

EYE PIGMENT RELATIONSHIPS IN THREE SPECIES GROUPS OF DROSOPHILA

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INTRODUCTION

The system of pigment cells in the compound eyes of *Drosophila melanogaster* has been described by Nolte (1950), Gersh (1952) and Clayton (1952). In these cells the pigment granules are laid down and they carry deposits of two pigment components, a red and a brown. These pigments can be extracted differentially (Ephrussi and Herold, 1944; Nolte, 1952); the red pigment is extracted in an acidified ethyl alcohol (AEA) and the brown pigment in acidified methyl alcohol (AMA). Quantitative estimations of the two pigments have been made by means of spectrophotometric analysis of the two extracts, and a comparison has been made between the wild type and a large number of mutant strains (Nolte, 1952-1955).

In a later study (Nolte, 1958b) the degree of dominance of wild type alleles of various mutant eye-color genes was investigated, and quantitative data were included for two South African strains of *D. melanogaster*; these were found to differ greatly from the data for the Canton-S strain which had mainly been used as a basis for the quantitative comparison of mutant strains of this species. This fact led to a consideration of the status of this quantitative characteristic in the various species and strains of *Drosophila* in South Africa. Since it then appeared that these pigment measurements might be useful characters for the analysis of natural populations, and could possibly be of interest in the tracing of phylogenies, the investigation was extended to include foreign species and strains. Since it is possible that the melanogaster and willistoni species groups are directly de-

rived from the obscura group (Sturtevant, 1940), the investigation was extended to include species and mutant strains of these three groups of the subgenus *Sophophora*.

MATERIAL AND METHODS

The melanogaster group consisted of South African strains of *D. melanogaster*, *D. simulans*, *D. séguyi* (Burla, 1954) and *D. opisthomelaina* (Nolte and Stoch, 1950). The latter species has later been described as *D. yakuba* (Burla, 1954). For purposes of comparison two American strains of *D. melanogaster*, i.e., Canton-S and Oregon-R, were included in this study. The designations of the South African strains are given in table 1, together with the place and year of collection. Most of these strains were derived from several females but have been inbred since the time of collection. The geographic distribution of these strains is indicated in figure 1.

The willistoni group consisted of three Brazilian species, *D. willistoni*, *D. nebulosa* and *D. capricorni*, the designations of the strains being given in table 3.

The obscura group consisted of three North American species, *D. pseudoobscura*, *D. persimilis* and *D. miranda*, and some mutant eye-color strains of these species, the designations being given in table 4.

The methods applied in this study were similar to those of previous investigations (Nolte, 1952-1955) and consisted of the histological examination of the eye pigment cells, the differential extraction of the two pigments, their spectrophotometric analysis, and their photometric quantitative estimation.

TABLE 1. *The strains of the four South African species*

Strain	Collection year	Locality
<i>D. melanogaster</i>		
Cape Town—1	1948	Domestic
Stellenbosch—1	1947	Deciduous fruit orchard
Stellenbosch—2	1954	Deciduous fruit orchard
Graaff-Reinet—1	1949	Domestic
Graaff-Reinet—2	1949	Domestic
Cedara—1	1947	Domestic
Cedara—2	1947	Domestic
Witwatersrand—1	1947	Domestic
Witwatersrand—2	1944	Domestic
Witwatersrand—3	1950	Domestic
Nelspruit—1	1947	Subtropical fruit plantation
Nelspruit—2	1947	Subtropical fruit plantation
Nelspruit—3	1950	Subtropical fruit plantation
Inhaca—1	1947	Domestic, on Island
Inhaca—2	1947	Domestic, on Island
Inhaca—3	1953	Domestic, on Island
Inhaca—4	1954	Domestic, on Island
Inhaca—5	1955	Domestic, on Island
Zoutpansberg—1	1947	Citrus plantation
Zoutpansberg—2	1947	Citrus plantation
Zoutpansberg—3	1947	Citrus plantation
Zoutpansberg—4	1947	Citrus plantation
Limpopo—1	1957	Rubbish dump in camp
<i>D. simulans</i>		
Stellenbosch—3	1954	Deciduous fruit orchard
Witwatersrand—4	1944	Domestic
Witwatersrand—5	1950	Domestic
Witwatersrand—6	1955	Domestic
Mkuzi—1	1947	Game Reserve
Zoutpansberg—5	1952	Pine plantation
Zoutpansberg—6	1952	Pine plantation
Zoutpansberg—7	1952	Citrus plantation
Limpopo—2	1957	Rubbish dump in camp
<i>D. opisthomelaina</i>		
Nelspruit—4	1947	Subtropical fruit plantation
Mkuzi—2	1947	Game Reserve
Inhaca—6	1955	Domestic, on Island
Limpopo—3	1957	Rubbish dump in camp
<i>D. ségyi</i>		
Limpopo—4	1957	Rubbish dump in camp
Inhaca—7	1957	Forest, on Island

EYE PHENOTYPE AND HISTOLOGY

The macroscopic eye color phenotype of the four species of the melanogaster group is red in *D. melanogaster*, a slightly brighter red in *D. simulans*, and a still brighter red (nearly scarlet) in *D. opisthomelaina* and *D. ségyi*. In the last three species the various strains collected do not appear to show much variation from the normal phenotypes of the species, but in *D. melanogaster* some strain variation is evident. For example, the Zoutpansberg-3 strain has a slightly darker eye than the Zoutpansberg-4 strain, the Inhaca-1 strains has a more garnet-like eye than the other strains from this locality, and the eyes of the Stellenbosch-1 strain have a more brownish tone than the normal type.

The three species of the willistoni group have an eye color which is brighter than that of the foregoing species. In *D. willistoni* and *D. capricorni* it may be called orange red, while in *D. nebulosa* the color is even brighter.

All the wild type strains of *D. pseudoobscura* and *D. persimilis* are very similar in their eye color phenotype, this being a dull garnet red, while in *D. miranda* the color is somewhat darker. The eye colors of the mutants of *D. pseudoobscura* were as follows. In *v* and *or* the eye is bright red; in *pr* it is purplish brown; in *se* the colour is darker than in the previous mutant; in the combination *v se* the eye is yellow on emergence but darkens through brownish yellow to sepia; in *ca* the color is a dull red; in the combination *or pr* the eye is colorless; in *gl* the eye is a translucent yellow with fine red stipples. The three mutants of *D. persimilis* have the following eye colors. In *or* the color is bright red, but perhaps somewhat duller in tone than in the same mutant of the previous species. The mutant *cd* has a dull reddish garnet eye color which darkens to a duller tone with age. The mutant *ras* was received under the name of sepia, but is not allelic to the sepia of *D. pseudoobscura*; the color is

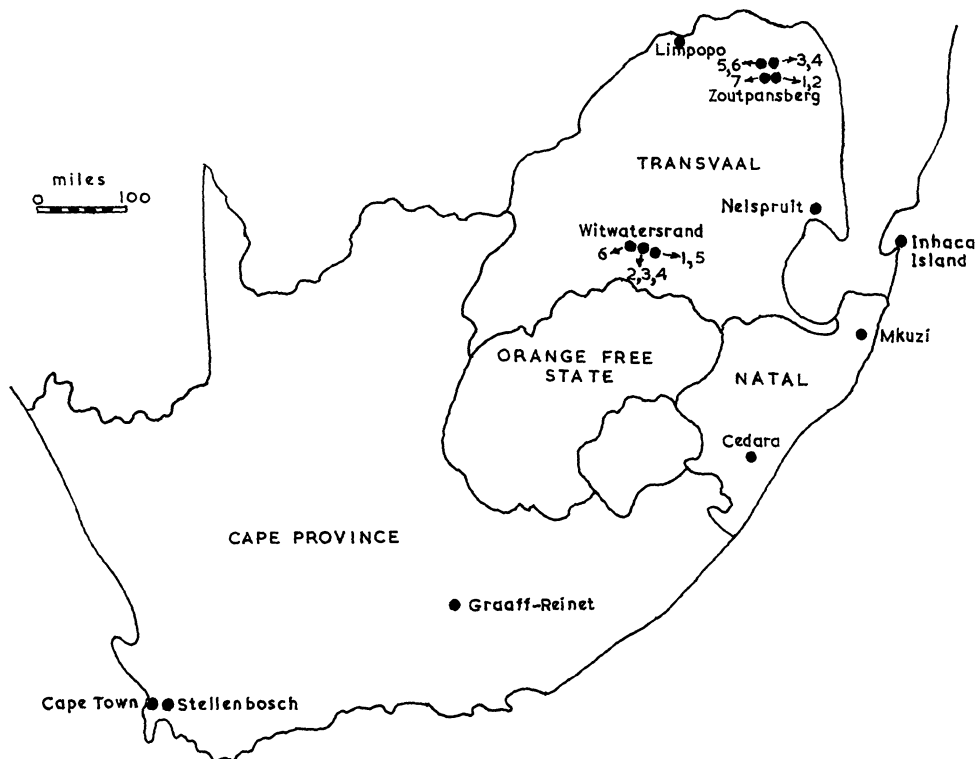


FIG. 1. The geographical distribution of the South African strains of the *Drosophila* species designated in table 1.

a darker sepia than that of the latter species.

In all essentials the histology of the compound eye of the other nine species is identical with that described for *D. melanogaster* and *Zaprionus vittiger* (Nolte, 1950). Exceptions noted were the following: the nuclei in the fenestrated zone of the eye of *D. capricorni* are located next to the layer of monopolars, instead of near the middle of the zone as in the other species, while in the species of the obscura group the post-retinal masses of pigment granules seem less continuous, being more stringlike in a radial direction. The mutant *gl* of *D. pseudoobscura* has a totally aberrant eye histology. The number of facets is reduced, the size of the individual facet is reduced and the shape is often distorted, or the facets are coalesced in pairs so that

large spaces separate them. In these spaces hairs are found growing in clusters, sometimes up to seven in a group. The ommatidia are shorter but thicker than normal, and nuclei often seem to be distributed throughout the width of the secondary pigment cell region; in this region no pigment granules could be seen. Below the basement membrane large empty spaces often appear to separate the outer region of the eye from the innermost nerve tissue. The layer of monopolars as well as the external optic glomerulus are reduced in extent. Only the primary pigment cells seem to be more or less normal in appearance, the basal and post-retinal regions being absent.

In all the species the four pigment regions occur—primary, secondary, basal and post-retinal. For the species of the *melanogaster* and *willistoni* group the

gross color of sections of the eye is very similar to that described for *D. melanogaster* (Nolte, 1950), the primary pigment cells and the outer region of secondary cells having a salmon red color, whereas the remaining pigment regions are a purplish red. Slight differences are: the outer region appears to be more reddish in *D. opisthomelaina* and *D. willistoni* than in the other species; the post-retinal pigment masses in *D. capricorni* are more elongated radially, and separated to a greater extent than in the other species; the basal pigment masses in *D. capricorni* and *D. nebulosa* are smaller than in the other species. Of the species of the obscura group, *D. pseudoobscura* and *D. persimilis* have primary pigment cells with large brown granules, as was found by Cochrane (1936). The aggregate color of the other three regions is more reddish in hue than the purplish red of *D. melanogaster*; the basal pigment masses are smaller, and the pigment granules in the post-retinal region are separated into more discrete groups or masses than in the latter species, that is, they are seen to be contained in cells (cf. Cochrane, 1936). In *D. miranda* the primary cells contain a heavy concentration of brown granules, while the other regions are a darker red than in the other two species.

The mutants *v* and *or* of *D. pseudoobscura* have red granules only in all the pigment regions, excepting in the primary cells in which no pigmented granules are visible; the general impression of red is lighter than that of the wild type. The mutant *ca* is lighter red than the foregoing mutants but also with no pigmented granules in the primary cells; in the other regions the red color is due to granules which are reduced in number but increased in size, many granules having 2-4x the normal size. In the mutant *pr* the primary cells contain a heavy concentration of brown granules, while the aggregate color of the other pigment regions resembles that of the mutant brown

of *D. melanogaster* but is more mauve purplish in hue; the granules in these regions are indistinct in outline as in the case of the latter mutant. The mutant *se* also resembles sepia of the latter species in general histological color (Nolte, 1954b), the primary cells having dark brown granules and the other pigment regions having smaller granules giving a general color impression of purplish wine; the post-retinal region is very narrow and the rhabdomeres are yellow. In the combination *v se* the aggregate color of the pigment regions is yellow, with no color in the primary cells. In the mutant *gl* only the primary cells contain pigmented granules which are brown in color and less in number, but larger in size, than in the wild type.

In *D. persimilis* the mutants *or* and *cd* resemble the bright red mutants of the previous species, the primary cells also being without pigmented granules. The mutant which has been named raspberry resembles the raspberry mutant of *D. melanogaster* (Nolte, 1950) in some respects. The primary cells are heavily pigmented with brown granules, while the remaining color is found in the secondary cells and is a reddish violet. The secondary pigment cells, or the granular masses thereof, seem to be more or less normal at the outer ends, but appear as stringlike masses throughout the main part of their length and then seem to peter out, often not reaching the basement membrane. The rhabdomes are yellow in color and around their bases groups of pigmented granules occur, each group being widely separated from the others. Post-retinal pigment masses are absent.

THE TWO PIGMENTS

The red and brown pigments are present in the eyes of all ten species. The spectrophotometric curves of the two pigments, dissolved in AEA and AMA respectively, agree with those obtained for the wild type of *D. melanogaster* (Nolte, 1954a). In the brown pigment there is

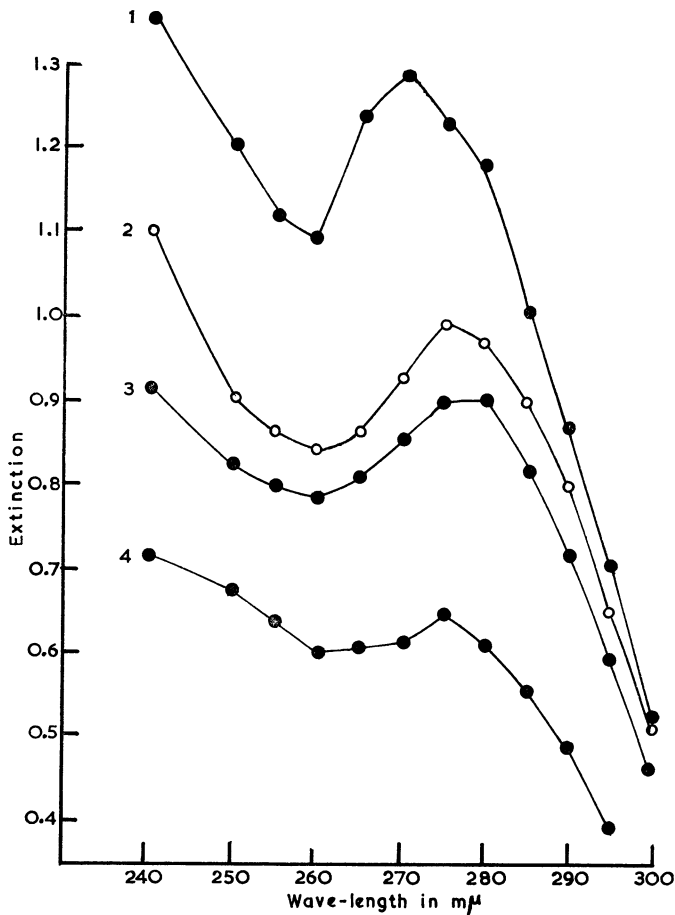


FIG. 2. Ultraviolet absorption curves of the red pigment extracted in AEA, for 10 heads per 1 cc. 1, *D. pseudoobscura*, the mutant sepia. 2, *D. melanogaster* wild type. 3, *D. pseudoobscura*, the Texas strain. 4, *D. pseudoobscura*, the Aspen strain.

a more or less continuous drop in light absorption in the ultra-violet and the visible spectrum, but with a slight peak at 444 $m\mu$. For the red pigment of the species of the melanogaster and willis-toni groups a valley occurs in the absorption at 260 $m\mu$, followed by a sharp rise with a peak at about 280 $m\mu$, then a sharp drop to the visible range, followed again by a sharp rise from about 400 $m\mu$ to form the bell-shaped curve with the peak of absorption at 480 $m\mu$. However, for the various wild type and mutant strains of the three species of the obscura group, some variation occurs in the ultraviolet range. The valley and

peak of absorption in this region seem somewhat flattened, for various strains, due to the low absorption varying between 255 and 260 $m\mu$, and the high absorption varying between 275 and 280 $m\mu$ (fig. 2). In addition, the height between the valley and peak of absorption is less for these species than for the species of the melanogaster and willis-toni groups. The curve for the red pigment of the Aspen strain of *D. pseudoobscura* appears to be atypical in the skewed peak it gives at 275 $m\mu$.

The mutant *se* of *D. pseudoobscura* has a modified red pigment identical with that of the same mutant of *D. melano-*

TABLE 2. *Relative amounts of red and brown eye pigments of various strains of four species of the melanogaster group*

Strain	Red pigment	Brown pigment
<i>D. melanogaster</i>		
Canton—S	0.9162 ± 0.0109	0.0870 ± 0.0013
Oregon—R	0.9188 ± 0.0021	0.1043 ± 0.0036
Cape Town—1	1.0063 ± 0.0097	0.0991 ± 0.0038
Stellenbosch—1	0.5996 ± 0.0106	0.0858 ± 0.0012
Stellenbosch—2	0.9765 ± 0.0083	0.1137 ± 0.0016
Graaff-Reinet—1	0.9667 ± 0.0123	0.0966 ± 0.0017
Graaff-Reinet—2	1.1321 ± 0.0182	0.1227 ± 0.0022
Cedara—1	1.1070 ± 0.0225	0.1113 ± 0.0037
Cedara—2	1.0719 ± 0.0147	0.1268 ± 0.0026
Witwatersrand—1	1.0377 ± 0.0162	0.0950 ± 0.0012
Witwatersrand—2	1.0362 ± 0.0111	0.1050 ± 0.0016
Witwatersrand—3	0.9071 ± 0.0109	0.1098 ± 0.0017
Nelspruit—1	1.0982 ± 0.0073	0.1150 ± 0.0025
Nelspruit—2	1.0749 ± 0.0131	0.1112 ± 0.0018
Nelspruit—3	1.1062 ± 0.0166	0.1327 ± 0.0036
Inhaca—1	0.7107 ± 0.0162	0.1552 ± 0.0028
Inhaca—2	1.1577 ± 0.0128	0.1040 ± 0.0015
Inhaca—3	0.9014 ± 0.0090	0.1016 ± 0.0011
Inhaca—4	1.0656 ± 0.0144	0.1193 ± 0.0027
Inhaca—5	1.0550 ± 0.0173	0.0982 ± 0.0022
Zoutpansberg—1	1.0698 ± 0.0132	0.1026 ± 0.0038
Zoutpansberg—2	1.1034 ± 0.0101	0.1092 ± 0.0024
Zoutpansberg—3	0.9694 ± 0.0114	0.1253 ± 0.0018
Zoutpansberg—4	0.8768 ± 0.0074	0.0996 ± 0.0017
Limpopo—1	1.0637 ± 0.0076	0.1143 ± 0.0036
Mean of South African strains	1.0041 ± 0.0278	0.1110 ± 0.0031
F ₁ Canton-S × Graaff-Reinet—2	0.9444 ± 0.0182	0.1102 ± 0.0070
Canton-S × Stellenbosch—1	0.8382 ± 0.0134	0.0838 ± 0.0023
<i>D. simulans</i>		
Stellenbosch—3	1.1163 ± 0.0460	0.1229 ± 0.0035
Witwatersrand—4	1.0705 ± 0.0096	0.1075 ± 0.0019
Witwatersrand—5	1.0139 ± 0.0095	0.1078 ± 0.0017
Witwatersrand—6	1.0708 ± 0.0315	0.1069 ± 0.0070
Mkuzi—1	1.0374 ± 0.0140	0.0968 ± 0.0031
Zoutpansberg—5	1.0614 ± 0.0207	0.1007 ± 0.0045
Zoutpansberg—6	1.0062 ± 0.0214	0.0938 ± 0.0021
Zoutpansberg—7	1.0531 ± 0.0081	0.1196 ± 0.0022
Limpopo—2	1.1250 ± 0.0176	0.1194 ± 0.0030
mean of South African strains	1.0616 ± 0.0143	0.1084 ± 0.0033
<i>D. opisthomelaina</i>		
Nelspruit—4	1.1128 ± 0.0237	0.1053 ± 0.0020
Mkuzi—2	1.1165 ± 0.0456	0.1174 ± 0.0050
Inhaca—6	1.1042 ± 0.0480	0.1038 ± 0.0028
Limpopo—3	1.1469 ± 0.0225	0.1230 ± 0.0027
Mean of South African strains	1.1201 ± 0.0083	0.1124 ± 0.0047
<i>D. séguyi</i>		
Limpopo—4	1.1352 ± 0.0095	0.1334 ± 0.0021
Inhaca—7	1.2036 ± 0.0076	0.1343 ± 0.0024
Mean of South African strains	1.1694 ± 0.0339	0.1339 ± 0.0005

gaster (Nolte, 1954b). The AEA solution has a greenish yellow color which becomes paler on standing and ultimately nearly colorless. In the ultraviolet the valley of absorption is at 260 m μ and the peak at 270 m μ , while in the visible region absorption drops, with, however, a small peak at 415 m μ . The mutant called raspberry of *D. persimilis* gives a normal absorption curve for the red pigment, that is, unlike the modified curve found for the raspberry mutant of *D. melanogaster* (Nolte, 1958a); however, the peak of absorption in the ultraviolet was much flattened.

PIGMENT QUANTITIES

The quantitative photometric estimations of the red and brown eye pigments in the three species groups are given in tables 2–4, the data being for extinction (E) and its standard error, at 480 m μ for the red pigment and at 444 m μ for the brown pigment, for concentrations of 10 heads per 1 cc. of the solvents AEA and AMA respectively. In each test 40 heads were extracted, and 10–12 experiments were made for each strain.

DISCUSSION

The differences in eye pigmentation between the ten species of *Drosophila* which were studied appear to be mainly of a quantitative nature for the red and brown pigments, differences being noted between groups, species and strains. However, some qualitative differences appear to exist.

(1) The brown pigment appears to be qualitatively identical in all the species.

TABLE 3. *The relative amounts of red and brown pigments in the eyes of the three species of the willistoni group*

Species	Strain	Red pigment	Brown pigment
<i>D. willistoni</i>	Idana	1.1436 ± 0.0152	0.1134 ± 0.0047
<i>D. nebulosa</i>	Belem	1.2988 ± 0.0348	0.1812 ± 0.0058
<i>D. capricorni</i>	Mogi	1.4824 ± 0.0246	0.2215 ± 0.0098

TABLE 4. *The relative amounts of red and brown eye pigments in the three species of the obscura group*

Strain	Red pigment	Brown pigment
<i>D. miranda</i>		
Big Bend	0.8138 ± 0.0226	0.2379 ± 0.0148
<i>D. pseudoobscura</i>		
Texas	0.8557 ± 0.0089	0.1940 ± 0.0050
Arrowhead, Aspen	0.6670 ± 0.0112	0.1877 ± 0.0081
Standard, Piñon		
Flats 340	0.8497 ± 0.0132	0.1766 ± 0.0024
Standard, Piñon		
Flats 345	0.7970 ± 0.0098	0.2023 ± 0.0050
Arrowhead, Piñon		
Flats 122	0.8234 ± 0.0059	0.1871 ± 0.0025
Arrowhead, Piñon		
Flats 126	0.9167 ± 0.0101	0.1843 ± 0.0035
Chiricahua, Piñon		
Flats 334	1.0425 ± 0.0185	0.2067 ± 0.0047
Chiricahua, Piñon		
Flats 352	0.9769 ± 0.0166	0.2039 ± 0.0062
Mean of Piñon		
Flats strains	0.9010 ± 0.0387	0.1935 ± 0.0050
vermillion (<i>v</i>)	0.7910 ± 0.0100	0.0049 ± 0.0004
orange (<i>or</i>)	0.9088 ± 0.0109	0.0053 ± 0.0005
claret (<i>ca</i>)	0.4439 ± 0.0097	0.0363 ± 0.0018
purple (<i>pr</i>)	0.0275	0.1714 ± 0.0030
sepia (<i>se</i>)	0.0549 ± 0.0019	0.2030 ± 0.0055
sepia at 415 m μ	0.2921 ± 0.0057	
vermillion sepia (<i>v se</i>)	0.0404 ± 0.0018	0.0133 ± 0.0008
vermillion sepia at 415 m μ	0.1753 ± 0.0123	
glass (<i>gl</i>)	0.0100	0.0114
<i>D. persimilis</i>		
Standard, Sequoia	0.9004 ± 0.0177	0.1667 ± 0.0028
Arrowhead, Porcupine Flat	0.9625 ± 0.0158	0.1902 ± 0.0035
orange (<i>or</i>)	0.8933 ± 0.0241	0.0053 ± 0.0008
cardinal (<i>cd</i>)	0.8826 ± 0.0130	0.0282 ± 0.0017
raspberry (<i>ras</i>)	0.1253 ± 0.0054	0.1738 ± 0.0053

The red pigment shows certain qualitative differences. Firstly, this pigment in the species of the obscura group appears to be more variable in its light absorption in the ultraviolet than in the other species. In these the valley of absorption is at 260 m μ and the peak at 280 m μ ; for *D. miranda*, *D. pseudoobscura* and *D. persimilis* the lowest point of the valley varies from 255 m μ to 260 m μ , independently in various strains from the variation at the peak of from 275 to 280 m μ . For the pupae of *D. melanogaster* the valley has been found at 255 m μ and the peak at 280 m μ (Nolte, 1954a), so that qualitative changes do seem to occur in

ultraviolet absorption by the red pigment. Secondly, the Aspen strain of *D. pseudoobscura* has been found to show a sharper, more skew, peak in the ultraviolet, i.e., a sharper rise in absorption from 270 m μ to 275 m μ than for the other strains of this species; it should also be noted that for the smaller quantity of red pigment in the eyes of this strain the peak should rather have been flattened.

(2) The quantitative comparison of the ten species must be based on relative sizes of the eye. A rough estimate has been made of the diameter of the eye, and in table 5 these estimates are given on a relative arbitrary scale. Whereas the species of the willistoni group have, totally and relative to body and eye size, larger amounts of both pigments than the species of the melanogaster group, the three species of the obscura group have a smaller amount of red pigment but a larger amount of brown pigment than the species of the other two groups. In table 5 are also given the ratios (R/B) between the amounts of red and brown pigment, computed from the data of tables 2-4. Generally for these ten species, the orderly decrease in the ratio is the result of three different factors. First, for species 1-4 the total amount of red pigment decreases in that order; secondly, for species 5-7 the increase in

TABLE 5. *The relative diameters of the eyes of the ten species, and the ratios between the amounts of red and brown pigments (R/B)*

Species	Relative diameter of eye	R/B
1 <i>D. willistoni</i>	8	10.1
2 <i>D. opisthomelaina</i>	8 $\frac{1}{4}$	9.9
3 <i>D. simulans</i>	8 $\frac{3}{4}$	9.8
4 <i>D. melanogaster</i>	8 $\frac{3}{4}$	9.0
5 <i>D. séguyi</i>	8 $\frac{3}{4}$ +	8.7
6 <i>D. nebulosa</i>	9	7.2
7 <i>D. capricorni</i>	9 $\frac{1}{2}$	6.7
8 <i>D. persimilis</i>	9	5.1-5.4
9 <i>D. pseudoobscura</i>	9	4.0-5.0
10 <i>D. miranda</i>	9 $\frac{1}{2}$	3.4

brown pigment content is relatively greater, in that order, than the increase in red pigment content; thirdly, for species 8-10 there is a decrease in red and an increase in brown pigment content. The two pigments thus vary differentially in their content in these species.

Relative to eye size, the three more tropical species, i.e., the species of the willistoni group, have a higher total eye pigment content than the temperate to subtropical species of the melanogaster group, and these again have a higher total content than the three more temperate species of the obscura group. The three species groups can thus be differentiated on their eye pigment content. During the independent evolution of the melanogaster and willistoni groups from the obscura group (Patterson and Stone, 1952) the red pigment seems to have increased in both groups, but the brown pigment has decreased in the melanogaster group. The red pigment increase may partly be due to the deposition of red pigment also in the primary cells, this pigment being absent in these cells in the obscura group.

(3) Of the four South African species of the melanogaster group, the strains of *D. melanogaster* show a greater quantitative variation in eye pigment than the strains of the other three species. Among these strains a large number show significant deviations from the species mean. Of the 23 strains studied, at least 7 showed deviations in the red pigment content, 5 in the brown pigment content, and 4 in both pigments. Increase, or decrease in content of both pigments might be ascribed to differences in body size. Of the abovementioned strains, however, only the Graaff-Reinet-2 strain is appreciably larger in body, and eye size, than any other strain. The reasons for quantitative variation in eye pigment content in these various strains must, therefore, be sought for in genetic factors conditioning characteristics other than that of size. Although environmental factors, such as

climate and food, in the various habitats of the strains may differ greatly, these could no longer have affected the quantitative data since most of the strains have been inbred under laboratory conditions for many years. The adaptation, genetically, of strains differing in this quantitative character, to different conditions of climate and habitat, has, however, to be considered.

In the first instance, it appears that general climatic factors do not play a part in the origination of differences in pigment content, since no significant trend is shown on comparing strains from the southwestern part of the country, which lies in the temperate winter rainfall region, with strains from the northeastern part, in the subtropical summer rainfall region. Secondly, although the habitats vary from a domestic environment in a temperate deciduous fruit region, to a subtropical fruit region, there is no consistent increase or decrease in one or both of the pigments in the strains from any particular locality. However, in nine out of thirteen strains from the hotter areas the amount of red pigment is greater than the mean for the species, although not significantly so in all cases.

On the other hand, inspection of the data of table 2 shows that variation between strains from one locality is often greater than the variation for the species; for example, the standard error for the red pigment content of the five Inhaca Island strains is ± 0.0787 as compared with the species standard error of ± 0.0278 . In every single case in which two or more strains have been collected from a locality, significant differences occur in one or more of them in regard to one or both of the pigments. The fact that it is generally only one pigment which varies, proves that size differences are not implicated, and that such variation is genetic.

Some characteristics of this variation are the following: (i) The range of variation for both pigments is large; the red

pigment content ranges from 0.5996 to 1.1577, and the brown pigment content from 0.0858 to 0.1552. (ii) Often there is a differential quantitative effect on the two pigments. (iii) In certain cases, e.g., Nelspruit-1 and Nelspruit-2, which are derived from collections very close together, a population in one locality appears to have no quantitative variation. In other populations, e.g., Zoutpansberg-3 and -4, Graaf-Reinet-1 and -2, Inhaca-1 and -2, significant differences occur in both pigments, although each of these pairs of strains is derived from collections taken side-by-side. (iv) Quantitative differences are found in strains from collections taken during different years from a population of one particular locality, e.g., Nelspruit-2 and -3, Inhaca-3, -4 and -5, Witwatersrand-2 and -3. (v) The two American strains show a significant difference in their brown pigment content; in general, the South African strains have a higher content of both pigments than these two strains.

To test the type of genetic difference between strains several F_1 hybrids of crosses between Canton-S and wild type South African strains were tested for eye pigments. The results in table 2 show that in the hybrids of crosses between strains intermediate amounts of both pigments are found.

In a previous study (Nolte, 1958b), in connection with the heterozygous effect of eye-color genes, the question was put as to whether many of the different wild type eye-color genes do not exist in multiple allelic series. Arising from the data of that investigation, it also appeared, however, that modifying genes of a different type are active in affecting the final quantitative results of the eye pigmentary processes. In one wild type strain these genes appeared to be of a dominant nature as regards the red pigment but of a recessive nature as regards the brown pigment, in comparison with a second strain. A survey of the quantitative data of the present investigation

favors the second possibility rather than the first. It appears as if two series of polygenes are segregating in most wild populations of *D. melanogaster*, and they affect the total content of red and brown pigments respectively. The fluctuations found in a particular population from year to year would merely depend on the genotypes of the sample collected.

(4) Strain differences in eye pigment content also occur in both *D. pseudoobscura* and *D. persimilis*, the most striking being in the chromosomal types of the former species. In the Piñon Flats collection of strains from neighbouring areas, the two Standard strains show significant differences in the amounts of both pigments, while in the Arrowhead and Chiricahua pairs of strains the differences lie only in the red pigment. On the other hand the Chiricahua strains have higher amounts of both pigments than the three Arrowhead strains studied. It is postulated that in this species, as in the South African strains of *D. melanogaster*, two series of polygenes, affecting the total content of red and brown pigments respectively, are segregating in wild populations.

It is interesting to speculate on the possible interrelationships of these genes with the gene arrangements of the chromosomal types, Standard, Arrowhead and Chiricahua. That these types have adaptive values has been demonstrated in various ways, the following being some of the findings for the populations of Piñon Flats. Dobzhansky (1943) found the Standard arrangement most frequent in winter and early spring, the Chiricahua type most frequent in early summer, and the Arrowhead type showing the least change in frequency. In population cage work Dobzhansky (1948) found Standard homozygotes, at 25° C, much superior in fitness to Arrowhead homozygotes, and the latter much superior to Chiricahua homozygotes. The relative adaptive values of Standard and Chiricahua homozygotes, and their heterozy-

TABLE 6. *The amounts of the two pigments in the eyes of various mutants, expressed as percentages of the amounts found in wild type strains*

Species	Mutant strain	% Red pigment	% Brown pigment
<i>D. pseudoobscura</i>	<i>v</i>	87	2.5
	<i>or</i>	101	2.8
	<i>ca</i>	49	18
	<i>pr</i>		89
	<i>se</i>	6	105
<i>D. persimilis</i>	<i>or</i>	96	3
	<i>cd</i>	95	16
	<i>ras</i>	13	98
<i>D. melanogaster</i>	<i>v</i>	95	5.2
	<i>cn</i>	96	5
	<i>ca</i>	27	29
	<i>p</i>	40	33
	<i>bw</i>		97
	<i>se</i>	6	142
	<i>cd</i>	108	15
	<i>ras</i> ²	15	103
	<i>ras</i>	25	110

gotes, were found to depend on the microorganisms used in the food of the flies and the larvae (da Cunha, 1951). Spassky (1951) found Chiricahua homozygotes showing higher viability at high temperatures and in dry food, while Arrowhead homozygotes show higher viability at cooler temperatures and in humid media. In the collections tested for eye pigment the Chiricahua type does seem to possess more of the postulated effective polygenes conditioning increased eye pigment than does the Arrowhead type.

(5) Gene homologies between different species of *Drosophila* have been worked out or postulated (Sturtevant and Tan, 1937; Sturtevant and Novitski, 1941; Spencer, 1945; Patterson and Stone, 1952). Due to absence of hybridization and to the fact that the mode of biochemical action of most eye color genes is unknown, it is somewhat difficult to precisely homologize some of these mutant genes. However, to supplement the evidence of known loci for these genes in some particular chromosome, the various

secondary properties of mutant eyes may be compared for two species, as well as the amounts of the two pigments, expressed as percentages of the normal amounts in the wild strains of each species. In table 6 some calculations of the latter type are given for the species *D. pseudoobscura* and *D. persimilis* as compared with *D. melanogaster*, the figures for the latter species being taken from previous investigations (Nolte, 1952, 1954a, 1954b, 1955). The mutants *v* of *D. pseudoobscura* and *D. melanogaster* are homologous, judging by the results of eye transplantation work (Gottschewski and Tan, 1938), by their localization in the X chromosome, and by their percentages of pigments in table 6; in both species this mutant is somewhat smaller in size than the wild type. According to the same criteria the mutants *or* of *D. pseudoobscura* and *D. persimilis* are homologous with *cn* of *D. melanogaster*; the loci of *or* in chromosome 3 and *cn* in chromosome 2R agree well with the presumed chromosome homology. The mutant *pr* of *D. pseudoobscura* certainly seems equivalent to *bw* of *D. melanogaster* when judged on the distances of their loci from *or* and *cn* respectively; the histological picture of pigmentation in the eyes of these two mutants is identical, and so also is the percentage of brown pigment compared with the wild type of the two species. The mutant *se* of these two species is also identical if judged on their loci, the presence of the yellow modification of the red pigment, and the percentage of this pigment as compared with the red pigment of the wild type, and also the fact that the brown pigment content is higher than in the wild type. The mutant *ca* of *D. pseudoobscura* is homologous with *ca* of *D. melanogaster* if judged on the localization of these genes in chromosomes 2 and 3R respectively, and on the decrease in number but increase in size of the pigment granules. This particular strain of mutant of *D. pseudoobscura* could equally well be ho-

mologous with the chromosome 3 mutant p of *D. melanogaster* which also has a reduced number but increased size of granules and which is more nearly equivalent in regard to the percentage of red pigment; this ca differs from both mutants in the latter species in having no pigmented granules visible in the primary pigment cells.

The locus of the mutant of *D. persimilis* received as cd could not be determined in chromosome 2; the percentages of the two pigments, however, correspond to those of the mutant cd of *D. melanogaster*, a mutant located in chromosome 3R. The strain received as se has been renamed raspberry (ras) because of its properties: it is sexlinked, the pigment cells are disarranged, and the percentages of pigments compare well with those of the raspberry mutants of *D. melanogaster*—it is primarily a structural gene and not a pigment gene, as in the latter species (Nolte, 1952). The mutant gl of *D. pseudoobscura* is also a structural gene.

These data on the comparison of eye color mutants of *D. pseudoobscura*, *D. persimilis* and *D. melanogaster* indicate that in *Drosophila* species the eye color genes are homologous, whether conditioning eye structure or the production of pigments, but that the pigmentary process as a whole has become modified in various groups and species during the course of the evolutionary sequences. Any such gene mutating will, however, produce a mutant with very similar effects in different species genotypes.

SUMMARY

A comparative study was made of the compound eye and its pigments in four South African species of the melanogaster group of the Sophophora (*D. melanogaster*, *D. simulans*, *D. opisthomelaina*, *D. séguyi*), three Brazilian species of the willistoni group (*D. willistoni*, *D. nebulosa*, *D. capricorni*), and three North American species of the obscura group

(*D. pseudoobscura*, *D. persimilis*, *D. miranda*). The following were the most important findings.

(1) The histology of the eye is very similar in all ten species, there being four pigment regions—primary, secondary, basal and post-retinal—with granules containing red and brown pigments. The species of the obscura group differ from the other groups in that brown pigment only occurs in the primary pigment cells. During the evolution of the melanogaster and willistoni groups from the obscura group the process of eye pigmentation was apparently modified by the addition of red pigment deposition in the primary cells.

(2) Spectrophotometric analysis of differential extracts of eyes in AEA and AMA show that the eyes of all ten species possess the same two pigments, a red and a brown. The spectrophotometric curve of the red pigment of the species of the obscura group shows slight deviations from that of the other two groups, specifically in the ultraviolet absorption.

(3) Quantitative photometric estimations of the red and brown pigments show that the three species of the willistoni group, relative to eye size, have a higher total pigment content than the four species of the melanogaster group. The three species of the obscura group have, totally and relative to body and eye size, a smaller amount of red pigment but a larger amount of brown pigment than the species of the other two groups. During the evolution of the latter groups the red pigment content has increased, perhaps due to deposition in the primary as well as the other pigment cells, but in the melanogaster group the brown pigment content has decreased.

(4) Of the four South African species, wild type strains of *D. melanogaster* show a greater quantitative variation than wild type strains of the other species. This variation occurs in either the red or the brown or in both pigments. Generally this variation cannot be ascribed to size

differences, nor to differences in the natural habitats of the strains, but must be based on genetic differences. This genetic variation indicates the presence of two series of polygenes independently affecting the amounts of red and brown pigment, and these polygenes seem to be segregating in most of the populations studied.

(5) Strain differences in eye pigment content also occur in wild type strains of *D. pseudoobscura*. Various Standard, Arrowhead and Chiricahua chromosomal type strains show significant differences in the amounts of one or both of the pigments. It is postulated that in this species also two series of polygenes affecting the total amounts of red and brown pigments respectively are segregating in wild populations.

(6) Evidence is used, from the histology of the eye pigment regions and the percentages of red and brown pigments in mutants as compared with the wild type, to demonstrate the homology of various eye color genes in *D. pseudoobscura*, *D. persimilis* and *D. melanogaster*. These genes have apparently exactly similar expressions in the three species, although the eye pigmentary system as a whole has become slightly modified during evolution.

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