THE EYE-PIGMENTARY SYSTEM OF *DROSOPHILA*

IX. HETEROZYGOUS EFFECTS OF EYE-COLOUR GENES

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1. INTRODUCTION

DOMINANCE, as a basic fact of genetics, is intimately bound up with the problem of genic action in the aspects of the kinetics and thresholds of action and the quantities of end-products. Goldschmidt (1955) has fully discussed dominance as a potency effect which should be explained in terms of genic action controlling in a quantitative way some phase of the kinetics of production of active end-products. During the course of investigations on the eye-structure and pigments of a large number of eye-colour mutants of *Drosophila melanogaster* it was noted that some heterozygotes, although phenotypically normal in eye-colour, appeared to possess amounts of red and brown pigments differing from those occurring in the wild-type. It was consequently decided to institute an inquiry into the quantitative relationships of these pigments in a randomly selected series of heterozygotes. Those selected were the heterozygotes of the following mutants: vermilion (*v*), vermilion^{36f} (*v*^{36f}), scarlet (*st*) and cardinal (*cd*) of the vermilion group of mutants; brown (*bw*), garnet² (*g*²), garnet³ (*g*³), carnation (*car*) and purple (*pr*) of the ruby or garnet group; sepia (*se*) and clot (*cl*) of the dark group; apricot³ (*w*^{a3}) of the white series of multiple alleles. These heterozygotes were obtained by crossing the named mutants with the Canton-S strain of wild-type. In the meantime, however, it was discovered that some South African strains of wild-type show significantly different amounts of eye pigments. One such strain is a high-pigment strain, called Graaff-Reinet, and obtained from the Eastern Cape Province; another strain is a low-pigment strain, called Stellenbosch, and obtained from the Western Cape Province. Heterozygotes of some of the above-named mutants were obtained by crossing with these two wild strains.

The quantitative data of pigment extracts from these heterozygotes were compared with the relative amounts of red and brown pigments, obtained for the homozygous strains in previous studies, in which the methods of quantitative estimation are fully described (Nolte, 1954, 1955, 1958).

2. OBSERVATIONS

In none of the heterozygotes could any deviation from the normal red colour of the wild-type eye be detected. The red and brown

pigments produced in the eyes of these heterozygotes were also normal in regard to the spectrophotometric curves obtained for the extracts

TABLE 1

Relative amounts of red and brown pigments in the eyes of the series of mutants and their heterozygotes with the Canton-S wild-type

Strain	Red pigment	Brown pigment
+	0.8993 ± 0.0052	0.0866 ± 0.0007
st/st	0.8910 ± 0.0043	0.0049 ± 0.0002
st/+	0.9015 ± 0.0125	0.0814 ± 0.0013
v/v	0.8574 ± 0.0034	0.0045 ± 0.0001
v/+	0.8708 ± 0.0044	0.0991 ± 0.0017
v ^{36f} /v ^{36f}	0.8641 ± 0.0055	0.0030 ± 0.0003
v ^{36f} /+	0.9521 ± 0.0021	0.0837 ± 0.0019
cd/cd	0.9672 ± 0.0026	0.0131 ± 0.0002
cd/+	0.9844 ± 0.0060	0.0893 ± 0.0016
bw/bw	0.0110	0.0750 ± 0.0006
bw/+	0.8791 ± 0.0050	0.0824 ± 0.0007
pr/pr	0.2282 ± 0.0010	0.1183 ± 0.0050
pr/+	0.8743 ± 0.0112	0.0908 ± 0.0011
car/car	0.2999 ± 0.0044	0.0403 ± 0.0011
car/+	0.9290 ± 0.0095	0.0811 ± 0.0015
g ³ /g ³	0.1900 ± 0.0033	0.0409 ± 0.0012
g ³ /+	0.9888 ± 0.0125	0.0952 ± 0.0011
g ² /g ²	0.1384 ± 0.0044	0.0274 ± 0.0004
g ² /+	0.9625 ± 0.0125	0.0897 ± 0.0004
se/se	0.0556 ± 0.0006	0.1233 ± 0.0013
se/+	0.8669 ± 0.0042	0.0963 ± 0.0018
cl/cl	0.3603 ± 0.0013	0.1367 ± 0.0019
cl/+	0.9883 ± 0.0188	0.1006 ± 0.0018
w ^{a3} /w ^{a3}	0.0542 ± 0.0008	0.0090 ± 0.0002
w ^{a3} /+	0.7711 ± 0.0103	0.0989 ± 0.0038

TABLE 2

Relative amounts of red and brown pigments in the eyes of some strains and their heterozygotes with the Graaff-Reinet wild-type

Strain	Red pigment	Brown pigment
+	1.1321 ± 0.0182	0.1227 ± 0.0022
Canton-S/+	0.9444 ± 0.0120	0.1102 ± 0.0070
v/+	0.9547 ± 0.0090	0.1094 ± 0.0022
cd/+	1.0175 ± 0.0368	0.1275 ± 0.0017
bw/+	0.0446 ± 0.0156	0.1088 ± 0.0035
pr/+	0.9065 ± 0.0151	0.1331 ± 0.0010
car/+	0.9269 ± 0.0118	0.1033 ± 0.0031
g ³ /+	1.0083 ± 0.0216	0.1225 ± 0.0022
g ² /+	0.9333 ± 0.0330	0.1155 ± 0.0069
se/+	0.8480 ± 0.0051	0.1232 ± 0.0019
cl/+	1.0708 ± 0.0041	0.1327 ± 0.0021

in the two solvents AEA and AMA. The quantitative data for the pigments are given in tables 1-3, the measurements being of extinction (*E*) for 10 heads per 1 c.c. extract. The AEA extract was measured at 480 mμ, although for the mutant sepia the peak in absorption is

at 415 m μ for the modified red pigment, and for the mutant clot it is at 430 m μ for the mixture of normal and modified red pigment (Nolte, 1954*b*). The AMA extract was measured at 444 m μ .

TABLE 3
Relative amounts of red and brown pigments in the eyes of some strains and their heterozygotes with the Stellenbosch wild-type

Strain	Red pigment	Brown pigment
+	0.5996 $\pm$ 0.0106	0.0858 $\pm$ 0.0012
Canton-S/+	0.8382 $\pm$ 0.0134	0.0838 $\pm$ 0.0023
<i>cd</i> /+	0.9312 $\pm$ 0.0312	0.1063 $\pm$ 0.0013
<i>bw</i> /+	0.7792 $\pm$ 0.0242	0.0853 $\pm$ 0.0004
<i>se</i> /+	0.8228 $\pm$ 0.0098	0.1113 $\pm$ 0.0028

3. DISCUSSION

As in previous investigations of this series, the norm in eye-size was used as a basis for the evaluation of quantitative pigment differences. It is evident that eye-size is difficult to measure accurately, and consequently small differences in the diameter of the eye may result in appreciable differences in pigment content as measured photometrically. None of the strains were isogenic for eye-size genes, neither for any other genes which might affect pigment quantities, and thus the standard error of the quantitative data is not always a reliable tool for estimating the significance of differences. In the case of the heterozygotes any occurrence of a pigment content higher than normal could be ascribed to an increase in eye-size as the result of heterosis. It should be noted that although heterosis is to be expected in the heterozygotes between different inbred strains, micrometric data of eye-size in all strains and their heterozygotes show no appreciable differences, and in most cases fell within the limits of the norm.

As has been established in previous studies, most eye-colour mutants have a lower content of one or the other or both of the pigments than the Canton-S wild-type. On the other hand, the mutant *cd* has a higher red pigment content, while the mutants *pr*, *se* and *cl* have a higher brown pigment content. The Graaff-Reinet strain of wild-type has a red pigment content higher than that of any other strain and a brown pigment content equal to that of *se* but less than that of *cl*. The Stellenbosch wild-type strain has a red pigment content lower than that of the Canton-S strain but an equal amount of brown pigment. The inspection and comparison of the quantitative data of tables 1-3 show a constant trend of interaction of eye-colour genes in heterozygotes: in no instance does it seem as if the dominance of the normal allele is complete for both pigments. If, however, the presence of amounts of pigment slightly in excess of those of the wild-type should be ascribed to heterosis, some of the strains should be classified as mutants of normal alleles with full dominance.

Three main types of quantitative interaction appear to occur in these data. (1) The first type is an additive effect resulting in intermediate quantities of pigment in the heterozygote, although the effect may be differential on the two pigments. Such cases in which the amounts of a pigment are more or less equal in the two strains, but with a slight increase or decrease in their heterozygote, are included in this type; so also are those cases in which the mutant has a higher content of a pigment than the wild-type, but in the heterozygote of which the amount is intermediate. The three wild-type strains show this type of interaction in combinations, and so do the mutants *st*, *bw*, *pr* and *se* in their heterozygotes with Canton-S, as also the mutants *v*, *bw*, *car*, g^2 , g^3 , *se* and *cl* in their heterozygotes with Graaff-Reinet.

TABLE 4
The dominance ratios of the normal alleles in the three wild-type strains

Strain	Dominance ratio of normal allele in					
	Canton-S		Graaff-Reinet		Stellenbosch	
	Red	Brown	Red	Brown	Red	Brown
<i>st</i>	1.80	0.38	...	...	...	...
<i>v</i>	-0.36	1.31	-0.29	0.78	...	...
<i>v</i> ^{36f}	4.00	0.93	...	...	...	...
<i>cd</i>	-1.51	1.08	-0.39	1.09	-0.40	1.57
<i>bw</i>	0.96	0.28	0.67	0.42	1.61	0.91
<i>pr</i>	0.93	0.74	0.50	5.73	...	...
<i>car</i>	1.10	0.77	0.51	0.53	...	...
g^3	1.25	1.28	0.74	1.00	...	...
g^2	1.17	1.10	0.60	0.85	...	...
<i>se</i>	0.92	0.47	0.47	-0.67	1.82	-0.36
<i>cl</i>	1.33	0.44	0.89	-0.43	...	...
<i>w</i> ^{a3}	0.69	1.32	...	...	...	...

(2) The second type of interaction in the heterozygote may be called superadditive in that the amounts of both pigments are higher than in the two parental strains. In this class would fall *cd* and the garnet alleles in their heterozygotes with Canton-S. (3) In the third type of interaction there appears a quantitatively differential effect in that only one of the two pigments is present in an amount in excess of that of wild-type, while the other shows an additive effect. In this class would fall *v*, *v*^{36f}, *car*, *cl* and *w*^{a3} in their heterozygotes with Canton-S, *cd* and *pr* in their heterozygotes with Graaff-Reinet, and *cd*, *bw* and *se* in their heterozygotes with Stellenbosch.

Another method of approach to the problem of the evaluation of the strength of dominance of eye-colour genes is to use, as a measure of average dominance the ratio: deviation of the heterozygote from the midparental value/half the parental difference. These ratios have been calculated for the normal alleles in the three wild-type strains and are given in table 4. The data of this table may be classified

under three types in regard to the strength of dominance of normal alleles. First, unity indicates full dominance of the normal allele if positive, and of the mutant gene if negative; secondly, any ratio greater than unity indicates overdominance; thirdly, any ratio smaller than unity indicates partial dominance. Some reservations must, however, be made if the significance of differences in the quantitative data of wild-type and heterozygote is taken into consideration, and if it is kept in mind that general heterosis in heterozygotes may increase body size and cause a concomitant increase in eye pigment content. Taking these factors into account, we find that in some genes a dominance ratio somewhat lower than unity may still be accepted as an indication of full dominance, and that in other cases a ratio somewhat greater than unity may result from heterosis in size, so that these genes should also be accepted as showing full dominance.

If the above-mentioned exceptions are granted as valid, we still find a series of mutant genes which exert some influence in the heterozygote. Examples are the following:—the mutants *cd* and *v* for the red pigment, and the mutants *bw*, *pr*, *car*, *se* and *cl* for the brown pigment; in addition, the mutants *v*^{36f} and *w*^{a3} in the Canton-S heterozygote show interaction for the red pigment, while for this pigment all the mutants show an interaction in the Graaff-Reinet heterozygotes. In general, however, it may be noted that, especially in relation to the Canton-S strain, those mutants causing large decreases in brown pigment content show heterozygous interaction for the red pigment, while mutants causing greater deviations in red pigment content show heterozygous interaction for the brown pigment. To this must be added that the first-named class is composed mainly of those mutants which, in addition, either decrease or increase the red pigment content to a slight extent, while in the second class the mutants, in addition, affect the brown pigment content, in three cases increasing the amount above that of the normal.

All these data may provide some evidence on the nature of dominance and the functions of various genes.

(1) Superior heterozygotes for monogenic differences are known in *Drosophila* (Oliver and Green, 1944). In the present investigation, however, some cases of overdominance such as occur in the heterozygotes of the garnet alleles with Canton-S wild-type, are difficult to distinguish from expressions of heterosis in size. It is doubtful whether any instance of a dominance ratio not more than 30 per cent. greater than unity can be regarded as an index of a specific interaction between the normal and mutant alleles.

(2) Different effects were observed for the two series of multiple alleles which were investigated. In the garnet alleles the relative quantities of each of the two pigments in the two homozygotes show ratios equivalent to the dominance ratios in the heterozygotes. In the vermilion alleles, however, the types of heterozygous interaction for the red pigment are of a contrasting nature: in the *v* heterozygote

the mutant allele shows slight dominance, while in the v^{36f} heterozygote overdominance occurs. It may be that this difference is related to the differential functions of the alleles if the hypothesis of Green (1952) is valid, viz., v and v^{36f} are pseudo-alleles, and while v may act during the conversion of tryptophane to an intermediate in the brown chromogen reaction chain, v^{36f} may be active during the conversion of the intermediate product into formylkynurenine.

(3) The fact, which has been mentioned above, that mutants mainly affecting the brown pigment content show heterozygous interaction for the red pigment content, and that mutants mainly affecting the red pigment content show heterozygous interaction for the brown pigment content, may be related to the origin of dominance of the normal alleles of these specific genes. For these genes it may be said that the wild-type alleles show dominance in what may be regarded as the main function of the locus, *i.e.* an action in either the red or the brown pigment chains. Any secondary action of the gene, any effect on the other pigment, may then be the result of interactions between various gene-controlled processes which have arisen subsequently to the fixation of dominance. Relative dominance is a property not of the gene but of the sum of gene-controlled processes. To what extent does this concept apply in the case of the white locus which is assumed to affect both pigment chains to a more or less similar extent? Muller (1935) has demonstrated the incomplete dominance of the normal allele of white, and in the present investigation of the w^{a3} allele of white there appears to be heterozygous interaction for both pigments. From the evidence of the quantitative relations between the two pigments in the series of alleles of white (Nolte, 1958) it seems that the balance between the two substrates is easily disturbed during their differentiation from a common precursor. Presumably during the evolution of dominance of the normal allele of this locus this unstable balance resulted in the fixation of dominance only in a relative way for the two pigments respectively.

(4) Some evidence may be adduced for the concept of relative dominance, or the dependence of dominance of a specific gene on the genotypic milieu. First, cases of conditioned dominance of eye-colour genes have been reported for *D. subobscura* by Crew and Lamy (1932) and for *D. melanogaster* by Schultz (1935); genes at known loci in the latter species, *e.g.* facet and purple, have been shown to be dominance modifiers of vestigial (Blanc, 1946). In such instances the presence of a specific pair of genes lowers the threshold for another pair so that the latter has some expression in the heterozygote. Secondly, the data on the heterozygotes with three different wild-type strains in the present study may be considered. These data in table 4 show some striking differences. In general, the same group of mutants (those affecting mainly the red pigment content) show interaction for the brown pigment in the heterozygotes with the Canton-S, Graaff-Reinet and Stellenbosch strains. However, these same mutants, with the addition

of the garnet alleles which show more or less full dominance of the normal allele for the red pigment in the Canton-S heterozygote, cause a more intermediate quantitative effect in the Graaff-Reinet heterozygotes. Viewed from another angle, the above-named mutants, especially *pr*, *car*, g^3 , g^2 , *se* and *cl*, do not show appreciable differences in relative red pigment content between their heterozygotes with Canton-S and Graaff-Reinet. On the other hand, the brown pigment content in the series of heterozygotes with Graaff-Reinet is greater than in the heterozygotes with Canton-S by amounts more or less equivalent to the difference in brown pigment content between the two wild-type strains. The mutants which affect mainly the brown pigment, for example, *v* and *cd*, show a similarity of effect in the two different heterozygotes. The mutants *se* and *cl* have normal alleles in the Canton-S strain with some degree of dominance for the brown pigment, but in the Graaff-Reinet strain the mutant alleles show a degree of dominance, also in the Stellenbosch strain for *se*.

These facts have to be reconciled with some of the theories on the origin of dominance. According to the theory of Haldane (1931) selection may have resulted in the establishment of more potent wild-type alleles, *i.e.* alleles with a considerable margin of safety. In the case of the above-mentioned series of genes the normal alleles in the Graaff-Reinet are not able to raise the amount of red pigment in the heterozygote any higher than do those of the Canton-S strain, although the Graaff-Reinet strain has appreciably more red pigment than the Canton-S; indeed, though quantitatively inferior, Canton-S strain seems superior in the potency of its normal alleles. For these eye-colour genes there seems to be a maximum in potency which is not affected by raising the quantitative reaction very far above the threshold level. The relative dominances of these normal alleles, for this quantitative character, are dependent on the genotype. The change in the degree of dominance for the brown pigment, of the normal alleles of *se* and *cl* in Canton-S and Graaff-Reinet strains, is another example of this dependence. A third example is the quantitative differences for the brown pigment in the interactions between the group of mutants under consideration in their heterozygotes with the two wild-type strains.

The theory of the evolution of dominance by the selection of modifiers which raise the level of action in the heterozygote nearer to that in the homozygote (Fisher, 1931) could fit the facts discussed above. In this case it would be tempting to speculate on whether a modifier of a specific eye-colour gene could not be one or more of the other essential genes in the system of eye-pigment reactions, *i.e.* the whole complex of reactions is integrated through reciprocal effects of the genes on each other in the role of modifiers. If in the wild-type phenotype many degrees of normality may exist, all appearing alike on account of threshold conditions, many of the wild-type eye-pigment genes could be presumed to exist in multiple allelic forms (isoalleles).

The selection of a specific allele as a modifier of the effect of another gene could then be assumed to raise the potency of the latter in its heterozygous state—this specific allele could, in its turn, be regarded as a more potent allele in its relation to the efficiency of the whole reaction-system. Thus the selection of more potent alleles and of modifiers is interrelated, and the theories of Haldane and Fisher are integrated into a single concept.

It seems, however, as if modifying genes of another type are active in effecting the quantitative pigment differences between the wild-type strains. Since the heterozygotes of crosses between these strains possess intermediate amounts of the eye pigments, these genes may act quantitatively as systems of polygenes; this problem is being investigated in a series of inbreeding experiments.

4. SUMMARY

An investigation on the quantitative relationships of the red and brown pigments in twelve eye-colour mutants of *Drosophila melanogaster* and their heterozygotes with three wild-type strains, viz. Canton-S from the United States and the South African high-pigment Graaff-Reinet and low-pigment Stellenbosch strains, have yielded the following results.

(1) In no case is there an absolute dominance of the normal alleles concurrently in the three wild-type strains. The quantitative interactions which generally appear in the heterozygotes are of three types: an additive effect for both red and brown pigments, a super-additive effect for both pigments, and a combination of the two effects, the first for one pigment and the second for the other pigment.

(2) If the average dominance of a normal allele is expressed as the ratio: deviation of the heterozygote from the midparental value/half the parental difference, more instances of nearly complete dominance become evident, but still a series of mutant genes is left with some expression in the heterozygote, generally only for one pigment in Canton-S heterozygotes but mostly for two pigments in Graaff-Reinet heterozygotes.

(3) Cases of overdominance often are difficult to distinguish from the effects of heterosis for body size in heterozygotes.

(4) Those mutants which affect mainly the red pigment show heterozygous interaction for the brown pigment, and those which affect mainly the brown pigment show heterozygous interaction for the red pigment. It is postulated that the normal alleles of these genes are dominant for the main function of the locus, but may even be recessive for secondary effects, so that dominance is relative.

(5) This relative dominance is the property of the sum of gene-controlled processes, and it is suggested that, in the case of the genes of the eye-pigmentary system, the dominance must be regarded as standing in relationship to the efficiency of the whole interaction-system. The whole complex of reactions may be integrated through

the reciprocal effects of the genes on each other as modifiers, a concept which would unite the theories of Haldane and Fisher on the evolution of dominance, that is, by the selection of more potent wild-type alleles and of modifiers respectively.

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