THE EYE-PIGMENTARY SYSTEM OF *DROSOPHILA*

VI. THE PIGMENTS OF THE RUBY AND RED GROUPS OF MUTANTS

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The fourth and fifth groups of eye-colour mutants of *Drosophila melanogaster* are based on a somewhat arbitrary classification, since these mutants form a more or less graded series, though in general colour phenotype the members of each group are more nearly alike. In the fourth group or the ruby class are included purple (*pr*), rosy (*ry*), pink (*p*), purpleoid (*pd*), claret (*ca*), maroon (*ma*), prune (*pn*); with these mutants should also be included the group carnation (*car*), ruby (*rb*), garnet^3 (g^3), carmine (*cm*) and raspberry^2 (ras^2) which has been studied in previous investigations (Nolte, 1950, 1952). In the fifth group or the red class are included safranin^2 (sf^2), sepiaoid (*sed*), dark eye (*dke*), mahogany (*mah*), bordeaux (*bo*) and Moiré (*Mé*). These mutant eye colours are all conditioned by point mutations excepting Moiré which is associated with an inversion, *In* (3*L*), and is homozygous lethal so that the strain must be maintained in the heterozygous condition.

The methods applied in this study are similar to those of previous investigations, viz. mainly the histological examination of the eye pigment cells and the differential extraction and photometric measurement of the pigments.

OBSERVATIONS

(1) *Phenotype*

An objective comparison of the differences in eye-colour phenotypes between some of the members of these two groups of mutants is difficult because of the mimic qualities found in each group, and in the following descriptions of the strains maintained some deviations will be found from the phenotypes as given by Bridges & Brehme (1944).

In the ruby group the eye colours just after emergence are all of the ruby type with that of claret perhaps most clearly ruby; the mutant pink is the lightest or a pinkish ruby, and purpleoid a fairly light ruby; rosy, maroon and purple are a more brownish ruby; prune is the darkest, a purplish ruby. After about a week of ageing the eyes of all these mutants have darkened but those of pink are still the lightest, being a dull ruby with a purplish tone; maroon is a dull ruby (more or less like the mutant carnation), while rosy is a darker or duller ruby or garnet than carnation; purple is a purplish ruby; purpleoid, claret and prune now appear to be darker though in the case of the first two this might be due to the duller tone, while prune is a dark brown purplish garnet.

At the borderline between this group and the mutants with more reddish eyes we find safranin^2, with eyes of a duller colour than the members of the red group. On emergence mahogany is a darker ruby than that of rosy, safranin^2 even darker, dark eye a dull dark ruby red, sepiaoid with a more reddish tone and bordeaux has a ruby red colour. After about a week of ageing safranin^2 has a duller garnet tone than the other mutants of which the general colour is a dark red or wine; bordeaux is the lightest of these, sepiaoid slightly

duller, while the darker red of dark eye and mahogany is more nearly alike. The mutant Moiré has an eye of the reddish type though more translucent and with a slight brownish tinge; an iridescent effect is caused by a ring of six flecks around the normal central fleck.

In the case of the mutant prune strain maintained in this laboratory a structural abnormality occurs in a small, but varying, percentage of individuals in every culture, this abnormality being a reduction in size of one or both eyes, the reduction appearing round the circumference of the eye or more generally along the lower margin which then has a half-moon-shaped deleted part on one side only or on both sides, leaving the ventral margin of the eye pointed.

(2) *Histology*

The group of mutants classified as having the ruby type of eye colour shows great variation in regard to the histological bases for the macroscopic phenotypes. In the mutant purple the type of coloration is that of the wild-type, but the outer salmon red of the primary pigment cells and the distal third of the secondary cells is due to more brownish granules which appear yellowish in transmitted light and which form a region more permanent than in the wild-type in which, with ageing, this region assumes more nearly the colour of the other pigment regions; in purple these regions have red granules giving in aggregate a purplish red tone which appears brighter red than in the wild-type. In the mutant rosy the general impression of colour is like that of the carnation mutant (Nolte, 1950), but darker and with the primary cells and distal parts of the secondary cells more yellowish than the brown of carnation, and the basal cells (which are normal in size) and the post-retinal region a darker brown than in carnation; the four pigment regions are greatly alike as regards the distribution of granules, and the granules in the secondary pigment cells are more definitive in outline than in the case of carnation. In the mutant pink the general impression is again like that of carnation but in all four pigment regions a brown type of granule causes an aggregate colour that is more brown than the brownish red of carnation. As in carnation the granules of the basal cells are clumped into small rounded masses (though not so small as in the latter), but unlike the pigment granules of the secondary cells of carnation which seem smaller than normal or perhaps more indistinct in outline due to being less densely pigmented, the granules of these cells of pink are distinctly larger, about one-third having at least twice the normal diameter. In the mutant purpleoid the carnation type of pigmentation again occurs but also more brownish in general colour tone due to the brown type of granule. In the primary pigment cells more granules than normal occur or perhaps they are more deeply pigmented, being a dark brown; the secondary cells have the more indistinct or less densely pigmented granules of the carnation type, the basal cells also appear as small rounded masses of granules, while the post-retinal pigment region is narrow. The mutant claret has an eye which microscopically shows a medium brown tone, all regions having similar granules of a brown type; in the secondary cells the granules show a variable size increase, about one-sixth being from twice to four times the normal size. The eye of the mutant maroon has a similar type of microscopic appearance as claret with, however, all regions having a similar type of indistinct brown granule giving a general colour tone of medium brown, darker than claret. In the mutant prune, in addition to the abnormalities in external form of the eye a totally different picture appears in eye structure. In the primary cells brown granules occur as a dense mass with a general yellowish brown colour tone, while in the remaining

pigment regions the red type of granule gives a colour impression of a reddish brown. Of these regions the post-retinal granules occur only as a very narrow layer, while the secondary and basal cells show a high degree of disarrangement. Generally the basal cells appear as very small rounded masses, while the pigmented parts of the secondary cells are cut up into lumps along the length of the ommatidia, with the biggest lumps tightly apposed against the primary pigment cells. The picture is somewhat like that of raspberry[2] (Nolte, 1950) but often more aberrant, i.e. the pigment granule masses are more broken up into separate globular masses; in some eyes, however, this splitting up of the granular mass into several lobes is not so excessive, but instead gives the impression of the coagulation of granules into uneven stringlike masses surrounding the retinulae. The basal pigment cells appear as irregularly distributed bodies on the basement membrane. In pupal eyes of prune the development of these abnormalities may be followed up. At about 70 hr. after puparium formation the orientation of the secondary pigment cells seems more normal with most of the pigment granules located near the basement membrane so that it is difficult to observe the basal cells, while the post-retinal granules appear to be completely absent: in older pupae, i.e. about 12 hr. later, the aberrations are very evident, the general impression being as if the bases of the secondary pigment cells (or the masses of pigmented granules) were pulling outward, while the general colour of this region is more purplish red than in the adult.

In the group of mutants with a more reddish eye colour this general difference in gross phenotype to that of the former group is correlated with a different histological picture. In the mutant safranin[2] the appearance is like that of the wild-type but a darker red or more dull in colour, i.e. a dark purplish red with perhaps a tinge of brown; the primary pigment cells appear as brownish masses of granules. In the case of sepiaoid the wild-type histological appearance again occurs with, however, the primary pigment cells more yellowish or definitely salmon in colour. In the mutant dark eye the wild-type kind of coloration is modified by the more brownish primary cells. For the mutant mahogany the general impression is a dark red with the primary cells a salmon colour; in all regions the granules are larger than normal, most being three to eight times the normal size, and, in addition, the number of granules is decreased. In the pupa of mahogany, because of the large size of the granules, the problem of growth of granules and of sequence of deposition of the pigments could be followed up more clearly than in the case of any other mutant. In the eyes of pupae about 70 hr. after puparium formation it was found that the granules of the primary and secondary cell regions are already larger than normal, and in the latter region are mostly congregated near the basement membrane; all granules are a medium brown with those in the primary pigment cells showing a more yellowish tinge. In older pupae, viz. at 78–84 hr., the yellowish brown tinge remains in the primary cells and the distal parts of the secondary cells, while most of the granules of the latter region are still clumped near the basement membrane, and at different stages it became evident that a purplish red colour advanced outward from the basement membrane. At 84 hr. after puparium formation the granules are only slightly smaller than in the adult; due to clumping of granules at the basement membrane end of the secondary cells the basal cells could not be observed, while very few granules are evident in the post-retinal region.

In the mutant bordeaux the colour is less purplish than in the wild-type and all regions are more nearly alike in their coloration. In the mutant Moiré the gross phenotype is based on histological abnormalities in that the primary pigment cells seem to be retracted

proximally so that most of their cell-bodies appear to lie at the level of the pseudo-cone nuclei, and only here and there a string-like projection of pigmented granules reaches the lenses. In addition, the basal pigment cells are not clearly visible as definite masses of granules but appear as small bodies on the basement membrane. In the secondary cell region large open spaces appear in many eyes. The general impression of colour of Moiré eyes is like that of the wild-type though somewhat lighter and with all regions being more nearly alike in colour.

(3) *Photometric analysis*

The light absorption curves of the two pigments in the eyes of these two series of mutants are those normally obtained for these pigments, so that the only differences from the wild-type are in the amounts of these pigments. The quantitative determinations of the red and brown pigments in the two groups of mutants are given in Tables 1–4, the data being in 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. of the solvents AEA and AMA respectively.

Table 1. *Relative amounts of red pigment in the eyes of pr, ry, p, pd, ca, ma, pn*

Exp. no.	*pr*	*ry*	*p*	*pd*	*ca*	*ma*	*pn*
XXXIX	0·2180	0·3240	0·3680	0·1780	0·2324	0·2781	0·2400
	0·2213	0·3144	0·3760	0·1700	0·2414	0·2857	0·2372
	0·2366	0·3276	0·3893	0·1734	0·2348	0·2671	0·2330
	0·2200	0·3080	0·3822	0·1800	0·2290	0·2597	0·2315
XL	0·2410	0·3136	0·3680	0·1696	0·2320	0·2708	0·2316
	0·2220	0·2976	0·3624	0·1720	0·2580	0·2894	0·2339
	0·2196	0·3325	0·3696	0·1568	0·2440	0·2760	0·2280
	0·2350	0·3176	0·3650	0·1720	0·2480	0·2813	0·2264
XLI	0·2400	0·3020	0·3488	0·1640	0·2320	0·2781	0·2308
	0·2086	0·3126	0·3320	0·1976	0·2368	0·2841	0·2261
	0·2340	0·3100	0·3384	0·1976	0·2576	0·2780	0·2323
	0·2424	0·3092	0·3300	0·1840	0·2600	0·2858	0·2302
Mean E	0·2282	0·3141	0·3608	0·1762	0·2422	0·2786	0·2318
S.E.	±0·0010	±0·0029	±0·0057	±0·0035	±0·0033	±0·0026	±0·0012

Table 2. *Relative amounts of brown pigment in the eyes of pr, ry, p, pd, ca, ma, pn*

Exp. no.	*pr*	*ry*	*p*	*pd*	*ca*	*ma*	*pn*
XXXIX	0·1144	0·0721	0·0304	0·0533	0·0260	0·0455	0·0931
	0·1123	0·0700	0·0289	0·0540	0·0245	0·0479	0·0958
	0·1262	0·0735	0·0285	0·0520	0·0224	0·0525	0·0982
	0·1156	0·0756	0·0291	0·0524	0·0231	0·0525	0·0976
XL	0·1200	0·0714	0·0301	0·0545	0·0243	0·0467	0·0903
	0·1133	0·0700	0·0294	0·0530	0·0231	0·0476	0·0967
	0·1141	0·0700	0·0315	0·0532	0·0245	0·0525	0·0951
	0·1151	0·0686	0·0266	0·0546	0·0274	0·0531	0·0980
XLI	0·1178	0·0665	0·0252	0·0511	0·0256	0·0511	0·0973
	0·1165	0·0700	0·0259	0·0525	0·0266	0·0533	0·0987
	0·1260	0·0693	0·0273	0·0553	0·0266	0·0525	0·0928
	0·1280	0·0700	0·0280	0·0511	0·0252	0·0530	0·0980
Mean E	0·1183	0·0706	0·0284	0·0531	0·0249	0·0507	0·0960
S.E.	±0·0050	±0·0007	±0·0005	±0·0004	±0·0005	±0·0008	±0·0008

Some remarks may be added to the data of these tables. First, the general size of eye of the mutants prune, purple, rosy, maroon is below the norm which was established in a previous study (Nolte, 1953*a*). Secondly, the strain safranin[2] appears to grow more slowly than most mutant strains, the formation of puparia starting on the average 24 hr.

later than in most strains. Thirdly, in the case of Moiré which is maintained in the heterozygous condition, the bodies and heads, and consequently the eyes, are slightly smaller than those of their wild-type sibs.

Table 3. *Relative amounts of red pigment in the eyes of sf², sed, dke, mah, bo, Mé*

Exp. no.	*sf²*	*sed*	*dke*	*mah*	*bo*	*Mé*
XLII	0·4000	0·7169	0·5700	0·6720	0·6120	0·7150
	0·3650	0·7061	0·6200	0·6960	0·6060	0·7000
	0·3850	0·7035	0·5680	0·7080	0·5880	0·6840
	0·4000	0·7035	0·5700	0·6600	0·5935	0·7200
XLIII	0·3600	0·7009	0·5880	0·6720	0·6000	0·7050
	0·3600	0·6960	0·5892	0·6939	0·5904	0·6900
	0·3640	0·7296	0·5600	0·7080	0·5880	0·7091
	0·3600	0·7032	0·5760	0·6900	0·6120	0·7125
XLIV	0·3560	0·7297	0·5760	0·7125	0·6240	0·6875
	0·3740	0·7101	0·5760	0·7080	0·6144	0·7000
	0·3520	0·7143	0·5880	0·6960	0·6228	0·7357
	0·3576	0·6912	0·5844	0·6744	0·6144	0·7333
Mean *E*	0·3695	0·7088	0·5805	0·6909	0·6055	0·7077
S.E.	±0·0047	±0·0035	±0·0044	±0·0050	±0·0038	±0·0048

Table 4. *Relative amounts of brown pigment in the eyes of sf², sed, dke, mah, bo, Mé*

Exp. no.	*sf²*	*sed*	*dke*	*mah*	*bo*	*Mé*
XLII	0·0882	0·0875	0·0827	0·0852	0·0864	0·0768
	0·0826	0·0854	0·0867	0·0858	0·0913	0·0723
	0·0852	0·0882	0·0867	0·0882	0·0852	0·0768
	0·0844	0·0847	0·0894	0·0937	0·0907	0·0710
XLIII	0·0829	0·0896	0·0830	0·0889	0·0834	0·0720
	0·0852	0·0854	0·0855	0·0852	0·0870	0·0710
	0·0864	0·0861	0·0832	0·0913	0·0852	0·0738
	0·0832	0·0875	0·0819	0·0858	0·0834	0·0783
XLIV	0·0854	0·0852	0·0853	0·0882	0·0864	0·0730
	0·0869	0·0889	0·0875	0·0913	0·0858	0·0730
	0·0826	0·0864	0·0865	0·0870	0·0882	0·0716
	0·0833	0·0864	0·0840	—	0·0864	0·0711
Mean *E*	0·0847	0·0868	0·0852	0·0882	0·0866	0·0734
S.E.	±0·0005	±0·0004	±0·0006	±0·0009	±0·0007	±0·0007

DISCUSSION

(1) If the mutant carnation (*car*) is taken as an example of the other members of the ruby or garnet group of mutants previously studied (Nolte, 1950, 1952), we find in the members of the group in the present investigation a similar type of mimic effect in the gross macroscopic eye colour, but based on different types of histological modification. The seven mutants of this group may be divided into four subgroups on comparing them with the wild-type and carnation. First, we find *pr* as a representative of the wild-type class of eye-histology, but lighter red in colour. Secondly, a subgroup consisting of *ry*, *p* and *pd* is more like carnation but with the colour more brownish. Thirdly, a subgroup consisting of *ca* and *ma* with a more medium type of brown coloration than the previous group. The fourth subgroup consists of *pn* in which gross abnormalities occur.

The red group of mutants may also be divided into four subgroups. First, we find *bo* more definitely like the wild-type in general pigment cell histology though brighter red in colour. Secondly, the three mutants *sf²*, *sed* and *dke* are also of the wild-type class but with the primary pigment cells more definitely brown. Thirdly, there is the mutant *mah* with a dark red aggregate colour but with the granules much larger than normal and

the number greatly reduced. Fourthly, the mutant *Mé* which has gross histological abnormalities.

In general one must again conclude that similar eye-colour phenotypes may be based on very different histological modifications.

(2) In a comparison of the quantities of red and brown pigments present in the eyes of the two series of mutants it is again a striking fact that the reductions in the amounts of the two pigments in different cultures of the same strain are not directly correlated, i.e. a greater reduction of the brown pigment content in a culture may be accompanied by a lesser reduction in the red pigment content and vice versa. A study of the data in Tables 1–4 gives some justification for the classification of the mutants of this study into a ruby and a red type based on their gross phenotype. If the data obtained in a previous investigation (Nolte, 1953*a*) for the pigment content in the eyes of the Canton-S wild-type strain are utilized for purposes of comparison the percentages of pigments remaining in the eyes of the two series of mutants may be given as approximate figures as in Table 5; the amounts of pigment in the wild-type were found to be $0{\cdot}8993 \pm 0{\cdot}0052$ for the red pigment and $0{\cdot}0866 \pm 0{\cdot}0007$ for the brown pigment. In the ruby group as well as in the

Table 5. *The proportions of red and brown pigments, expressed as approximate percentages of the amount in the wild-type, in the eyes of the two series of mutants*

Mutant	Red pigment	Brown pigment
pr	25	137
ry	35	82
p	40	33
pd	20	61
ca	27	29
ma	31	59
pn	26	111
sf^2	41	98
sed	79	100
dke	65	98
mah	77	102
bo	67	100
Mé	79	85

mutant connecting this group with the red group, viz. safranin^2, a great reduction in the amount of red pigment as compared with the wild-type is effected, but with the exception of pink and claret the reduction in the amount of brown pigment is much less, while in the case of purple and prune there actually occurs an increase in the amount of brown pigment. In the case of the red group of mutants, however, there is a moderate reduction in the red pigment content, while with the exception of Moiré the content of brown pigment does not differ statistically from that of the wild-type. The reduced amounts of both pigments in the eyes of Moiré might be ascribed, in the first instance, partly to the decreased size of the eye and, secondly, to the cellular modifications in the pigment regions.

(3) An evaluation of all the data obtained in this investigation may lead to some inferences on the probable functions of the wild-type alleles of the various mutant genes. Considering the fact that all these genes affect the amounts of one or both of the pigments and/or the size and number of the granules it is evident that their normal alleles condition the production and supply of various basic substances necessary for, or utilized in, the eye-pigmentary system.

As in the case of the other members of the ruby group of eye-colour mutants previously investigated (Nolte, 1952) and also the mutants of the light group (Nolte, 1953*b*), we find no simple relationships between the amounts of reduction in the content of the two pigments resulting from the mutations to *ry*, *p*, *pd*, *ca* and *ma*; as in the case of the other series of mutants these also act differentially on the two pigments. These disproportionate effects would bring these mutants into the same category as that which was assumed as the function of the other series, so that it is also postulated that the normal alleles of these genes direct enzyme specificities utilized during metamorphosis. If during this stage in the life of the organism various proteins (and other constituents) are broken down, a large series of amino-acids and other histolytic products may be set free. Most of the amino-acids, peptides, etc., will be utilized during the synthesis of proteins for the developing imago, but some of these substances and other residues may well be required for the development of the eye-pigment cells, their granules and the pigments. If the mutant genes of this series are hypomorphic in their action the substances utilized in the eye may be deficient in amount and consequently a reduction in the amount of pigments would result. This postulate presupposes that in the case of this particular series of mutants the substances are of such a nature that their presence is required as basic constituents for the production of both pigments. The most probable level at which their normal alleles would therefore act, would be in the production of precursors to the common substrate required by the w^+ gene which differentiates substrates for combination with the brown and red chromogens. On the other hand these genes may provide tryptophane for the formation of brown chromogen and a similar precursor (possibly a peptide or amino-acid) for the ultimate production of the red chromogen, or all three chains of reactions may be supplied with basic substances for further differentiation; the hypomorphic action of the mutant alleles could result in deficiencies of the requisite substances. If histolytic and/or synthetic enzyme activities result from the action of these genes it is reasonable to assume that some of the products of such action may have manifold uses. One such use could be in the production of the protein carrier in the pigment granules. In the series of mutants under consideration most of the members show a reduction in the extent or size of either or both of the basal and post-retinal pigment regions. This reduction could in most instances not be conclusively ascribed either to a reduction in granule number or to a decrease in total granule volume, but one or both of these phenomena may well occur. A second use for the products of these genes, in fact perhaps the principal use, may be in the synthesis of proteins and other substances in one or other body system: a pointer to this possibility is found in the fact of the decrease in size of many of these mutants.

The cause of the increase in the size of the granules in the secondary pigment cells of *p* and *ca* is not evident but may well be an unequal supply of basic substances consequent on the modified action of the mutant alleles, i.e. an action disproportionate to that which occurs in the wild-type, so that more of a particular substance is deviated to the reaction chain leading to the formation of the granules; these granules would then contain proportionately less of the pigments since the number of granules does not appear to be reduced. All these modifications in the production of basic substances may be correlated with primary processes of histolysis and synthesis, i.e. they constitute epigenetic aspects of a general modification in protein metabolism.

In the case of the mutants *pr* and *pn* the position in regard to their functional category is somewhat more difficult to evaluate since an approximate three-quarter reduction in

red pigment content is concomitant with increases in the amount of the brown pigment. In the mutant *pr* no histological modifications are evident so that this gene may act in a way similar to that of the mutant *Hn*^{r} (Nolte, 1935*b*), i.e. it appears to affect the common basic precursor or constituent which is ultimately differentiated by the w^{+} gene so that some of this substance is diverted from the red pigment pathway to that of the brown. On the other hand the action of this mutant is the converse of that of *cd* (Nolte, 1953*a*) so that if the normal allele of the latter is postulated to act in the further preparation of the differentiated substrate for combination with the brown chromogen, the normal allele of *pr* may be postulated to prepare the other differentiated substrate for combination with the red chromogen. In that case the modified action of the mutant gene may cause a partial blocking of this process, resulting in more of the common substrate being diverted, through the action of the w^{+} gene, to a differentiated substrate for combination with the brown chromogen.

A similar premise for the probable function of *pn* is perhaps not tenable if the effect of this mutant on pigment content is considered in conjunction with the histological modifications of the pigment cell regions and the decrease in the size of the eye as found in many individuals. These modifications by the action of the mutant appear to place the function of the normal allele of *pn* during the differentiation of the eye, apparently during the development of the pigment cell regions, but possibly at an earlier stage when the ommatidial elements are laid down. The decrease in the amount of red pigment and increase in the amount of brown pigment could then be secondary effects of the mutant gene resulting epigenetically from disturbances in the method or rather time sequence of deposition of the two pigments.

The mutants *sf*^{2}, *sed*, *dke*, *mah* and *bo* result in the reduction in amount of the red pigment to from one-half to about three-quarters of the normal amount, but the amounts of brown pigment do not differ significantly from that of the wild-type. The chain in the production of eye pigments wherein these mutants may be fitted is that for the providing of precursors for the red chromogen, so that it is postulated that the action of the normal alleles of these genes result in the production of such substances which initially may be in the form of peptides or amino-acids resulting from proteolytic action during the histolytic processes of metamorphosis. Whether some of these genes stand in a sequential relation to each other is not evident since no combinations have been attempted between these mutant strains. The principal action of sf^{+} may be in the provision of a peptide or amino-acid essential for growth since it appears that the mutant strain develops more slowly than the normal.

The gene *mah* may, in its action, fall in this class of genes, but will then have a secondary effect in the reduction of the number of granules though an increased granule size. Since in this mutant the outward spread of red pigment during the elongation of the secondary pigment cells is clearly seen to take place in granules which are already increased in size and already possess a deposit of brown pigment, it is possible that the normal allele of this gene acts in the production of such granules, so that *mah* effects a reduction in number but secondarily causes the granules to develop to a larger size from the materials made available by other genes. The reduced amount of red pigment in this mutant may then also be a secondary effect due to the fact that the prior adsorption of brown pigment on a decreased surface area of granules could block the subsequent adsorption of the full amount of red pigment.

The reduction in the content of both pigments resulting from the action of *Mé* seems more nearly equivalent, and if this fact is taken in conjunction with the histological abnormalities caused in the primary cells, as also in the secondary and basal regions, it seems reasonable to place the function of the normal allele of this mutant in the processes of differentiation or orientation of the pigment cells.

SUMMARY.

The fourth and fifth groups of eye-colour mutants of *Drosophila melanogaster* studied histologically and photometrically, are the ruby group with the mutants purple (*pr*), rosy (*ry*), pink (*p*), purpleoid (*pd*), claret (*ca*), maroon (*ma*) and prune (*pn*), and the red group with the mutants safranin^2 (*sf*^{2}), sepiaoid (*sed*), dark eye (*dke*), mahogany (*mah*), bordeaux (*bo*) and Moiré (*Mé*).

(1) The mimic effects of the members of each group in the gross phenotype is in general based on differential histological modifications and changes in the amounts of either or both of the pigments. The ruby group may be divided into four subgroups: *pr* as a representative of the wild-type class of coloration histologically but a reduced amount of red pigment and an increased amount of brown pigment; *ry*, *pd* and *p* with the type of coloration and pigment cell histology of *car* of a previous study, all three with disproportionate reductions in the amounts of red and brown pigments, with, in addition in the case of *p*, a large percentage of the granules in the secondary pigment cells increased in size; *ma* and *ca* with a more medium type of brown coloration than carnation and with a small percentage of granules in the secondary cells of *ca* increased in size, and both mutants showing greatly reduced amounts of both pigments; *pn* with a greatly reduced amount of red pigment and increased amount of brown pigment, but with gross abnormalities in eye-structure, i.e. reduction in eye size in some individuals, reduction in the size of the pigment granule masses in the basal and post-retinal regions, and a disarrangement of the pigment granule masses in the secondary cells into lumps or irregular strings.

The red group may also be divided into four subgroups but with only the amount of red pigment reduced in the members of the first three subgroups: *bo* more nearly like the wild-type; *sf*^{2}, *sed* and *dke* also like the wild-type histologically but with the primary pigment cells more brownish; *mah* with the granules much larger than normal but with a reduced number; *Mé* with slightly reduced amounts of both pigments and with gross histological abnormalities, mainly the retraction of the primary pigment cells or their granular masses proximally.

(2) An attempt is made to indicate the possible modes of action of these genes in eye pigmentation. The normal alleles of *ry*, *p*, *pd*, *ca* and *ma* seem to act at the same level as that postulated for *car*, i.e. during the histolysis of metamorphosis these genes direct the specificities of enzymes with the result that some of the breakdown residues are utilized during the pigmentary process. The action of the normal allele of *pr* fits into the reaction chains connected with the w^+ gene, i.e. either during the production of a common precursor for the differentiating action of the latter gene or during the combination of the differentiated substrate with the red chromogen. The normal allele of *pn* seems to act during the processes of differentiation of the eye, e.g. those in connexion with the development of the pigment cells. The normal alleles of *sf*^{2}, *sed*, *dke* and *bo* appear to act during the production of the precursor/s for the red chromogen, while that of *mah* acts at the level of pigment granule production or differentiation, and that of *Mé* during the processes of differentiation or orientation of the pigment cells.

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