THE EYE-PIGMENTARY SYSTEM OF *DROSOPHILA*
II. PHENOTYPIC EFFECTS OF GENE COMBINATIONS

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(With Three Text-figures)

Gene-combination analysis has been one of the most important experimental techniques to contribute evidence for the fact that the colour of the compound eyes of *Drosophila*, as conditioned by a mutant gene, is often the result of a differential quantitative modification of the two pigments of the normal eye, viz. the red and the brown pigments. Based on the work of Wright (1932), Schultz (1935), Mainx (1938) and Ephrussi & Herold (1944), the general conclusion has been reached that the mutant genes scarlet (*st*) and brown (*bw*) respectively suppress the production of the brown and the red pigment, and that other mutant genes reduce the production of one or both of the pigments. The most extensive work on the macroscopic and microscopic effects of gene combinations has been that of Mainx (1938). Since, however, the present author (Nolte, 1950) has advanced a new interpretation of the structure of the eye, of the orientation of the pigment granules, and of the deposition of the pigments, it has been found essential to examine eye pigmentation, macroscopically and microscopically, in a range of gene combinations which include some of those studied by Mainx, but also various new combinations which were thought to be relevant to the main problem, viz. the photometric measurement of eye-pigment concentrations in the attempt to obtain some indication of the action of, and the reasons for, the occurrence of the many eye-colour genes in *Drosophila*.

MATERIAL AND METHODS

The incentive to this study was the discovery of a strain of what was thought to be the white-eyed mutant of *D. melanogaster*, but was later found to be a combination of eosin (*w^e*) and ruby (*rb*), both sex-linked genes (Nolte, 1944, 1948). During the development of ideas engendered by this and other types of interaction of genes a large series of gene combinations was built up. Since most of the mutant genes were sex-linked, the general technique was that of backcrossing heterozygous females to cross-over males; compound mutant strains were tested for homozygosity, in cases where the phenotypes were only slightly or not visibly different to the mutants singly, by backcrossing to the individual single mutants. The main series tested were the following:

(i) Six members of the multiple allelic series of the white (*w*) locus (satsuma, coral, blood, cherry, eosin and apricot) were individually combined with the four sex-linked mutant genes ruby (*rb*), carmine (*cm*), garnet³ (*g³*) and carnation (*car*)—twenty-four different combinations.

(ii) The four genes *rb*, *cm*, *g³* and *car* were combined *inter se* in all possible ways—eleven different combinations.

(iii) The same four genes were individually combined with the mutant genes *st* and *bw*— eight different combinations.

(iv) The mutant gene raspberry[2] (ras^2) was combined with st and bw and also with each of the six multiple alleles of w—eight different combinations.

(v) The position-effect white-mottled 4 (w^{m4}) was combined with each of the genes st, bw, rb, cm, g^3 and car—six different combinations.

(vi) The gene car, extracted from an inversion stock called roughest[3] (rst^3), was combined with coral (w^{co}) by means of double crossing-over, and also with st and bw—three combinations.

(vii) The combination $w^c g^3$ was combined with the car of the rst^3 stock by double crossing-over—one combination.

Although it has been thought essential to describe the macroscopic phenotypic expressions of the various mutant eye-colour genes, it is difficult to give comparative values for these colour phenotypes, due to the fact that a curved surface within an overlying transparent corneal layer has to be compared with the flat plates of the colour atlas. In addition, the colour as judged externally by reflected light is not correlated directly with the colour of the pigment granules; on the contrary, the colour of the eye depends on the number of pigmented granules, their distribution in the different regions of pigment cells, the concentration of pigment in the granules, and in general on the hue, chroma and value of the pigments. Hue is the name used for the colour in the spectrum range, value indicates the amount of light in the colour, viz. its brightness, and chroma is the degree of strength or purity or saturation.

Unfortunately, different workers on the description of eye colours in *Drosophila* have used the colour names rather loosely, or described colours under different systems of lighting, or used different kinds of colour atlas or standards for comparison, with the result that discrepancies appear in the literature. In this study the eye-colour phenotypes of all the single mutants and their different combinations, as judged macroscopically with the eye in reflected light, were compared, under similar conditions of lighting and magnification, with the graded plates of the *Munsell Book of Color* (1929). This provides a most useful system of colour grades, as is evidenced by the fact that many of its patterns have had their tristimulus specifications computed on the C.I.E. system. In the Munsell system each hue is graduated into a vertical scale for value, extending from a lower 0 or dark to an upper 10 or light, and a horizontal scale for chroma extending outward from weak to strong. Consequently each colour grade is specified by hue, value and chroma, and an example of such a specification is that of 7·5 R 4/12 for the wild-type eye; 7·5 R being the yellowish red hue, the value 4 being a medium value or brightness, and the chroma 12 being the deepest red at the foregoing value.

OBSERVATIONS

The range of hues in which all eye colours tested fall is as follows: 7·5 R = yellowish red, 10 R = red yellow-red, 2·5 YR = reddish yellow-red, 5 YR = yellow-red, 7·5 YR = yellowish yellow-red, 10 YR = yellow-red yellow, 2·5 Y = reddish yellow. The values vary from 3 for the darkest eyes to 8 for the lightest eyes. By means of these specifications a fair guide could be obtained to the colour grades of the eyes of the wild-type, mutants and combinations of mutants used in this investigation. Because of the fact that a magnification of × 10 only was used for viewing the eyes, and the daylight source of light was specific for the room wherein the examination was carried out, no absolute values were obtained; on the other hand, the different specifications were all obtained under more or less identical

conditions and can thus be taken as comparable. Another difficulty is that the eyes of different mutants and mutant-combinations darken at different rates on ageing; the specifications obtained are, however, for ages of 5–7 days after eclosion, for individuals from cultures under identical conditions of food container, food mass, and at a temperature of 25° C.

In Table 1 the data are given as obtained by this method of comparison, together with a general name for the colour and notes on the darkening of the eye. The following remarks must be added to the data of the table. First, all eye colours darken after eclosion, at least to some extent. Sometimes the amount of observable darkening reaches the maximum after a few hours; in other cases the darkening continues for a period longer than the 5 days after eclosion at which most of the stocks were examined, though in histological sections this continued darkening cannot be observed in the colour of the granules. Secondly, due to the type of distribution of the pigment granules, and also to transparency, some colours do not appear to fall into the grades as given in the system; these are then noted as falling between certain grades or are mentioned as being near a certain specification. Thirdly, the darker colours are often duller and thus actually with less chroma than some of the brighter colours. Fourthly, some of the mutant genes condition eye colours which are variable in expression; an outstanding case is that of cherry (w^{ch}), this variability also being expressed in its combination with other eye-colour genes.

Table 1. *The eye-colour phenotypes of single and compound mutants of*
Drosophila melanogaster

Genotype	Sex	Descriptive term	Munsell specification	Darkening
Wild type	Both	Red	7·5 R 4/12	—
st	Both	Bright red	7·5 R 5/12	—
bw	Both	Brown-garnet	10 R–2·5 YR 3/6	Brown to garnet
bw st	Both	White	2·5 Y 8/2	—
w	Both	White	2·5 Y 8/2	—
rb	Both	Ruby	7·5 R 3/8–10	To garnet
cm	Both	Reddish ruby	7·5 R 3/8–10	To reddish garnet
g^3	Both	Ruby	7·5 R 3/8–10	To garnet
car	Both	Ruby	7·5 R 3/8–10	To garnet
rb cm	Both	Ruby	7·5 R 3/8–10	To garnet
rb g^3	Both	Light ruby	10 R 4/10	To ruby garnet
cm g^3	Both	Light ruby	10 R 3/10	To ruby
rb car	Both	Yellowish pink	10 R 5–6/10	To light yellowish ruby
cm car	Both	Yellowish pink	10 R 5/10	To yellowish ruby
g^3 car	Both	Yellowish pink	10 R 5/10	To yellowish ruby or garnet
rb cm g^3	Both	Pinkish ruby	7·5–10 R 5/10	To ruby
rb cm car	Both	Pink-yellow	2·5 YR 5–6/10	To yellowish pink
rb g^3 car	Both	Pink-yellow	2·5 YR 5–6/10	To yellowish pink
cm g^3 car	Both	Pink-yellow	2·5 YR 5–6/10	To yellowish pink
rb cm g^3 car	Both	Pink-yellow	2·5 YR 5–6/10	To yellowish pink
rb st	Both	Pinkish yellow	5 YR 6/12	To orange
cm st	Both	Orange vermilion	Near 7·5 R 5/12	To dull ruby
g^3 st	Both	Orange	Near 2·5 YR 6/14	To yellowish ruby
car st	Both	Orange vermilion	Near 7·5 R 5/12	To dull ruby
rb bw	Both	Pinkish tan-yellow	2·5 YR 6/8	—
cm bw	Both	Pinkish tan-yellow	2·5 YR 6/8–10	—
g^3 bw	Both	Yellowish brown	Near 2·5 YR 5/8	—
car bw	Both	Yellowish brown	Near 2·5 YR 4/8	—
w^{sat}	Both	Deep ruby	7·5 R 3/6	—
w^{sat} rb	Both	Yellowish ruby	10 R 4/8–10	—
w^{sat} cm	Both	Yellowish ruby	10 R 4/8–10	—
w^{sat} g^3	Both	Yellowish ruby	10 R 4/8–10	—
w^{sat} car	Both	Yellowish ruby	10 R 4/8–10	—
w^{co}	♂	Yellowish ruby	10 R 4/8	To garnet
	♀	Yellowish ruby	10 R 4/9	To garnet
w^{co} car	♀	Yellowish pink	Near 2·5 YR 5/10	—

Table 1 (cont.)

Genotype	Sex	Descriptive term	Munsell specification	Darkening
$w^{co}g^3$	♀	Light yellowish pink	5 YR 5/10	—
$w^{co}cm$	♀	Pinkish yellow	5 YR 6/10	—
$w^{co}rb$	♀	Buff-yellow	7.5 YR 7/8	—
w^{bl}	♂	Yellowish ruby	10 R 4/8–10	—
	♀	Yellowish pink	10 R 5/10	—
$w^{bl}car$	♂	Yellowish pink	2.5 YR 5/10	—
	♀	Pinkish yellow	2.5 YR 6/12	—
$w^{bl}g^3$	♂	Pinkish yellow	5 YR 6/10–12	—
	♀	Pinkish yellow	5 YR 6/9	—
$w^{bl}cm$	♂	Light pinkish yellow	7.5 YR 6–7/10	—
	♀	Buff-yellow	7.5 YR 7/8	—
$w^{bl}rb$	♂	Cream-yellow	10 YR 7–8/6	—
	♀	Cream-yellow	10 YR 8/6	—
w^{ch}	♂	Light yellowish pink	5 YR 5/10	—
	♀	Yellowish pink	10 R 5/10	—
$w^{ch}car$	♂	Buff-yellow	10 YR 7/6	—
	♀	Pinkish yellow	5 YR 6/10–12	—
$w^{ch}g^3$	♂	Cream-yellow	10 YR 7–8/6	—
	♀	Light pinkish yellow	7.5 YR 6–7/10	—
$w^{ch}cm$	♂	Tinged white	2.5 Y 8/4	—
	♀	Buff-yellow	10 YR 7/7	—
$w^{ch}rb$	♂	White	2.5 Y 8/2–4	—
	♀	Tinged white	10 YR 8/4	—
w^{e}	♂	Pinkish yellow	5 YR 6/10	—
	♀	Yellowish pink	10 R 5/10	—
$w^{e}car$	♂	Cream-yellow	10 YR 7–8/6	—
	♀	Pinkish yellow	5 YR 6/10–12	—
$w^{e}g^3$	♂	Cream	2.5 Y 8/6	—
	♀	Buff-yellow	7.5 YR 7/8–10	—
$w^{e}cm$	♂	Creamy white	2.5 Y 8/4	—
	♀	Buff-yellow	10 YR 7/6	—
$w^{e}rb$	♂	White	2.5 Y 8/2–4	—
	♀	Tinged white	10 YR 8/4	—
w^{a}	♂	Yellowish pink	2.5 YR 5/10	—
	♀	Pinkish yellow	2.5 YR 6/10–12	—
$w^{a}car$	♂	Light pinkish yellow	5 YR 6/8–9	—
	♀	Light pinkish yellow	5 YR 6/8	—
$w^{a}g^3$	♂	Buff-yellow	7.5 YR 7/8–10	—
	♀	Buff-yellow	10 YR 7/8	—
$w^{a}cm$	♂	Cream	2.5 Y 8/6	—
	♀	Cream	2.5 Y 8/6–8	—
$w^{a}rb$	♂	Tinged white	2.5 Y 8/2–4	—
	♀	Tinged white	2.5 Y 8/2–4	—
ras^2	Both	Dark ruby	7.5 R 3/6	—
$ras^2 st$	Both	Reddish orange	2.5 YR 6/14	—
$ras^2 bw$	Both	Brown-garnet	10 R–2.5 YR 3/6	From brown to garnet
car from rst^3 stock				
car	Both	Ruby	7.5 R 3/8–10	To garnet
$w^{co}car$	Both	Pinkish yellow	5 YR 6/10–12	—
$w^{e}g^3 car$	♂	Cream-white	2.5 Y 8/4–6	—
	♀	Cream-yellow	10 YR 7–8/6	—

The following additions may be made to the data of Table 1.

(1) The eye colours of white-mottled (w^{m4}) and its compounds:

w^{m4}. The eye colour conditioned by this inversion is very variable. Sometimes the eye is red with an apparently pitted effect due to unpigmented ommatidia; often the colour is garnet with mottles of a more yellowish colour. The eye of the male is often more dull in colour and seems more variable than that of the female.

$w^{m4} st$. In this combination the colour is also variable. Sometimes it is vermilion in the female, with the pitted effect and darkening to dull ruby; often it is an orange-vermilion with mottles of a more yellowish colour. In the male it is often more mottled and lighter yellowish in ground-colour.

$w^{m4} bw$. This combination too shows variation, an average type of colour being a mottled light brown, the male generally showing lighter yellow mottles. Sometimes the colour is dull brown.

$w^{m4} rb$, $w^{m4} cm$, $w^{m4} g^3$, $w^{m4} car$. In these four combinations the range of variation is great, so that no differentiation is possible between them. In general the amount of mottling is great, and the eye colour is diluted to a yellow with the mottled areas a brownish red.

(2) The eye colours of the compounds of ras^2:

$w^{sat} ras^2$, $w^{co} ras^2$, $w^{bl} ras^2$, $w^{ch} ras^2$, $w^{e} ras^2$, $w^{a} ras^2$. In these combinations with the alleles of the w locus the eye colours are nearly indistinguishable from those of the multiple alleles singly.

(3) The eye colours of some combination heterozygotes:

$rb/rb st/+$ and $rb/rb bw/+$. Both have a lighter ruby eye colour than rb.

$cm/cm st/+$ and $cm/cm bw/+$. Both have a lighter reddish ruby eye colour than cm.

$w^{co}/w^{co} car/+$. Has a yellowish brown eye colour, lighter than the yellowish ruby of w^{co} and darker than the yellowish pink of $w^{co} car$.

$rb g^3 car/+ + car$. Has a lighter colour than the ruby of car.

$rb g^3 car/rb g^3 +$. Has very light brownish eyes, these being lighter than the light ruby of $rb g^3$.

$cm g^3 car/cm + +$. Has a more brownish eye than the reddish ruby of cm.

$cm g^3 car/+ + car$. Has an eye colour which is somewhat lighter than the ruby of car.

$cm g^3 car/cm g^3 +$. Has very light pale brownish eyes, these being lighter than the light ruby of $cm g^3$.

Some pertinent histological data with relation to the pigment granules of various mutants and compounds will be given under the next heading.

DISCUSSION

From the data of the macroscopic study of the eye colours of mutants of *Drosophila*, singly and in combination, the following general facts emerge. First, the range of the grades of colour or hues in which the red and brown pigments show up, singly and in mixture, are the following: red, brownish red, yellowish red, orange and yellow. Secondly, most colours fall in a restricted range with respect to value and chroma, the values being mainly the higher or brighter values, and the chroma in general being the higher grades for each value.

In addition to a discussion on the diluting effects of the various mutant genes, some observations may be made regarding the various factors which affect the amount of colour expressed by a specific genotype during different periods of imaginal life.

(1) The darker grades of eye colour are often the result of a reduced amount of pigment; if judged on the basis of the pigmentation of the granules in histological sections such eyes often have less pigment than is present in the brighter grades. A typical example is that of ruby (rb) which macroscopically is one of the darkest grades, yet in sections show granules which are much more dilute than in the wild type (Nolte, 1950); a factor contributing to this darkened phenotype is the presence of large brown granules in the primary pigment cells. Other examples of a reduced amount of pigment in the granules but a dark eye colour are cm, g^3, car, ras^2, $rb cm$, $rb g^3$.

(2) Darkening of the eye colour occurs in the case of all the tested genotypes after eclosion, although this process is not always easily distinguishable. The amount of darkening, judged macroscopically, varies greatly among the different stocks, being less noticeable in the case of the more reddish eyes such as of the wild type and scarlet, also of raspberry and the multiple alleles satsuma, cherry, eosin and apricot, as well as in the case of combinations with highly diluted eye colour. In the case of ruby, garnet, carmine and carnation and some combinations of these genes, as well as in the case of the multiple alleles coral and blood, the amount of darkening is much more evident. In the previous publication (Nolte, 1950) it has been indicated that darkening is the result of an increase in the amount of pigment as evidenced by granule colour in histological sections. The periods of duration of darkening vary when judged on this basis, e.g. after 1–2 days the eyes of *bw* change from brown to garnet and the granules then appear to be fully pigmented; in the case of *rb* the darkening from ruby to garnet is apparently due to an increase in the amount of red, though macroscopically darkening is not always directly correlated with any apparent change in granule colour because in one series of sections at the ages of 1, 2, 10 and 18 days after eclosion only the eyes at the last-named age were distinctly more reddish in aggregate granule colour.

(3) The phenotypic expression of many genotypes is greatly influenced by many factors; the eyes of the mutants ruby and garnet[3] darken more slowly and even to a lesser extent in cultures which are overcrowded or which have too high a moisture content or which develop hyper-acidity. The multiple alleles of white are especially liable to great variation in their expression due to the action of the following factors. Temperature influences blood so that with increase from 19 to 30° C. during the stages of larval development the amount of pigment is reduced; through these grades of reduction this allele simulates in its expression the phenotypes of the other alleles of this multiple series. The allele cherry appears to be inherently variable in its action, since slightly different colours appear even in a single culture. In the case of coral, blood, cherry, eosin and apricot, factors such as overpopulation or underfeeding, excess moisture and hyper-acidity cause flies to emerge with eyes more dilutely coloured than under normal circumstances.

The position-effect white-mottled 4 is the most extreme genotype with regard to expressivity under various environmental and genetic conditions; this special case will be further considered in connexion with photometric measurement of pigment concentration.

(4) The characteristic histological features of certain mutant genes also appear in combinations of these genes with other mutant eye-colour genes. The large brown granules of the primary pigment cells conditioned by the gene ruby (Nolte, 1950) also occur in combinations with the mutant genes carmine, garnet[3], carnation and satsuma. The highly reduced basal pigment cells specific for the mutant carnation also occur in combination with the mutants ruby, carmine, garnet[3] and satsuma. The disarrangement of the secondary pigment cells correlated with the mutation raspberry[3] also appears in its combination with *st* but not in the combination with *bw*.

(5) The most striking fact emerging from the data of Table 1 is that mutant eye-colour genes act as dilutors of the normal red eye and that the types of dilution may be classified as resulting from the action of various non-alleles, of various groups of multiple alleles, and of various combinations between these two types.

(a) In a previous publication (Nolte, 1950) a discussion has already been given of the action of the mutant genes ruby, carmine, garnet[3] and carnation on the amount of

pigmentation. Though carmine conditions a more reddish phenotype, the genes ruby, garnet[3] and carnation condition phenotypes which, after the darkening of the eye during the first week of imaginal life, may be called mimics. As has been indicated, however, the histological pictures of these mutant eyes indicate that specific reactions must be ascribed to these genes. On the basis of their individual combinations with *st* and *bw* the four genes reduce the amounts of red and brown pigments to a differential extent. Judged macroscopically, the series in increasing order of reduction of red pigment would be carnation→carmine→garnet[3]→ruby, and in increasing order of reduction of brown pigment would be carnation→garnet[3]→carmine→ruby. It must be mentioned that there appears to be little difference between carmine and carnation with respect to their red pigment content, and between carmine and ruby with respect to their brown pigment content; the combinations carmine-brown and ruby-brown are macroscopically very similar, but histological sections show more pigment in the form of large brown granules to be present in the primary pigment cells of the latter combination. Mainx (1938), who made use of the allele garnet[2], placed the two series of reductions, viz. of red and brown pigments, both at carmine→carnation→ruby→garnet[2].

Though no multiple alleles of the gene carnation are given in the list of mutants of *Drosophila* drawn up by Bridges & Brehme (1944), the carnation occurring in the *rst*[3] inversion stock appears to be such an allele. Though it appears to condition a similar ruby eye colour on eclosion, darkening to the same garnet as in the case of the normal carnation stock, in combination with coral it causes a more pinkish yellow or orange eye colour than the yellowish pink of the other carnation-coral combination.

A condition of dominance change appears to occur in the combinations of carmine and ruby with *st* and *bw*, because carmine flies heterozygous for the last-named two genes respectively have lighter reddish ruby eyes than carmine flies homozygous for the normal alleles of the two genes; a similar condition occurs in the case of the gene ruby.

(*b*) The multiple alleles at the *w* locus act in series as dilutors of eye pigmentation. On the basis of macroscopic effect the series, at average room temperatures of about 20° C. and in ascending order of reduction of pigmentation, reads: satsuma→blood→coral→cherry→eosin→apricot, and for the males it reads: satsuma→blood→coral→apricot→cherry→eosin. At 25° C. the eye colours of the females of blood, cherry and eosin are nearly indistinguishable, while those of the males still remain in the series as blood→cherry→eosin. Mainx (1938) found that, on the basis of their combinations with *st* and *bw*, the alleles apricot, eosin, coral and blood cause increased production of both the red and brown components, in that order.

Histologically the genes of this series appear to reduce the production of both pigments, but that of the red to a greater extent; the general picture is that of a type very similar to *bw*. For example, satsuma greatly resembles *bw*, but is darker with more and darker brown granules in the primary pigment cells, and in addition the basal pigment cells are bigger in volume and the granules of the post-retinal region are in aggregate more a brown than a purplish brown.

(*c*) The combinations of ruby, carmine, garnet[3] and carnation *inter se* cause reduction in eye pigmentation, when in duplo, below that of the lightest member of the pair, and a further increased reduction when combined in triplo; the combination of all four is difficult to obtain and to differentiate, since it is apparently not much lighter than the lightest combination in triplo. In increasing order of reduction of pigmentation the various

combinations may be placed as follows, extending in colour from ruby to a pink-yellow: $rb\,cm \rightarrow rb\,g^3 \rightarrow cm\,g^3 \rightarrow rb\,cm\,g^3 \rightarrow g^3\,car \rightarrow cm\,car \rightarrow rb\,car \rightarrow rb\,g^3\,car \rightarrow rb\,cm\,car,\,cm\,g^3\,car$. In histological sections the following characteristics appear:

$rb\,cm$. Resembles rb but somewhat more dilute in granule colour.

$rb\,g^3$. Resembles rb but is more dilute, the granules in aggregate being a light purplish colour.

$cm\,g^3$. Resembles the above, but the granule colour is more reddish.

$g^3\,car$. A more dilute example of the ruby-garnet type, with the granules of the primary cells brown and those of the other pigment cells more purplish; the basal pigment cells are very small.

$cm\,car$. A type similar to the above, with the granules of the primary cells brown and those of the other pigment regions more purplish brown.

$rb\,car$. Also belongs to the above type but even more dilute in pigmentation of granules.

$rb\,g^3\,car$ and $cm\,g^3\,car$. These two combinations are more brownish than the others but very dilute, with apparently no pigmented granules in the primary pigment cells.
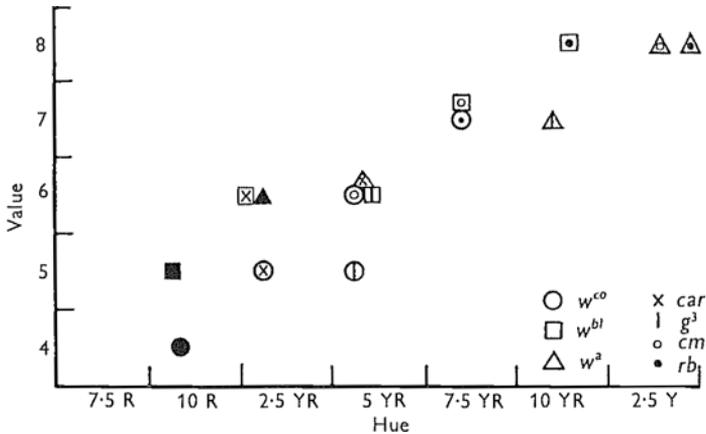

Fig. 1. The colour grades in the Munsell system of the eyes of females of some of the white alleles and their combinations with the genes of the ruby group. w^{co}, coral; w^{bl}, blood; w^a, apricot; car, carnation; g^3, garnet^3; cm, carmine; rb, ruby.

In certain combinations the four genes also show dominance changes, as has been indicated in the addenda to Table 1. This is especially the case with carnation which causes a lesser amount of reduction in pigmentation when combined in the heterozygous state with other genes than in the homozygous state; carnation thus shows partial dominance in the heterozygous condition. Actually the breeding of triple gene stocks was sometimes facilitated by distinguishing the heterozygotes required for crossing-over of genes as those individuals with more dilutely coloured eyes.

(d) In combination with the alleles of the white locus the genes ruby, carmine, garnet^3 and carnation also act as dilutors. The ascending order of reduced pigmentation in combination with each of the alleles, excepting with satsuma, runs carnation$\rightarrow$garnet$^3 \rightarrow$ carmine$\rightarrow$ruby. In the case of satsuma the four combinations are more or less equal in pigmentation, though more dilute than satsuma; in contrast, the combinations of ruby with cherry, eosin and apricot show nearly total suppression of pigmentation. This reduction of pigmentation is graphically illustrated in Figs. 1–3. As co-ordinates are taken

increasing value or brightness from 4 to 8 on the Munsell system, and a range of hues from reddish to more yellowish, i.e. with a decreasing amount of red, each of the hues is divided into descending grades of chroma from 12 to 2, and thus in effect the graph shows a range of decreasing colour. In Fig. 1 are given the specifications for females of three alleles of w, conditioning a dark, a medium and a light colour (at 25° C.), viz. coral, blood and apricot,
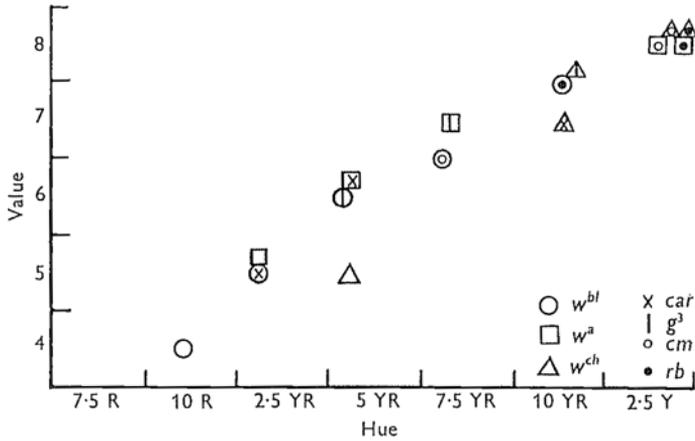

Fig. 2. The colour grades in the Munsell system of the eyes of males of some of the white alleles and their combinations with the genes of the ruby group. w^{bl}, blood; w^a, apricot; w^{ch}, cherry; car, carnation; g^3, garnet^3; cm, carmine; rb, ruby.
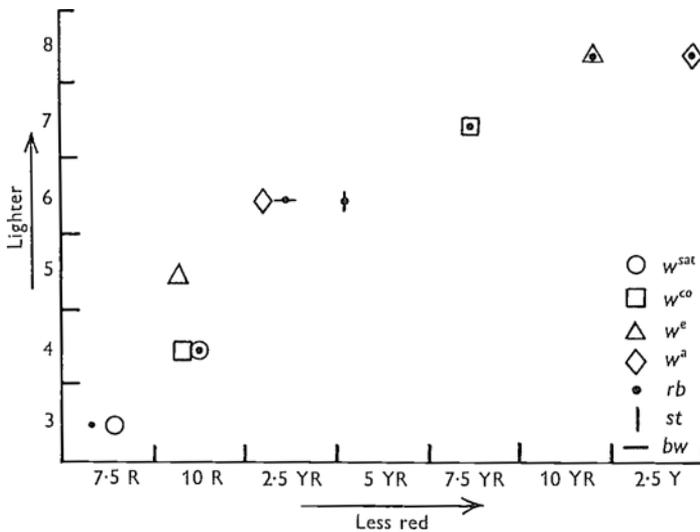

Fig. 3. The interactions of eye-colour genes, expressed as grades in the Munsell system. w^{sat}, satsuma; w^{co}, coral; w^e, eosin; w^a, apricot; rb, ruby; st, scarlet; bw, brown.

and of their combinations with the four dilutors ruby, carmine, garnet^3 and carnation. In Fig. 2 are given similar specifications for the males of three grades of colour among the alleles of w. In both cases the reduction of pigmentation is clearly indicated, with approximately equivalent effects of each of the dilutors on the series of multiple alleles. In Fig. 3 a diagrammatic representation is given of the diluting effect of the gene ruby on the eye

pigmentation of females of the alleles satsuma, coral, eosin and apricot in comparison with the dilution produced by the combination of ruby with st and bw. The grades of colour range from ruby to white. If the combinations ruby-scarlet and ruby-brown are taken as indicators of the actual, or at least approximate, amounts of red and brown pigment present in the mutant ruby, then interaction of the latter with the series of four alleles seems not to produce proportional amounts of red and brown pigment in the combinations, but to cause a geometric progressive or cumulative increase in the reduction of the total amount of pigment produced, so that in the most extreme case, viz. the combination apricot-ruby, pigment production is almost totally suppressed. This cumulative reduction due to interaction is also evident in the amount of pigmentation of the combination eosin-garnet^3-carnation which is distinctly lighter in eye colour than even the light-coloured combination eosin-garnet^3.

Histological sections of the eyes of various of the combinations mentioned above show the following characteristics:

$w^{sat}car$. The granules appear to be brown and the basal pigment cells are very small.

$w^{sat}g^3$ and $w^{sat}cm$. In these two combinations the aggregate colour of granules is brown.

$w^{sat}rb$. The colour is brown, with large brown granules in the primary pigment cells.

$w^{co}car$ and $w^{co}g^3$. In these two combinations the aggregate colour is light brown; the primary cells are more heavily pigmented than the rest, and the basal pigment cells are small.

$w^{bt}car$ and $w^{bt}g^3$. These combinations have brown granules only, are most heavily pigmented in the primary pigment cells, and the basal cells are small.

On the grounds of the similarity of the eye colours of the four dilutors and the fact that the order of their increasing distance in the chromosome from the locus of w is similar to the order of their decreasing effect in the reduction of pigmentation of the w alleles, an initial premise was that their decreasing effect was correlated with distance in the chromosome—a type of position effect. Attempts were made, by the use of the rst^3 inversion chromosome, to transfer one of the alleles of white, viz. w^{co}, by crossing-over to this chromosome which contains car in the inverted segment; the aim was to determine whether car caused an increased reduction of pigmentation due to a decrease in distance between it and w^{co}. These attempts failed, however, since the left break of the rst^3 inversion is immediately to the right of the w locus so that no cross-overs of w^{co} to the $rst^3 car$ chromosome could be obtained.

(e) The eye-colour change resulting from the inversion of the normal white locus to the heterochromatin, in the case of w^{m4}, is the most variable of all the observed phenotypes. With an optimum amount of pigment production the colour is red, but this is modified to a mottled garnet under the influence of various factors, e.g. hyper-acidity of the food culture, overcrowding and other unknown factors. In combination with the mutant genes scarlet, brown, ruby, carmine, garnet^3 and carnation, the colour is further reduced, the mottling becoming more noticeable and the variability much greater. In overcrowded cultures of the combination mottled-4-scarlet some flies are found with pinkish yellow eyes, some with orange eyes and others with scarlet eyes, all mottled; in similar cultures of the combination mottled-4-brown the eye colours vary from pinkish yellow to light brown, with some of the older flies having dull brown eyes, all mottled. In the other four combinations variation is generally characterized by a reduction of the reddish brown mottles, with the eye becoming more yellowish or pinkish yellow.

(*f*) The gene raspberry[2] in combination with *st* shows a small reduction in red pigment, while in combination with *bw* there results no apparent reduction in brown pigment. With the multiple alleles of *w* combinations are difficult to differentiate from the single mutants, consequently little interaction may be said to take place.

Summary

As a preliminary to the quantitative measurement of pigment concentration in the eyes of the various mutants of *Drosophila melanogaster*, in an attempt to obtain some indication of the action of, and the reasons for, the occurrence of the large number of eye-colour genes, eye colour was examined, macroscopically and microscopically, in a series of mutant sex-linked genes and their combinations, also of combinations with the autosomal genes scarlet and brown. The data obtained permit the following conclusions:

1. Eye colour, in its gross phenotype, is influenced by various factors. There is often a visible darkening after eclosion, with various periods of duration for different mutants, though continued darkening to the maximum extent is not always evidenced histologically by the colour of the granules. Environmental factors, such as overcrowding in larval cultures, too high a moisture content of the food, and hyper-acidity, cause a dilution of colour and affect especially the multiple alleles of *w* and their combinations with other mutant eye-colour genes.

2. Histological examination shows that the darker grades of eye colour are often correlated with reduced amounts of pigment, as tested by combination with the genes *st* and *bw*; examples are the genes *rb*, *cm*, *g*[3], *car* and *ras*[2], and some of their combinations.

3. The characteristic histological features of certain mutant genes appear in combinations of these genes with others. Examples are the large brown granules in the primary pigment cells conditioned by *rb*; the highly reduced basal pigment cells specific for *car*; the disarrangement of the secondary pigment cells caused by *ras*[2].

4. Mutant eye-colour genes always act as dilutors of the pigment carried by the wild-type eye, and in combinations of these genes the dilution is generally increased, but in different ways for different series of combinations. First, the genes *rb*, *cm*, *g*[3] and *car* condition very similar eye-colour phenotypes, but, while the various combinations between them show reduction in pigmentation when combined in pairs and increased reduction when combined in threes, the increasing order or reduction for these combinations shows no simple relation to the concentration of the pigments in the single mutants as postulated from the combinations of the latter with *st* and *bw*; the reduction in pigmentation is disproportionate during the interaction between these genes. Secondly, in the case of the combination of the genes *car*, *g*[3], *cm* and *rb* with the alleles of *w* the ascending strength of effect on reduction in pigmentation is in the order named, and this coincides with the determined order of increasing reduction in brown pigment content of the four mutants. This indicates that in the interaction with the *w* alleles the brown pigment is mainly involved, a conclusion strengthened by the fact that the mutant gene *ras*[2] which shows no visible interaction with *bw*, also exerts little effect in combination with the *w* alleles.

REFERENCES

BRIDGES, C. B. & BREHME, K. S. (1944). The mutants of *Drosophila melanogaster*. *Publ. Carneg. Instn*, no. 552.

EPHRUSSI, B. & HEROLD, J. L. (1944). Studies of eye pigments of *Drosophila melanogaster*. I. Methods of extraction and quantitative estimation of the pigment components. *Genetics*, **29**, 148–75.

MAINX, F. (1938). Analyse der Genwirkung durch Faktorenkombination. Versuche mit den Augenfarbenfaktoren von *Drosophila melanogaster*. *Z. indukt. Abstamm.- u. VererbLehre*, **75**, 256–76.

NOLTE, D. J. (1944). White of *yw*, an interaction-product. *DIS*-18, 54.

NOLTE, D. J. (1948). A graded series of eye colors due to interaction of genes. *DIS*-22, 74–5.

NOLTE, D. J. (1950). The eye-pigmentary system of *Drosophila melanogaster*: the pigment cells. *J. Genet.* **50**, 79–99.

SCHULTZ, J. (1935). Aspects of the relation between genes and development in *Drosophila*. *Amer. Nat.* **69**, 30–54.

WRIGHT, S. (1932). Complementary factors for eye color in *Drosophila*. *Amer. Nat.* **66**, 282–3.