigment-cell regions of the latter are more or less normal in appearance, in the former three differences are discernible: first, the aggregate colour of the granules is more reddish brown; secondly, most granules are larger than normal; thirdly, a clumping of granules occurs in the main pigment regions. In the case of the dark group the two mutants clot and Henna-recessive have as phenotypes a very similar type of dull maroon and histologically resemble the wild-type in aggregate pigment granule colour. The mutants sepia and Plum^2 resemble the above-mentioned two mutants though the former is darker and the latter lighter in eye colour; however, both belong to the brown type in their aggregate granule colour.

In a previous study (Nolte, 1950) it was suggested that in the wild-type, for example, the brown pigment is deposited in its final or maximum quantity at or just after eclosion,

while the red pigment deposition is continued for a period of up to somewhat less than a week after eclosion resulting in the more densely pigmented granules of the inner layers of the eye. This method of pigment deposition results in the more yellowish or salmon red colour of the outer layers of pigment cells in young flies as opposed to the more fully pigmented or purplish red of the inner layers; it should be noted that after the red pigment was removed by solution in AEA it was found that all layers were more brownish (or

Table 1. *Relative amounts of red and brown pigments in the eyes of lt, ltd, pers*

	Red pigment			Brown pigment		
Exp. no.	*lt*	*ltd*	*pers*	*lt*	*ltd*	*pers*
XXXIII	0·1008	0·3467	0·3056	0·0076	0·0064	0·0054
	0·1027	0·3510	0·3024	0·0076	0·0070	0·0052
	0·1053	0·3233	0·3219	0·0082	0·0059	0·0056
	0·1040	0·3455	0·3241	0·0084	0·0058	0·0056
XXXIV	0·1033	0·3300	0·3178	0·0077	0·0059	0·0060
	0·1087	0·3433	0·3314	0·0082	0·0070	0·0060
	0·1039	0·3433	0·3222	0·0084	0·0055	0·0058
	0·1017	0·3400	0·3267	0·0073	0·0058	0·0067
XXXV	0·1060	0·3333	0·3040	0·0079	0·0055	0·0047
	0·1144	0·3533	0·3045	0·0079	0·0058	0·0054
	0·1024	0·3230	0·3130	0·0073	0·0070	0·0059
	0·1111	0·3213	0·3222	0·0072	0·0058	0·0061
Mean *E*	0·1054	0·3378	0·3163	0·0078	0·0061	0·0057
S.E.	±0·0012	±0·0034	±0·0029	±0·00012	±0·00017	±0·00015

Table 2. *Relative amounts of AEA-soluble and brown pigments in the eyes of* Hn^r, Pm^2, *se, cl*

	AEA-soluble				Brown pigment			
Exp. no.	Hn^r	Pm^2	*se*	*cl*	Hn^r	Pm^2	*se*	*cl*
XXXVI	0·5040	0·1680	0·3600	0·4330	0·1030	0·0749	0·1183	0·1393
	0·5200	0·1660	0·3600	0·4272	0·1071	0·0764	0·1181	0·1336
	0·5168	0·1840	0·3560	0·4291	0·1030	0·0757	0·1175	0·1313
	0·5224	0·1514	0·3620	0·4388	0·1023	0·0799	0·1304	0·1329
XXXVII	0·5200	0·1657	0·3800	0·4330	0·1071	0·0806	0·1316	0·1310
	0·5030	0·1867	0·3840	0·4310	0·1169	0·0770	0·1240	0·1461
	0·5360	0·1470	0·3920	0·4320	0·1004	0·0766	0·1217	0·1461
	0·5240	0·1600	0·3558	0·4306	0·1065	0·0735	0·1240	0·1491
	0·5360	0·1470	0·3920	0·4320	0·1004	0·0766	0·1217	0·1461
	0·5240	0·1600	0·3558	0·4306	0·1065	0·0735	0·1240	0·1491
XXXVIII	0·5160	0·1340	0·3932	0·4368	0·1065	0·0708	0·1239	0·1323
	0·5040	0·1376	0·3559	0·4291	0·1077	0·0768	0·1211	0·1323
	0·5120	0·1809	0·3520	0·4400	0·1035	0·0742	0·1253	0·1309
	0·5440	0·1600	0·3992	0·4600	0·1120	0·0730	0·1236	0·1337
Mean *E*	0·5185	0·1616	0·3708	0·4351	0·1063	0·0758	0·1233	0·1367
S.E.	±0·0036	±0·0050	±0·0050	±0·0026	±0·0013	±0·0008	±0·0013	±0·0019
Mean *E* at 480 mμ			0·0556	0·3603				
S.E.			±0·0006	±0·0013				

purplish brown) as in the mutant brown. If these data are taken as a basis for comparison, some conclusions may be reached in regard to the types of pigments and their regions of deposition in the two groups of mutants studied. In the case of light and persimmon the general histological picture, though the general impression of colour is that of a brownish tone, is indicative of that of the wild-type because of the more yellowish granules in the outer pigment regions. However, in the case of lightoid the general or aggregate colour impression is different, the reddish brown being correlated with structural differences,

e.g. increased granule size; the increase in size of granules may be the result of an amalgamation of normal-sized granules, in which case the clumping which starts in the primary pigment cells and terminates in the proximal parts of the secondary cells may be thought of as the continuation of this phenomenon of attraction. In the dark group of mutants the pigment deposition in Henna-recessive is evidently very similar to that of the wild-type, with the exception that the centrifugal method of deposition of red pigment does not reach the distal pigment regions to the extent that it does in the wild-type. In the case of Plum[2], on the other hand, the pigment and method of deposition greatly resemble that of the mutant brown, even to the small compact pigment masses in the basal cells. In the mutant clot the wild-type picture again appears, i.e. more red pigment in the inner than the outer layers, and after extraction in AEA the colour tone is similar to that of equivalent wild-type eyes but darker. In sepia the brown type of pigmentation occurs, with the exception that the yellowish colour tone of the outer layers of the secondary cells must be due to a deposit of red pigment (actually a modified form), since this is deposited to a full extent only a few days after eclosion and since it more or less disappears after extraction in AEA.

The size of granule in all pigment layers in the four mutants of the dark group is apparently normal, but in all three mutants of the light group an increase in size occurs. In a previous study (Nolte, 1950) this increase in size has been noted in the case of the mutants scarlet and ruby, and as cause an amalgamation of normal-sized granules has been proposed. In the case of scarlet, and in the present study of light and persimmon, this is difficult of proof, since the numbers of these modified granules are relatively low so that comparison with granule number in the wild-type is nearly impossible; in the case of the primary pigment cells of ruby, and in the present study of the secondary cells of lightoid, the numbers of these larger-sized granules are strikingly lower than in the wild-type so that an amalgamation of normal-sized granules may be postulated, though an initial production of fewer granules is not excluded. For ruby it was found that no noticeable difference in granule number occurred between 1- and 10-day-old flies, while for lightoid it appears that in the secondary pigment cells of the 84 hr. pupa and of the adult the granule number is more or less equivalent; however, the pupa already has a fairly large number of granules with increased size, and thus if an amalgamation of normal-sized granules occurs this must happen during an early stage. In the case of the mutant brown it appears as if the granules are smaller than in the wild-type, and thus it may be assumed that the granules increase in size during deposition of pigment; in brown only brown pigment is deposited, but in the wild-type the brown pigment is deposited first, or more quickly, and later accretion of red pigment increases the size of the granule. Taking all these facts into consideration it appears possible that the action of the mutant gene lightoid is a reduction in the number of granules resulting in the increase in size to a possible limiting maximum after which, though perhaps still available, no more pigment can be deposited. A secondary effect, perhaps correlated with the size of the granules, is the movement of these granules during the course of development of the eye, from clumps in the outer layers of the pigment cells to clumps in the inner layers.

(2) A comparison of the types of photometric absorption curves of the pigment extracts from the various mutants yields evidence on the types of pigment present. In the case of all mutants of both groups the brown pigment present is identical with that found in the wild-type and the mutant brown (Nolte, 1952*b*, 1953). For most of these mutants the

red or AEA-extracted pigment is identical with that found normally, e.g. in the wild-type and the mutants scarlet and vermilion. The mutants sepia and clot, however, show exceptions to this normality in their content of red pigment. The green-yellow extract in AEA of sepia eyes has also been noted by Mainx (1938), though for an extract in water; this yellow extract with green fluorescence he thought to be a qualitatively changed red component, i.e. he suggested that the red is oxidized to a yellow-green; in addition, he thought that the production of the red pigment is slowed down, beginning at the tip and working downwards along the ommatidia.

Several facts may be adduced to indicate the relationships between the normal red pigment and the modified form in sepia. First, a comparison may be made between the absorption curves of the AEA extracts of the eyes of sepia and clot, in Fig. 1, and between these and the curves for the wild-type and the mutant vermilion in a previous study (Nolte, 1953). The two curves in Fig. 1 follow each other closely from the drop in absorption in the lower ultra-violet range to a valley at 255 mμ, followed by a rise in absorption to a peak at 270 mμ, this again followed by a rapid drop in absorption to about 370 mμ, which in its turn is followed by a rise in absorption. This part of the absorption curve is very similar to that of the red pigment in the wild-type and the mutant vermilion, the main differences being that in the wild-type the valley is at 260 mμ and the peak at 275 mμ, though for the mutants vermilion and scarlet the valley is nearer 255 mμ. In the visible light range the curves of sepia and clot start deviating from the normal curve of the red pigment, since though a rise in absorption occurs the peak is reached sooner. On comparing the two curves and taking into account the indication of a peak at 480 mμ in the case of clot (which peak is actually attained after the AEA extract of clot has changed from yellow to orange) it appears that the peak of clot at 430 mμ is a composite, i.e. is caused partly by absorption by a modified pigment as found in sepia and partly by the normal red pigment—in other words the peak of the modified pigment has shifted from 415 to 430 mμ because of admixture with normal red pigment. In the second instance, whereas the normal red pigment may be reduced reversibly to a colourless product, the modified green-yellow pigment of sepia and clot may also be reduced to a colourless product but apparently irreversibly. Though this decolorization is accelerated by the addition of a reducing agent, it has, however, not conclusively been proved to be a real reduction, since it proceeds automatically in the extract and may be accelerated by shaking the latter. Another difference is that the decolorized products of the AEA extracts of sepia and, for example, scarlet differ in their absorption curves; whereas the curve of the decolorized normal red extract in the case of the mutant scarlet does not differ materially from the curve of the coloured product, the equivalent curve for sepia is seen in Fig. 1 to have the values in the ultra-violet shifted to higher wave-lengths, with the valley at 265 mμ and the peak at 285 mμ. A third comparison may be made between the normal and modified pigments extracted by AEA, i.e. in their method and time of deposition in, for example, the wild-type and sepia. In previous studies it has been noted that the red pigment deposition continues for at least 5 days after eclosion. In an experiment with a single culture of sepia the following values for E were determined: for newly emerged flies E was found to be 0·0344 at 480 mμ and 0·1576 at 415 mμ, while for 7-day-old flies the values were 0·0560 and 0·3840 respectively. It will be noted that the latter are more or less the normal values at the wave-lengths as calculated in Table 2, and it thus appears that the modified pigment also passes through a delayed period of deposi-

tion, even more so than the normal pigment. This modified pigment appears in the distal parts of the secondary pigment cells of sepia only some days after eclosion as has been described for the histological differences between newly emerged and old flies, and in this way resembles the normal red pigment of the wild-type; in the case of clot the deposition of the modified pigment is masked by admixture with normal red pigment. It has been observed, as also by Mainx (1938), that in compounds *se* sometimes acts independently, i.e. in its interaction with other genes related to red pigment production; for example, the compound *w*^{e} *rb* is white, or lighter than the yellowish pink of eosin, while the compound *w*^{e} *se* is brownish pink, i.e. darker than eosin (after ageing the eyes of this compound are brown). Taking all these facts into consideration it appears that the mutant sepia contains a pigment soluble in AEA and which is related to the normal red pigment, and is presumed to be produced from the same basic materials during pupal life. On comparing the reactions of the AEA extract of the eyes of the mutant clot it seems reasonable to postulate the production by this mutant of a mixture of this modified pigment with the normal red from the basic available substances.

The relation of eye pigmentation to body colour in sepia and clot is not apparent, since the darker body colour observed in these two mutants is presumably due to a melanin.

(3) A study of the relative amounts of red and brown pigments in the two series of mutants may lead to some conclusions in regard to the action of the mutant genes. In the first instance it must again be noted that the amount of brown pigment is not directly correlated with that of the red pigment from culture to culture of any particular mutant; secondly, although they contain relatively more brown pigment than the wild-type and are thus darker after extraction in AEA, the eyes of the mutants clot, sepia and Henna-recessive are white after the final extraction in AMA, and thus somewhat lighter than those of the wild-type.

In a previous study (Nolte, 1953) it was determined that the relative amounts of red and brown pigments in the eyes of the wild-type are $0{\cdot}8993 \pm 0{\cdot}0052$ and $0{\cdot}0866 \pm 0{\cdot}0007$ respectively. In comparison with this there has been a great reduction in the amounts of both pigments in the mutants of the light group, the relation between the pigments in regard to this reduction being, however, in inverse proportion, since in light the reduction in red pigment is greater but in brown pigment is less than in the other two mutants. In the dark group we find a reduction in the amount of red (or modified) pigment in all four mutants, but in three, viz. Henna-recessive, sepia and clot, the amount of brown pigment is significantly greater than in the wild-type, with increasing amounts in that order. In the previous study the mutant brown was found to have a relative brown pigment content of $0{\cdot}0750 \pm 0{\cdot}0006$ which is not significantly different from the brown pigment content of Plum[2]; the latter, however, has an appreciably higher content of red pigment, since for the mutant brown the AEA extract yielded an E of about 0·01.

An attempt has been made to evaluate the relative amounts of normal and modified red pigment in the eyes of clot by utilizing as average content the above-mentioned data for the wild-type and those of Table 2 for sepia and clot, as well as the relative amounts of absorption by extracts of the eyes of the wild-type, sepia and clot at the wave-lengths of 415, 430 and 480 mμ. If the absorptions at the latter wave-length are taken as 0·90, 0·36 and 0·05 respectively for the three strains, then it may be calculated that the absorption for clot at 480 mμ is due to the red pigment at the rate of approximately 36 % and to the yellow pigment at 64 %. Consequently an average peak of absorption of 0·43 at 430 mμ

for clot can result if the eyes of this mutant contain approximately 36 % of the red pigment found in the eyes of the wild-type and 84 % of the yellow pigment found in the eyes of sepia.

(4) Some of the foregoing facts may be adduced to give some indication of the possible modes of action of the genes of the two series of mutants. In the case of the light group we find that the red pigment in persimmon and lightoid has been reduced to less than one-half that found in the wild-type and the brown pigment to about one-fifteenth, while in light the red pigment has been reduced to less than one-eighth and the brown to about one-eleventh of the wild-type eye pigment content. In these three mutants we find no simple linear relationship between the normal amounts of pigment and their reduction, and thus the mutant genes act differentially on the two pigments; in the case of lightoid the reduction in amount of both pigments, though of the same order as in persimmon, is correlated with qualitative histological deviations in the eye. The differential effect of these mutant genes suggests a comparison with the genes of the ruby or carnation group which were investigated in a previous study (Nolte, 1952*b*), in which a similar type of differential or disproportionate reduction in the content of the two pigments was determined, the reduction being, however, to a lesser extent. If we accept the three mutant genes of the light group as extreme examples of the ruby or carnation type of mutant, it may be postulated that their mode of action falls in the same category as that assumed for the others, viz. the wild-type alleles of these genes direct the specificities of enzymes which are utilized during metamorphosis, possibly in catabolic processes of proteins. Some of the breakdown products will be used for reconstruction in the developing imago, but some of these residues may be used in the various reactions of eye pigmentation; the mutant genes are hypomorphic, their action resulting in a deficiency of the materials required during the formation of both pigments. On account of its effect on pigment granule size it appears as if the wild-type allele of lightoid acts at the level of granule formation, though this might still in effect be the deficiency of necessary basic material and thus this gene can be included in the above-mentioned category of genes.

Presumably the action of Plum^2 is due to a position effect since it is inseparable from an inversion and is allelic to brown (*bw*), the right break being at the *bw*$^+$ locus. As has been noted, the mutant Plum^2 has a brown pigment content nearly identical with that of the mutant brown but an appreciably higher red pigment content. If the action of Plum^2 is the result of a modified reaction by the dislocated *bw*$^+$ locus, the mutant locus and the transferred locus agree fairly well in their prohibitive action in the formation of red pigment; the lesser efficiency of Plum^2 in this respect might be due to the heterozygous action of the normal allele carried in the partner chromosome in heterozygous Plum^2 individuals. The effect of heterozygosity in general will be investigated in a future study.

The action of Henna-recessive may be narrowed down to one particular point in the interacting chains of reactions leading to eye pigmentation if one assumption is made. As compared with the wild-type this mutant has a red pigment content of about one-half but a statistically significant higher content of brown pigment; it has been postulated that the pigments have a stage in common during their formation, viz. in the kind of basic precursor or constituent which will ultimately be differentiated by the *w*$^+$ gene into specific substrates for combination with the specific chromophore groups. If it now be assumed that the wild-type allele of Henna-recessive acts at the level of production of this common precursor, the mutant may act in such a way that a qualitatively different product is

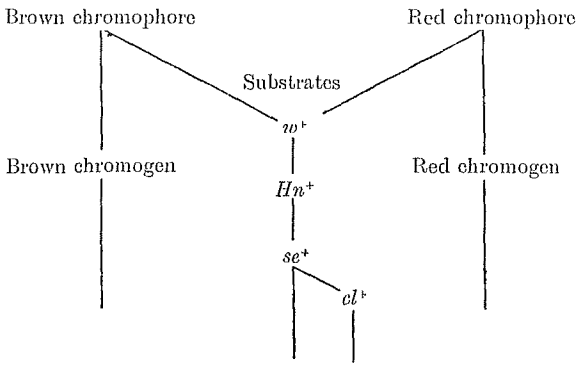
produced. In consequence the w^+ gene now has to differentiate a changed precursor, and it seems reasonable to assume that part of this common precursor originally destined for the red pigment may be diverted to the brown pigment reaction chain, enabling more of the available material to be used in the formation of brown chromogen for ultimate combination with the increased specific brown substrate.

In the case of the mutants sepia and clot the reasons have already been discussed for assuming their yellow AEA-soluble pigment to be related to the normal red eye pigment. Since the amounts of brown pigment in the eyes of these two mutants are considerably greater than that occurring in the wild-type, it may again be postulated, as for Henna-recessive, that more of a common basic precursor is diverted for use by the brown pigment reaction chain, the normal alleles of the two genes thus acting at the level of formation of this substance. One fact of possible support for this concept is that *se* is partly epistatic to the alleles of the white locus, e.g. w^e *se* is darker than w^e (this particular allele of eosin is a new allele about which more information will be given in a subsequent study). To confirm this epistasis and the fact that the wild-type alleles of sepia and white stand in a sequential relation to each other, a comparison was made between the photometric values of AEA and AMA extracts of the eyes of sepia, eosin and sepia-eosin. The AEA extract of w^e *se* is greenish yellow, with a valley at 255 mμ and a peak of absorption at 270 mμ, as for *se*, whereas in the case of w^e (which also has a modified type of red pigment) no definite valley or peak of absorption occurs in the ultra-violet. The AEA extracts of the three strains have the following relative amounts of pigment:

se at 415 mμ = 0·371, at 480 mμ = 0·56;
w^e *se* at 415 mμ = 0·081, at 480 mμ = 0·012;
w^e at 415 mμ = 0·028, at 480 mμ = 0·023.

The AMA extracts show the following relative amounts of pigment at 444 mμ: *se* = 0·123, w^e *se* = 0·04, w^e = 0·025. In the case of the brown pigment the amounts present in w^e *se* and *se* show a reduction in the former which is proportional to the reduction by w^e of the amount present in the wild-type. The AEA extracts show that at 415 mμ there is some epistatic action by sepia in the compound mutant, but at 480 mμ the amount of pigment in the latter is sub-additive as compared with the two single mutant strains; eosin reduces the larger amount of red pigment present in the wild-type, and in combination with sepia it reduces the lesser amount of pigment of the latter to an even greater extent. The concept is that the mutant gene sepia changes the basic precursor for the substrates to such an extent that the w^+ gene is forced to differentiate the precursor into a qualitatively different substrate for combination with the red chromogen (resulting in a qualitatively modified red chromophore group which is extracted by AEA) and leaving a part greater than normal to be modified into the specific brown substrate.

A complication arises in the case of the mutant clot in that part of the red pigment content consists of the modified sepia type, especially in view of the hypothesis that a specific gene is related to a specific chemical reaction. A possible place where the normal allele of clot may be fitted into the pigmentary reaction chains is during the formation of the common precursor, somewhere near the level of action of se^+, i.e. cl^+ may deliver a product for the use of the latter gene. In its mutant form it may deliver this product in such a form that the action of se^+ on this product is partly modified to that of the mutant *se*. The three genes Hn^+, se^+ and cl^+ may then be fitted as follows into the scheme as given in Fig. 9 of a previous study (Nolte, 1952*b*).



SUMMARY

The second and third groups of eye-colour mutants of *Drosophila melanogaster*, studied histologically and photometrically, are the light group with the mutants light (*lt*), lightoid (*ltd*) and persimmon (*pers*), and the dark group with the mutants sepia (*se*), clot (*cl*), Henna-recessive (Hn^r) and Plum^2 (Pm^2).

1. In *lt* and *pers* a small percentage of granules is larger than normal and the type of pigmentation histologically is a light brownish; in *ltd* the general colour is reddish brown, most granules are much larger than normal but less in number, and while the primary and basal pigment cells are not clearly delineated by their pigment granules, a clumping of granules occurs in the proximal parts of the secondary pigment cells. In *cl* and Hn^r the histological picture is like that of the wild-type, while in *se* and Pm^2 it resembles the brown mutant type in general colour.

2. In all the mutants, excepting two, the normal types of red and brown pigments occur. In *se* a modified red pigment is found, with a greenish yellow colour in solution and a changed spectrophotometric curve with a peak of absorption at 415 mμ instead of 480 mμ; in *cl* a mixture of this modified pigment and the normal red pigment occurs.

3. In *ltd*, *lt* and *pers* a great reduction in the amounts of both pigments as compared with the wild-type was determined; in Pm^2 the brown pigment content is equivalent to that of the mutant brown, but the red pigment content is appreciably higher; in Hn^r, *se* and *cl* the content of red pigment, or its modified form, shows a reduction as compared with the wild-type, but the amount of brown pigment is significantly higher. It is calculated that the eyes of *cl* contain about 36% of the red pigment found in the wild-type and 84% of the yellow pigment found in sepia.

4. An attempt is made to indicate the possible modes of action of these genes in eye pigmentation. It is postulated that the normal alleles of *lt* and *pers*, like those of the ruby and carnation mutants, are eye-colour genes indirectly only, their main function being the directing of enzyme specificities, especially for the breakdown of proteins during metamorphosis, some of the breakdown residues being utilized in the pigmentary process; the normal allele of *ltd* seems to play some part during pigment granule production. The action of Pm^2 seems to be due to a position effect of the bw^+ locus, its prohibitive effect on red pigment production being less efficient than that of the mutant brown. The normal alleles of Hn^r, *se* and *cl* seem to fit into the reaction chain for the production of a common basic precursor or constituent which is later differentiated by the w^+ gene into specific substrates for the formation of the red and brown chromophore groups. Hn^r seems to

divert some of this constituent from the red pigment to the brown pigment pathway, while *se* and *cl* seem to stand in a sequential relation to each other, their action (in the case of the latter only partly) being to modify the basic precursor in such a way that a modified red pigment results, and in addition a part of this substance destined for the red pigment substrate is diverted to the brown pigment production chain.

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