

ABSTRACT

The extracted eye pigments of a number of *Drosophila melanogaster* eye colour mutant stocks, of various combinations of such mutants, and of a number of laboratory cage populations, were measured according to a modification of the method of Ephrussi and Herold.

Full-sib studies and selection experiments showed that the brown eye pigment (xanthommatin) is a character of high heritability.

Allelism tests suggested that variation at loci recognised by mutations contributes to the variation in the content of both red and brown eye pigments observed in wild populations.

Studies of three of the intermediates of xanthommatin synthesis, namely tryptophan, kynurenine and 3-hydroxykynurenine at several stages of pupal development showed these to be very sensitive to mutations affecting eye colour. These intermediates are also affected by variation found in wild populations.

The red and brown eye pigments of a number of white locus alleles were studied in some detail in order to examine interactions between the two pathways at the level of the products of this locus.

VARIATION IN THE EYE PIGMENT PATHWAYS OF DROSOPHILA MELANOGASTER

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INTRODUCTION

Since Von Mainx (1938) demonstrated the existence of two types of eye pigment, red and brown, in Drosophila melanogaster, a great deal of research effort has gone into investigations of the properties and biosynthesis of these two pigments. As further details of the two pigment systems will be given where appropriate, it is sufficient to state here that the "red pigment" actually consists of a group of structurally similar pteridines collectively described as drosopterins while the brown pigment, xanthommatin, is an ommochrome derivative of tryptophan. Over sixty loci in melanogaster are known to affect eye pigmentation or the apparent eye colour.

In 1955, D.J. Nolte published a short note in Drosophila Information Service, briefly stating that the red and brown eye pigments of D. melanogaster appear to be under the control of two independently acting sets of polygenes. The evidence for this conclusion came from observations on two "wild type" strains. One strain had a significantly higher mean extractable red pigment content and a significantly lower mean extractable brown pigment than the other. In the F_1 , each of the two pigments tended to the higher parental value, suggesting some dominance and the independence of the genes controlling the two pigments.

On the basis of this report, Chauhan and Robertson (Chauhan, 1966; Chauhan and Robertson, 1966), undertook an investigation into the relationship between body size, eye size and red pigment content in eight laboratory cage populations of Drosophila melanogaster. They also carried out two-way, replicated selection for red pigment content. Barthelmess and Robertson (1970) later analysed the selected lines for the mean contents of a number of fluorescent pteridines thought to be

associated with the biosynthesis of the red pigment.

The results of this work may be summarised as follows: Body size (thorax length) and eye width were positively correlated. Red pigment content showed positive correlations with both eye size and body length. From analysis of the F_1 's of crosses between inbred lines derived by sib mating, an estimated 40% of the variance in red pigment content could be attributed to environmental factors. About 70 - 75% of the total genetic variance in red pigment was independent of eye size.

After eight generations of replicated two-way selection for red pigment content, the high lines diverged from the control by around 16%, and the low lines by approximately 25%. Selection on an index, namely the ratio of red pigment content to eye width, did not improve the response. Realised heritabilities ranging from 0.10 to 0.42 were obtained. Relaxation of selection led to a reduction in mean red pigment content towards the control value in the high lines, but brought about very little change in the low lines.

Analysis of the pteridine contents of the heads of flies drawn from the selected lines showed changes from the mean contents of the base population. One of the low lines was of particular interest, as its pteridine accumulation pattern was strikingly similar to that of the mutant Henna recessive. This mutant has lowered sepiapterin reductase activity, and accumulates sepiapterin (Taira, 1960). Unfortunately, Barthelmess and Robertson did not report any further studies on the enzyme activity in their sepiapterin accumulating line.

This work established the red eye pigment as the first quantitative character in melanogaster where it was possible to carry out any investigation of the underlying biochemistry.

Although there is the prospect of being able to investigate "quantitative" variation in the biochemical interactions leading to the final character "red pigment content", the system is far from ideal for this purpose. The main reason for this is, quite simply, that in spite of the mass of information available on drospterin biochemistry, no one has as yet succeeded in establishing the biosynthetic pathway(s) involved. A number of people (Hadorn and Mitchell, 1951; Ziegler, 1960; Schwink, 1971; Gregg and Smucker, 1965, and others) have extensively studied the fluorescent pteridines which may be separated from *Drosophila* heads using paper chromatography. Changes in the accumulation patterns of these pteridines have been shown for a large number of mutants affecting eye pigment content (see, for example, Gregg and Smucker, 1965). Ziegler (1960) is, however, of the opinion that some of the pteridines found on chromatography of flies' heads represent breakdown products which are not present in vivo. Conversely, some of the pteridines present in vivo may be sufficiently unstable as to be lost by breakdown on chromatography. The uncertainty as to the true pteridine profile in vivo and its relationship to the observed accumulation pattern on extraction makes it very difficult to assign mutants to a sequence in an ordered pathway.

Furthermore, out of more than sixty loci recognised as affecting eye colour in *melanogaster*, only two are known at which mutation can abolish red pigment synthesis. Alleles at one of these, namely white, also abolish brown pigment synthesis, and for reasons given later (see "The White Locus") is thought to be involved in the structure of the pigment granules. The mutant brown remains with an unknown function as being the only mutant which abolishes red pigment synthesis without doing the same for the brown pigment. This contrasts with the

situation for other biochemical pathways, such as that leading to brown pigment synthesis, where mutants which cause the failure to produce an active enzyme at most or all of the enzymatic steps in synthesis lead to a failure to synthesise end product.

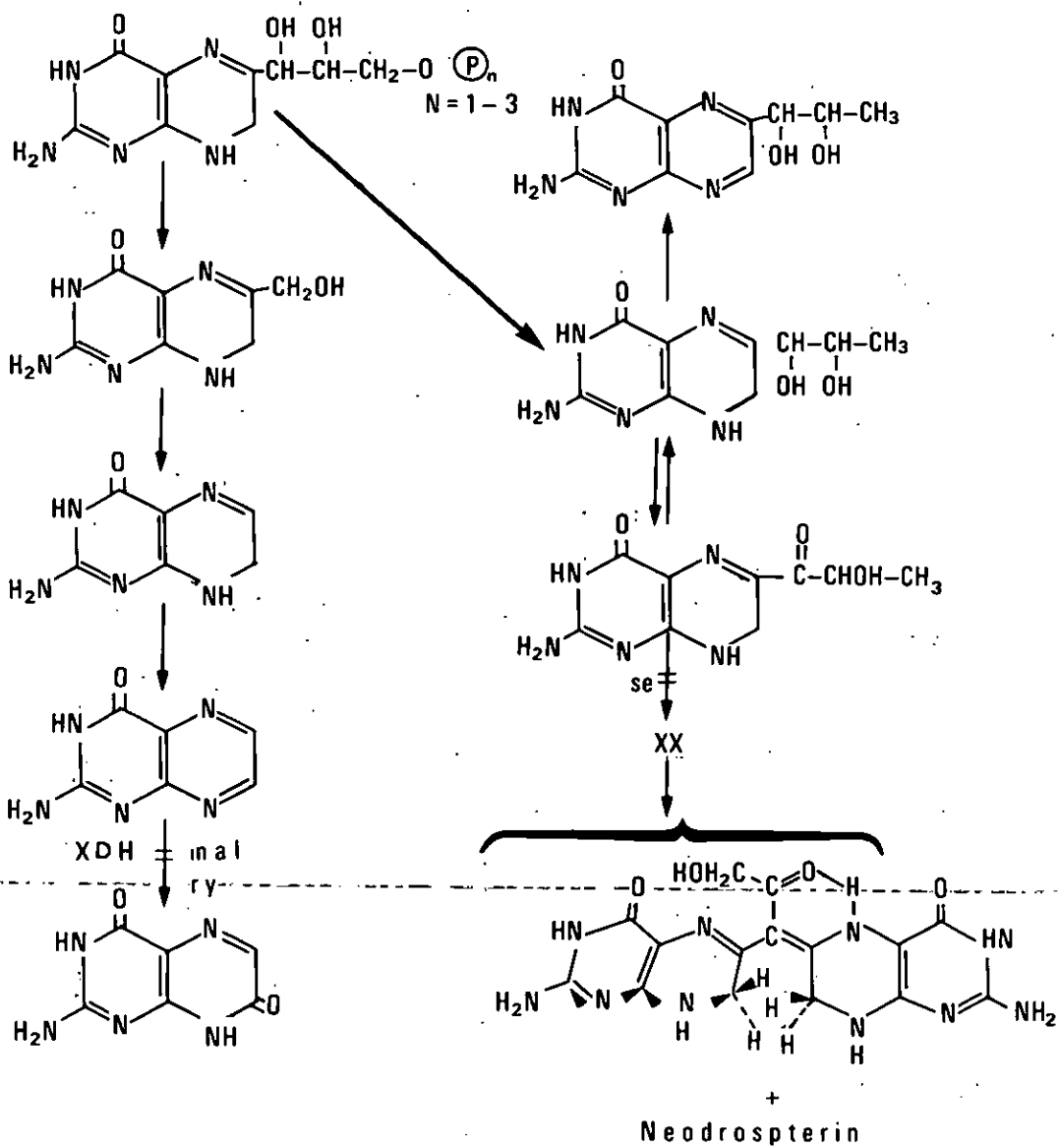
Taken in conjunction with the fact that there is growing evidence that the red pigment extracted from *Drosophila* eyes by the standard technique of Ephrussi and Herold (1944) consists of a group of structurally similar drosopterins (Hodern and Mitchell, 1951) this has led Parisi et al. (1977) to propose that red pigment synthesis proceeds by one or other of two parallel pathways which have only a few early steps in common (see Figure 1).

Certain enzyme-mediated steps which have been implicated in red pigment synthesis are shown in Figure 1. Xanthine dehydrogenase activity is dependent on the expression of a number of loci. Rosy is the structural locus for the enzyme, while maroon-like codes for an essential co-factor (Glassman, 1965). Cinnamon shows a similar maternal effect to ma-1, but has grave effects on viability which suggest that it affects other systems as well as XDH (Baker, 1973). Low xanthine dehydrogenase reduces the XDH activity recovered from flies carrying mutants at this locus (Lindsley and Grell, 1966), without any effect on visible pigmentation of the eye. Ry, ma-1 and cin flies all have similar, dull red-brown eyes, similar pteridine accumulation patterns and reduced amounts of extractable red pigment.

Taira (1960) has shown a correlation between reduced sepiapterin reductase activity and increased sepiapterin accumulation in a series of Henna recessive alleles. These mutants also markedly reduce the red pigment content.

Figure 1.

Model of drosopterin synthesis (red pigment pathway)
proposed by Parisi, Carfagna and D'Amoro (1976).



The association of guanosine triphosphate cyclohydrolase with the red pathway is rather more circumstantial. Evans and Howells (1978) have shown changes in the developmental profile of GTP cyclohydrolase paralleled by changes in the time of appearance of certain (probable) red pigment precursors in two mutants affecting eye pigment content (ras, pn). None of these mutants fulfils any of the criteria for the structural locus of this enzyme.

If Parisi's model of the pathway is correct (and further investigation of this is necessary), then attempts to understand the biochemical variations underlying the quantitative variation in red pigment content may prove disappointing. The complexity of a branched pathway situation would require far more accurate techniques of separation and measurement of the components of red pigment than could be currently applied to, for example, a selection experiment.

The red eye pigment content remains the first quantitative character in *Drosophila* where changes in the underlying biochemistry associated with selection have been demonstrated. Sadly, it would seem that our lack of detailed knowledge of, and the probable complexity of, the pathway(s) leading to the synthesis of red pigment may frustrate further investigations into the biochemical nature of the quantitative variation in this character.

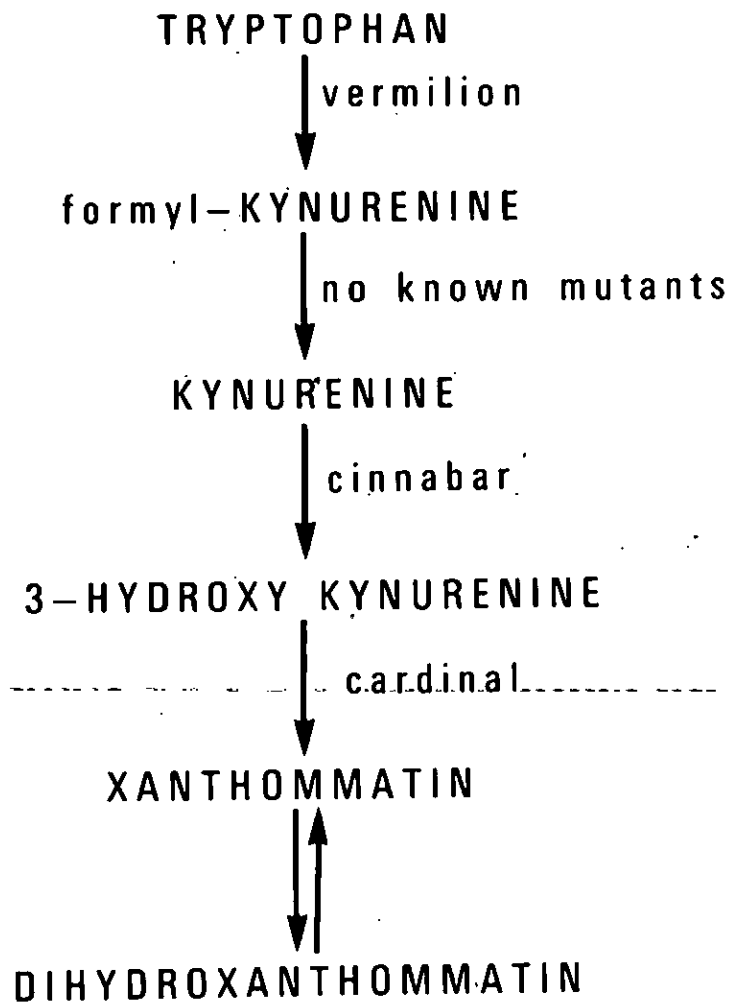
The prospects with regard to analysing the biochemical nature of the quantitative variation in brown pigment are rather more cheerful.

Current understanding of the biosynthesis of the brown pigment is far more advanced. Figure 2 shows the pathway (after Phillips et al., 1976). Beadle and Ephrussi (1936) showed by reciprocal transplants of eye anlagen that the mutants vermillion and cinnabar lacked diffusible

Figure 2.

A simplified version of xanthommatin synthesis
(brown pigment pathway).

THE XANTHOMMATIN PATHWAY IN
D.melanogaster.



substances necessary for brown pigment synthesis, and that vermilion precedes cinnabar in the pathway. The vermilion phenotype can be "cured" by feeding the larvae or injecting the pupae with kynurenine or 3-hydroxy kynurenine (Beadle and Tatum, 1946; Butenandt *et al.*, 1949), while cinnabar is only cured by feeding or injecting 3-hydroxykynurenine. It was later established that vermilion is the structural locus for tryptophan oxygenase (also described as tryptophan pyrrolase or tryptophan peroxidase-oxidase) (Baglioni, 1959, 1960; Kaufman, 1962), while cinnabar appears to be the structural locus for kynurenine 3-hydroxylase (Gosh and Forrest, 1966a).

Although both the mutants lacking brown pigment whose development in transplant is non-autonomous have been shown to abolish enzyme activity, it is not possible to conclude that those mutants whose development is autonomous are not "enzyme" mutants. In these cases it is possible that the product of a reaction in the pathway is not diffusible, or at least cannot be transported into the pigment granules. Such indeed appears to be true in the case of the mutant cardinal. Phillips *et al.* (1974) have shown that this mutant lacks a particle-bound phenoxazinone synthetase activity which is only recovered from the head of the fly. This enzyme appears to be responsible for catalysing the final step in the pathway.

Sullivan and Sullivan (1975) and Howells and Ryall (1975) have shown that the mutant scarlet appears to be defective in the transport of kynurenine and 3-hydroxykynurenine into the pigment granules. The absence of brown pigment in this mutant supports the contention that the final stages in synthesis take place in these granules.

Studies of changes in eye pigment content induced by mutants have shown that, as a general rule, single mutants affecting eye colour alter

the content of both pigments. Forrest (1958) suggested that this points to interactions between the two pathways, and a number of possible interaction mechanisms have been suggested. For example, Parisi *et al.* (1977) suggested that the end product of the brown pathway, xanthommatin, may be a co-factor in some reaction in the red pathway, becoming reduced to dihydroxanthommatin in the process. Forrest (1967) considered that tryptophan oxygenase might be inhibited by some pteridines associated with the red pathway. Gosh and Forrest (1967) raised the possibility that kynurenine 3-hydroxylase might require a pteridine co-factor. In order to evaluate the likelihood of such interactions occurring, it is necessary to consider the timing and the physical location of the reactions in the two pathways. In the red pathway, GTP cyclohydrolase shows a minor peak early in pupation. Isoxanthopterine, pterin and sepiapterin are present in chromatograms of heads from this stage onwards. The later stages of drosopterin synthesis show later onset. A second, major peak of GTP cyclohydrolase activity at, or near, eclosion coincides with the first appearance of drosopterin (Evans and Howells, 1978). Accumulation of drosopterin continues for up to seven days after emergence in the Oregon K strain used in the following experiments (see Materials and Methods).

The three easily detected intermediates of the brown pigment pathway, namely, tryptophan, kynurenine and 3-hydroxy kynurenine are present in larvae and can be detected throughout pupation. Xanthommatin first appears half-way through pupation, and a stable level has been reached in wild type stocks at, or shortly after, eclosion. Thus brown pigment synthesis is more or less complete before the final steps of drosopterin synthesis are detectable.

In a detailed histological study involving biochemical and electron microscope techniques, Shoup (1966) showed the presence of two types of pigment granules in melanogaster eyes. The first type appears to be "budded off" from the Golgi apparatus, as small vesicles which later fill with what is probably xanthommatin. These granules start to form approximately half-way through pupation, and are present both in primary and secondary pigment cells (see Figure 3). Shoup never observed these granules in the eyes of mutants lacking brown pigment (v, w, b, bw). Instead she occasionally found small, irregular granules which were rare in wild type eyes.

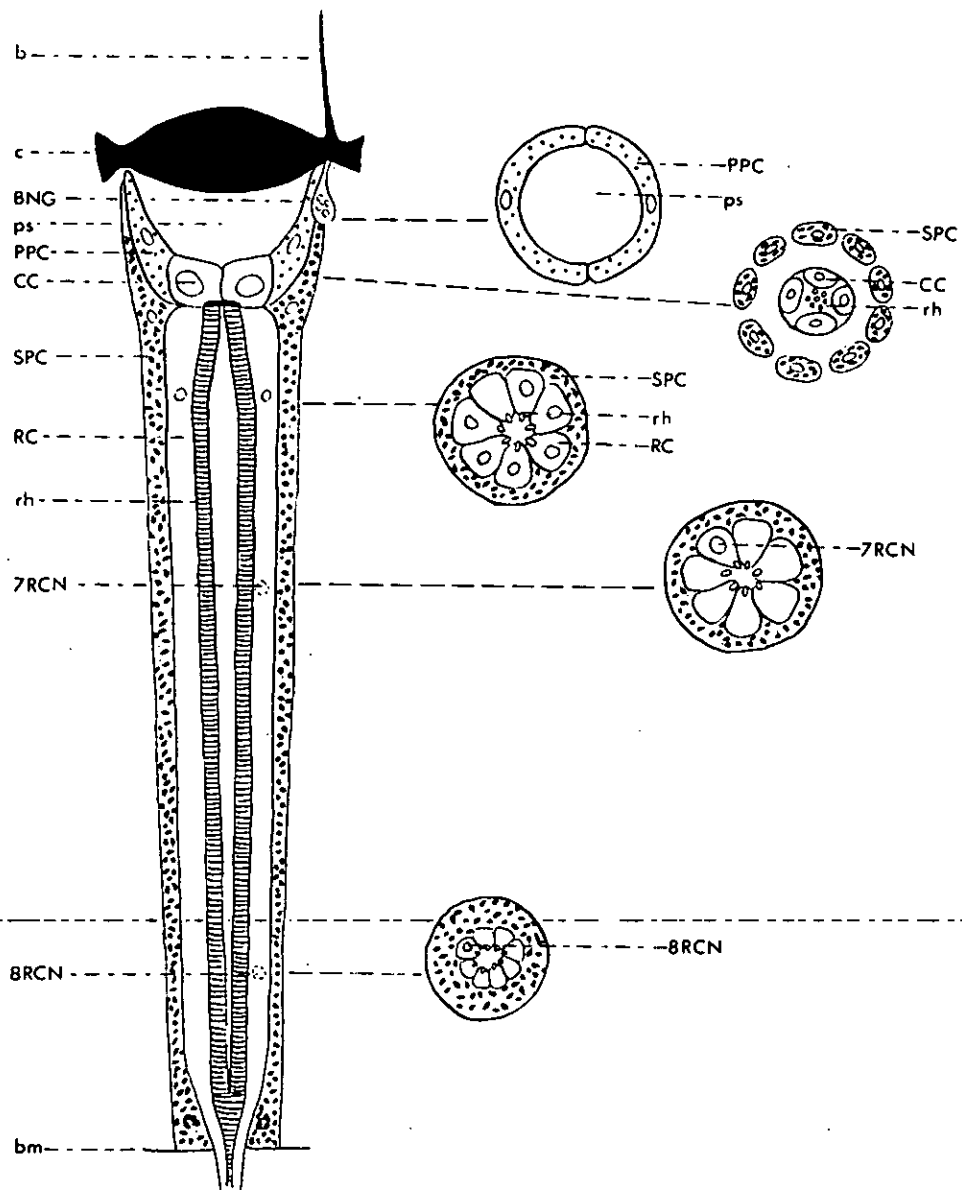
The granules which eventually contain the red pigment originate late in pupation, having the appearance at first of bundles of fibres, which gradually assume a normally granular morphology as the red pigment accumulates. These are restricted to the secondary pigment cells. Such granules were not found in the eyes of mutants lacking red pigment (w, bw).

In classical white mutants and in the epistatic white eyed vermilion; brown, Shoup noted the absence of both types of pigment granules and the presence of autophagic vacuolar structures which she suggested might be the remnants of pigment granules. It seems that the presence of the appropriate pigment is required to maintain granule integrity.

If, as the work of Phillips et al. (1974) suggests, xanthommatin is synthesised in the pigment granule from 3-hydroxykynurenine, and the two pigments are confined to separate granule types, as Shoup states, then it is difficult to envisage how interactions of the sort Parisi hypothesised could occur. Again the presence of 3-hydroxykynurenine

Figure 3.

Schematic diagram of an ommatidium, taken from
Shoup (1966).



①

FIGURE 1 Diagram of the adult ommatidium, after Clayton (15). *b*, bristle; *bm*, basement membrane; *BNG*, bristle nerve group; *c*, cornea; *CC*, cone cell; *ps*, pseudocone; *PPC*, primary pigment cell; *RC*, retinular cell; *7RCN*, *8RCN*, nuclei of seventh and eighth retinular cells; *rh*, rhabdomere; *SPC*, secondary pigment cell.

in larvae argues against the hypothesis of Gosh and Forrest that one of the red pathway pteridines is an essential co-factor for kynurenine 3-hydroxylase. This enzyme is active in the fly before the pteridines are present. However, there is very little experimental evidence on the existence or nature of between-pathway interactions.

Although the functions affected by four mutants in the brown pathway are reasonably well established (v coding for tryptophan oxygenase, cn for kynurenine hydroxylase, cd for phenoxazinone synthetase and st implicated in an active transport system), three further mutants totally lacking brown pigment, and hence having bright red eyes, are known. These are karmoisin, light and lightoid. So far it is not known how these mutants affect brown pigment synthesis. In addition, there are many other loci which affect the content of both pigments. Little or nothing is known about the mechanisms controlled by these fifty or so loci.

As a convenient system for investigating the relationship between the quantitative variation observed in the end-product of a short biochemical pathway and variation in the enzyme activities and pool levels within the pathway, the brown eye pigment content of melanogaster seems promising.

However, apart from Nolte's brief report (1955), there is no evidence that the brown pigment content does behave as a quantitative character. The first part of the project here reported concerns an investigation of the quantitative genetic aspects of the brown eye pigment content. The second part is a survey of a number of eye pigment mutants, examining their effects on the levels of both red and brown pigments in the adult eye and on the intermediate pool levels in the brown pathway.

Finally, the techniques used on the mutants are applied to a number of "wild type" flies which vary in pigment content.

MATERIALS AND METHODS

Stocks

With the exceptions of v', w, st, cn,se and bw which were already present in the laboratory, the mutant strains of Drosophila melanogaster used in this project were mostly obtained from the Mid-America Drosophila Stock Center in Bowling Green, Ohio. These strains, together with their catalogue numbers, are listed in Table 1. An Oregon K strain derived by ten generations of sib-mating from a highly inbred population maintained in Edinburgh was used as the standard "wild type" for the work with the mutants. Analysis of variance between and within families showed this strain to be lacking genetic variation in both red and brown eye pigments. Each eye colour mutant was backcrossed six times to the standard strain. At the end of this period of backcrossing, analyses of variance between and within families were carried out for each strain and revealed no genetic variation in the contents of either eye pigment in any strain. It was therefore assumed that most of the genetic background in each strain derived from the Oregon K strain, and that any variation between strains in eye pigment content might reasonably be ascribed to the mutants carried by the strains.

The cage populations used in heritability estimation had been present in the laboratory for periods ranging from eight months to fifteen years. In each case, the population number had been maintained at a level of between two and four thousand.

The "second chromosome" lines were kindly donated by J.C. McKay. These had been derived using marked inversion stocks from the males of a freshly trapped population of melanogaster supplied by Professor Prevosti. Each line carried Oregon K first and fourth chromosomes,

TABLE 1

Stocks received from Mid-America Drosophila Stock Center

Center No.	Stock	Eye Colour Mutant
b14	bo	bordeaux
b21	car	carnation
b23	cm	carmine
b121	g	garnet
b178	mal and yf: =	maroon-like
b179	mal ^b ₃	maroon-like ^{bronzy}
b200	pn ²	prune
b206	ras ²	raspberry ²
b209	rb	ruby
b300	v ^{36f}	vermilion ^{36f}
b301	v ^{48a}	vermilion ^{48a}
b302	v ^{51a}	vermilion ^{51a}
b303	v ^k	vermilion ^k
g111	cl	clot
g340	lt bw	light
g345	ltd	lightoid
g401	pd	purpleoid
h91	jv Hn ^r h	Henna ^{recessive}
h93	kar ²	karmoisin ²
h116	ma	maroon
h119	mah	mahogany
h128	p	pink
h192	sf ² cd	pafranin ² and cardinal
b327	w ^{bf2}	white ^{buff2}
b328	w ^{bl}	white ^{blood}
b329	w ^{BWX}	white ^{Brownex}
b330	w ^{cf} /Fm 4,y ^{3d} sc ⁸ dm B	white ^{coffee}
b331	w ^{ch} wy	white ^{cherry}
b332	w ^{co}	white ^{coral}
b335	w ^{cp}	white ^{copper}
b336	w ^e	white ^{eosin}
b340	w ^{ec}	white ^{ecru}
b341	w ^h	white ^{honey}
b351	w ^{sat}	white ^{satsuma}

Stocks received from Pasadena

cho	chocolate
a px or	orange
pr	purple
drb	dark red brown
ry	rosy

Stocks received from Oxford

dor	deep orange
red	red Malpighian tubules

marked third chromosomes (TM3 - contains Stubble, Serrate, ebony; Pr - Prickle, ebony) and homozygous second chromosomes derived from one of the Prevost1 males.

Culture Conditions

The flies used in experiments were raised on a standard yeast medium made up according to a recipe supplied by Professor Sang (personal communication). This is referred to as "Sang's yeast medium" and consisted of:

8% yeast (autolysed)
8% maize meal
1½% agar
0.6% "nipagen"
water to 100%.

These ingredients were premixed and then autoclaved for twenty minutes before the medium was dispensed into heat sterilised vials or bottles as required.

Stocks were maintained on a molasses medium routinely prepared in the laboratory. This consisted of:

22g dried yeast
150g maize meal
130g treacle
20g agar
1g "nipagen"
5ml propionic acid
1500ml water

The ingredients were mixed and boiled vigorously for ten minutes before being dispensed.

The flies were raised either in half pint milk bottles or in 3 inch x 1 inch glass vials, stoppered in both cases with cotton wool.

The "standard conditions" referred to in the text comprised Sang's yeast medium as a food source with constant light and a constant temperature of 25°C.

Pigment Extraction

The techniques of pigment extraction used were those of Ephrussi and Herold (1964). They showed that both red and brown pigments are soluble in the solvent used for brown pigment extraction, while only the red dissolved in the solvent used for red pigment extraction. The red pigment was therefore always extracted first.

Red Pigment (Drosopterins)

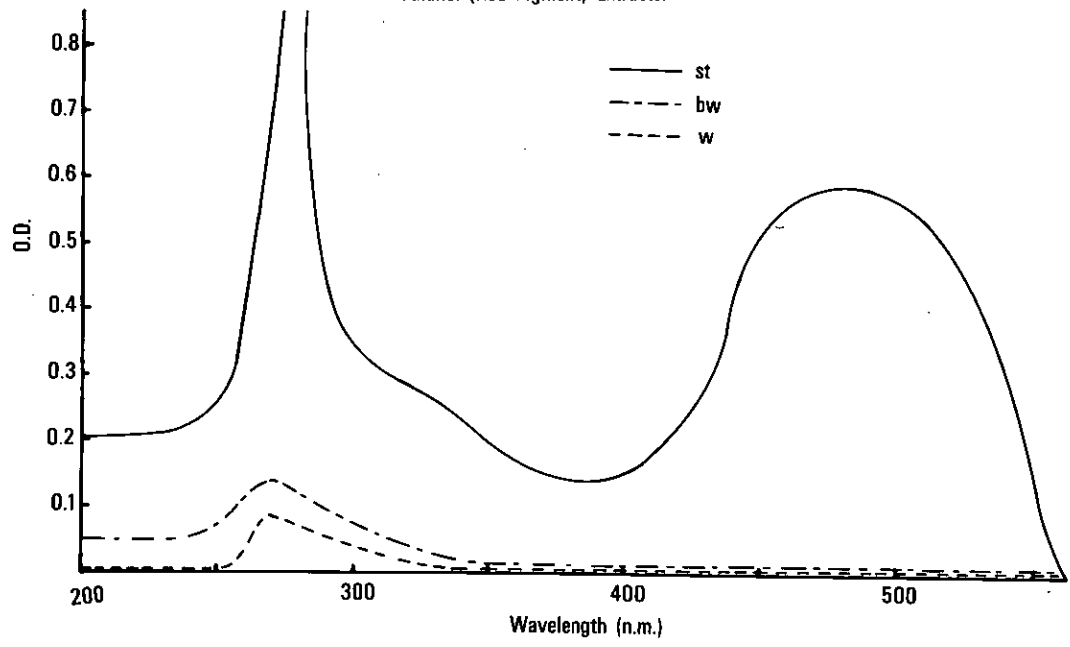
Adult flies were beheaded using a razor blade. The heads were then split longitudinally and individually heads were placed in stoppered plastic tubes containing 0.5 ml of solvent for 24 hours. This solvent consisted of 30% ethanol in distilled water acidified to pH 2 by the addition of concentrated hydrochloric acid. The absorbance of the pigment solution was measured at 470nm in a Beckman DB-GT spectrophotometer using a blank sample of solvent as the reference. Data were recorded as absorbance units $\times 10^{-2}$ per head per 0.5 ml of solution.

470nm was checked and found to be the wavelength of maximum absorbance for the red pigment (see Figure 4). The large peak in the U.V. region is common to extracts of brown and white mutants, which lack drosopterins. The linearity of optical density with pigment content is shown in Figure 5.

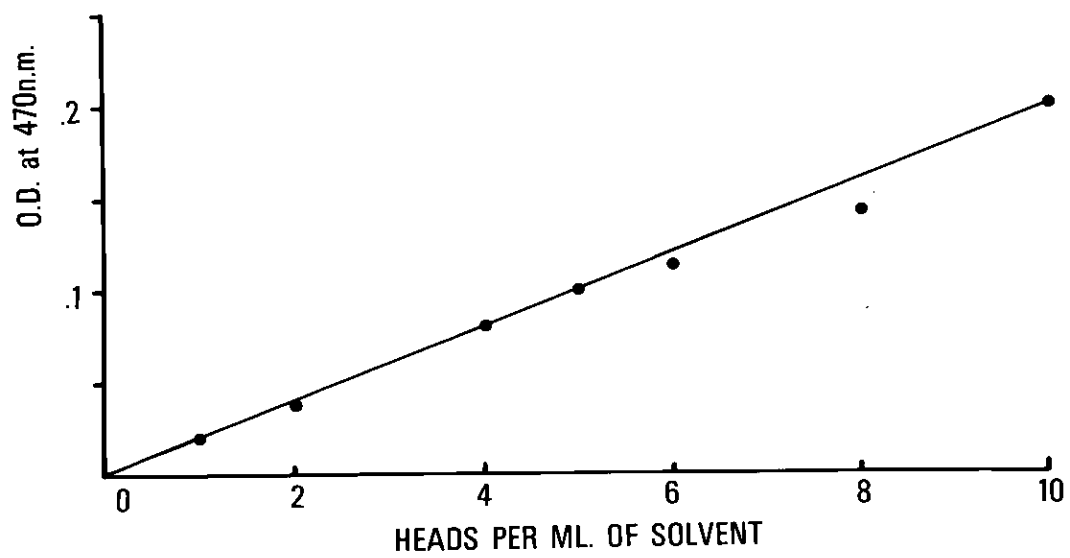
Figure 4.

Absorption spectrum of acidified ethanol extracts of *Drosophila* heads. 10 heads each of the mutants scarlet, brown and white were soaked in 1 ml of solvent for 24 hours to give the three solutions represented here.

Ethanol (Red Pigment) Extracts.



Linearity of Red Pigment O.D. with Concentration.



No further pigment could be detected in fresh solvent in which heads previously treated for 24 hours had been re-soaked for up to three days.

Brown Pigment (Xanthommatin)

After the red pigment solution had been drawn off, the heads were rinsed with a few drops of methanol and allowed to dry. 0.4 ml of a solvent consisting of dry methanol, acidified to 0.27N by the addition of dry, gaseous hydrogen chloride, was added to each head. Again, the heads were left for 24 hours to allow elution of the pigment to take place.

Ephrussi and Herold suggest that the pigment, which acts as a redox indicator, should be oxidised by the addition of hydrogen peroxide. Clancy (1942) preferred to reduce the pigment by the addition of sodium thiosulphate. Under the conditions obtained here in this laboratory, the addition of 0.3ml of distilled water was found to have the same effect as the addition of peroxide, and was used in preference. In order to determine the relative amount of pigment present, the absorbance of the solution was measured in a continuous flow system incorporating a Technicon colorimeter fitted with 440-nm filters. Solvent and water in the proportions of 4:3 (as in the pigment samples) provided a base line. Data were recorded in arbitrary units per head per 0.7 ml final volume. In order to correct for absorption due to "non-pigment" material, several white fly "brown pigment" extracts were measured along with each experiment and the absorbance value for the mean white extracts was subtracted from each experimentally recorded value.

As a further check that no red pigment remaining in the eyes was

extracted and thus augmenting the brown pigment value, a series of vermilion alleles were checked for the absorbance of "brown" pigment extract. The values obtained for these flies did not significantly exceed those of white mutants, therefore it was assumed that separation of the two pigments by the method of Ephrussi and Herold was satisfactory.

440 nm was checked and found to be the wavelength of maximum absorption of the brown pigment (Figure 6). Again, a large peak was found in the U.V. region. This was common to white mutant extracts and those of other xanthommatin-lacking mutants. The peak in the visible region (440 nm) was lacking in these extracts, therefore this was attributed to brown pigment. The linearity of optical density with pigment concentration is shown in Figure 7.

No further pigment could be detected when the heads were re-soaked in fresh solvent for periods of up to three days.

Soaking of untreated heads of scarlet, cinnabar and vermilion flies in acidified methanol resulted in the elution of red pigment, thus confirming Ephrussi and Herold's observations.

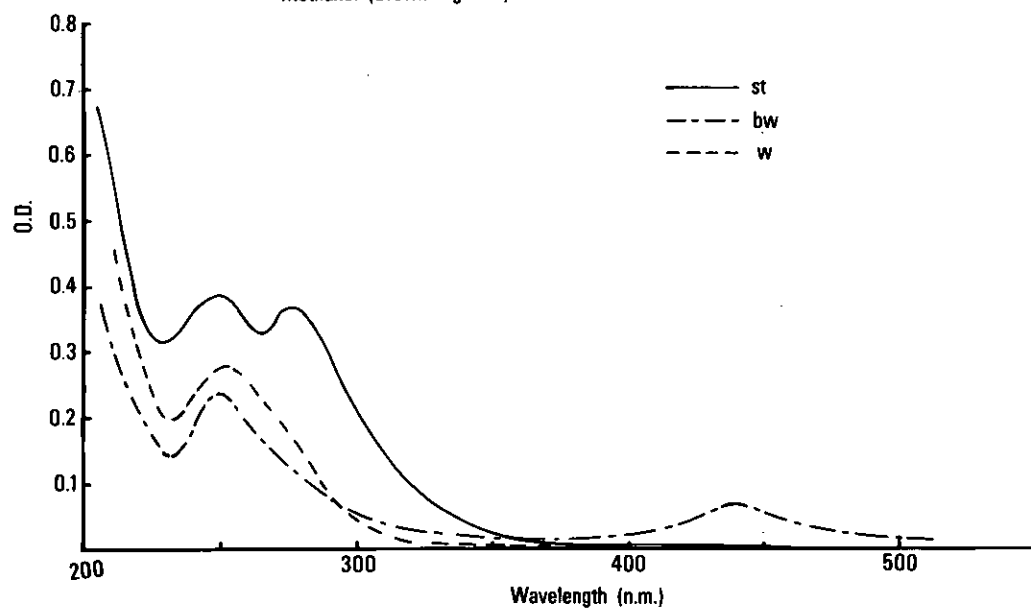
Timing of Pigment Extraction

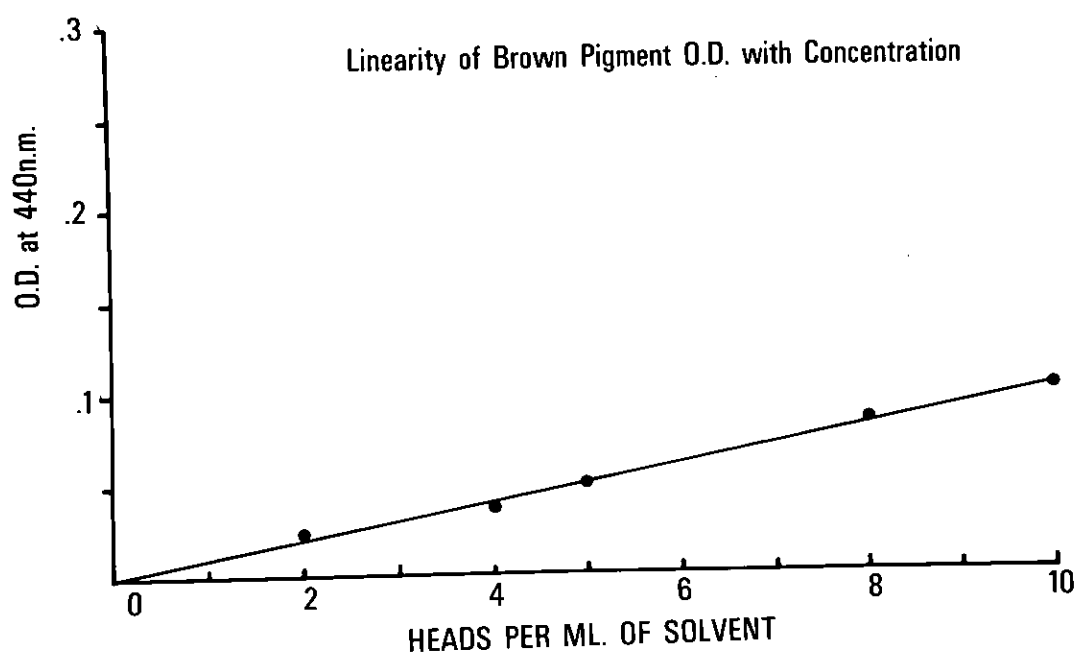
Samples of flies of the Oregon K strain reared under identical conditions were sacrificed for pigment extraction over periods ranging from three hours to ten days after emergence. The changes in pigment content over this period are shown in Figure 8. Brown pigment deposition appears to be complete at eclosion, while the red pigment content continues to increase for several days in the Oregon K stock. Flies were routinely aged for seven days after eclosion in an attempt to reduce the variation in red pigment score arising from sampling at

Figure 6.

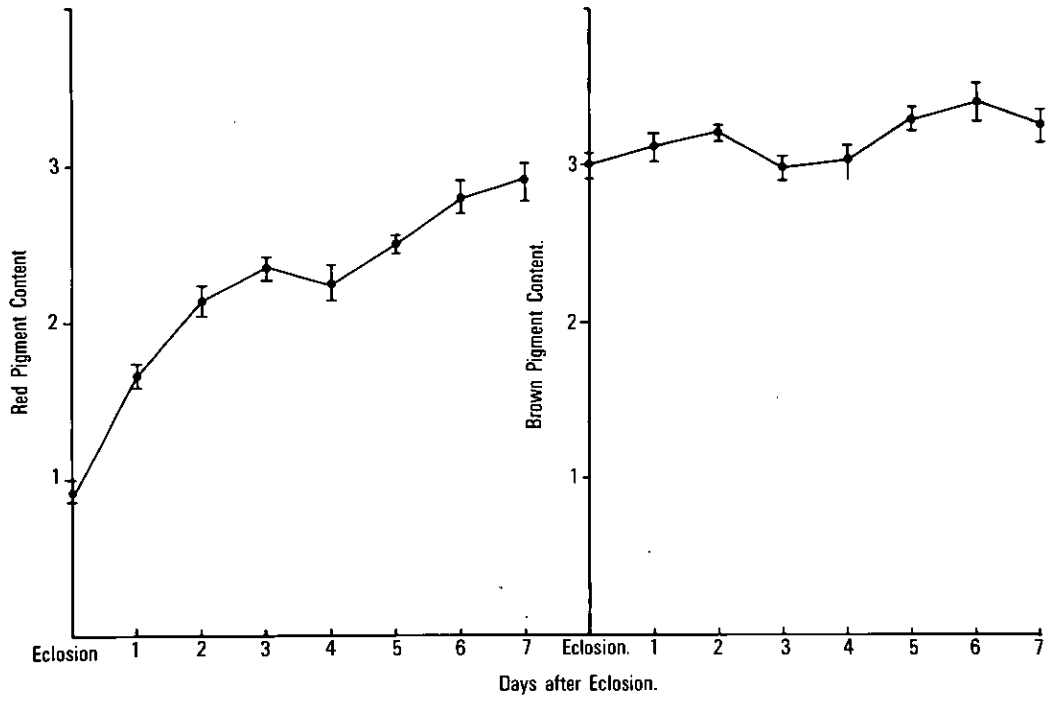
Absorption spectrum of acidified methanol extracts of *Drosophila* heads. 10 heads each of the mutants scarlet, brown and white, which had been presoaked in acidified ethanol were soaked in 1 ml of solvent for 24 hours to give the three solutions represented here.

Methanol (Brown Pigment) Extracts.





Time Course of Pigment Accumulation after Emergence.



different stages in pigment deposition. It should be noted that since it was not feasible to run time courses of pigment deposition for all the strains handled in this project, it is impossible to treat the seven-day point as anything more certain than an informed estimate of a reasonable time for pigment measurement.

Brown Pigment Pathway Intermediates

Tryptophan, kynurenine and 3-hydroxykynurenine contents of pupae were determined as outlined below. It was thought that as the formyl kynurenine pool is reported to be small in Drosophila melanogaster and the assay techniques are difficult to perform in the numbers required, that this pool could be ignored. Further support for this attitude comes from Glassman's failure to detect variation in kynurenine formamidase activity in a survey of a large number of mutant stocks.

Preparation of Pupae for Intermediate Assays

It was found possible to prepare the pupae in the same way for the determination of the contents of all three intermediates: tryptophan, kynurenine and 3-hydroxykynurenine. The procedure was as follows:

Pupae were homogenised in ten times their weight of 10% cold trichloroacetic acid in a Tri R homogeniser fitted with a Teflon pestle. This homogenate was centrifuged at 15,000 RPM for thirty minutes. The supernatant was drawn off and stored in a deep freeze at -20°C until required for assay and the pellet was discarded.

On thawing, the samples were spun in a bench centrifuge for a further ten minutes, and the supernatant was used for intermediate content determination.

Tryptophan determination

A scaled-down version of the method of Horn and Jones (1945) was used. 0.1ml of a 10 times weight by volume homogenate of flies or pupae in 10% trichloroacetic acid was pipetted into a 2ml tube and allowed to come to room temperature. To this was added 0.5ml of a 5% solution of p-dimethylaminobenzaldehyde in concentrated hydrochloric acid (S.G. 1.16). At five minute intervals, 0.1ml of distilled water, 0.5ml of concentrated hydrochloric acid and finally 0.01ml of 0.2% sodium nitrite in distilled water were added. The solution rapidly turns blue. The colour was found to be stable over a period of six hours at room temperature, or overnight at -20°C . Maximum absorbance was found to occur at 540nm (Figure 9). No interference was observed when kynurenine and 3 hydroxykynurenine were present at concentrations an order of magnitude higher than tryptophan. No effect of temperature on either the rate or the final absorption value was obtained, so for convenience, the assay was carried out at room temperature.

Absorbance of solutions of tryptophan was found to be non-linear with concentration. Repeat assays of the same standards using fresh reagents did not always give identical values. A set of standards was therefore measured with each batch of samples analysed, to give a curve such as those shown in Figure 10. Absolute values in terms of micromoles of tryptophan present in the assay mix were obtained from the appropriate standard curve and converted to micromoles per gram of starting tissue (pupae). Every set of experiments included the "standard Oregon K strain. Many repeats of intermediate determination on replicate cultures of this strain had been used to establish a "normal" range for tryptophan, kynurenine and 3-hydroxykynurenine at

Absorption Spectrum of Tryptophan

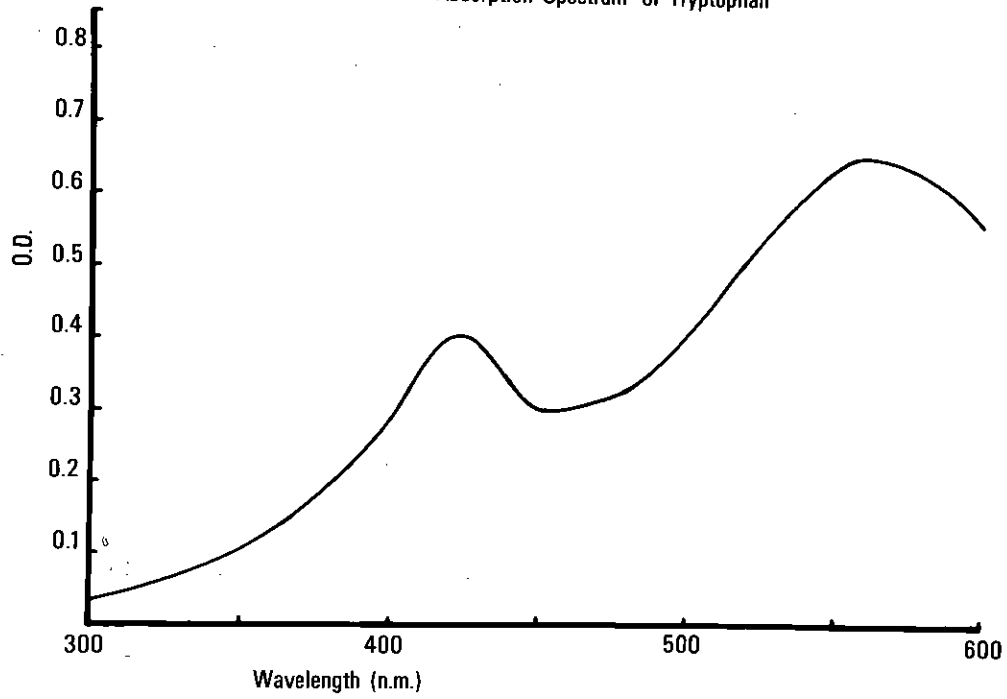
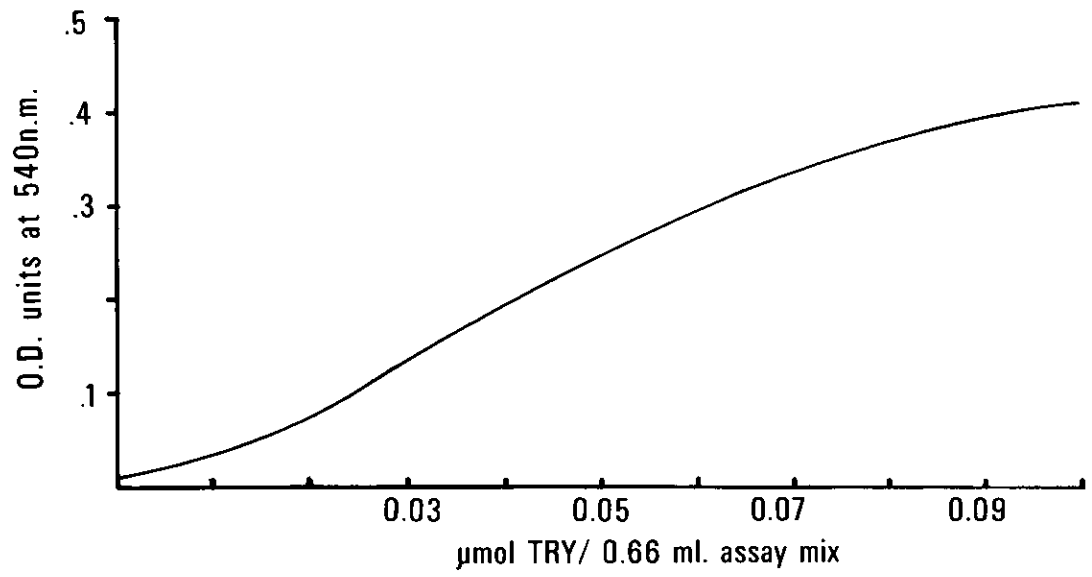


Figure 10.

Concentration curves such as this were determined using known concentrations of tryptophan each time that the tryptophan concentration of pupae was determined experimentally.

TYPICAL TRYPTOPHAN CONCENTRATION CURVE



each of the three time points. Thus if conditions of growth or assay conditions deviated seriously from those laid down, any consequent effect on intermediate values obtained could be detected in the Oregon strain. Fresh assays, and, if necessary, repeats of the cultures of flies, could then be carried out.

Kynurenine Determination

The method used was based on the Bratten-Marshall reaction (Bratten and Marshall, 1939). 0.1ml of pupal homogenate was pipetted into a 2ml plastic tube and placed on ice. When the sample was thoroughly chilled, 0.2ml of chilled 0.3% sodium nitrite was added, the mixture vortexed, and returned to the ice bath. After three minutes, 0.2ml of 12% ammonium sulphamate was added, the mixture vortexed, and left at room temperature for three minutes. Finally, 0.2ml of 0.2% N-1-naphthylethylene-diamine was added, the mixture vortexed, and placed in a water bath at 60°C for ten minutes. The absorption was read at 560nm (checked as the point of maximum absorbance, Figure 11). The absorbance values were non-linear with concentration (see Figure 12), so in all cases the absorbance value was converted to the absolute quantity of kynurenine per gram of pupae by reference to the standard curve determined for each experiment.

3-Hydroxykynurenine Determination

The method was basically that described by Phillips and Forrest (1970). 1.5ml of 1.5% NaNO_2 was added to 0.1ml of pupal homogenate. The sample was vortexed, and absorbance at 400nm read immediately. The value of a blank consisting of 0.1ml of homogenate plus 1.5ml of distilled water was subtracted to allow for any absorption caused by eye

Absorption Spectrum of Kynurenine.

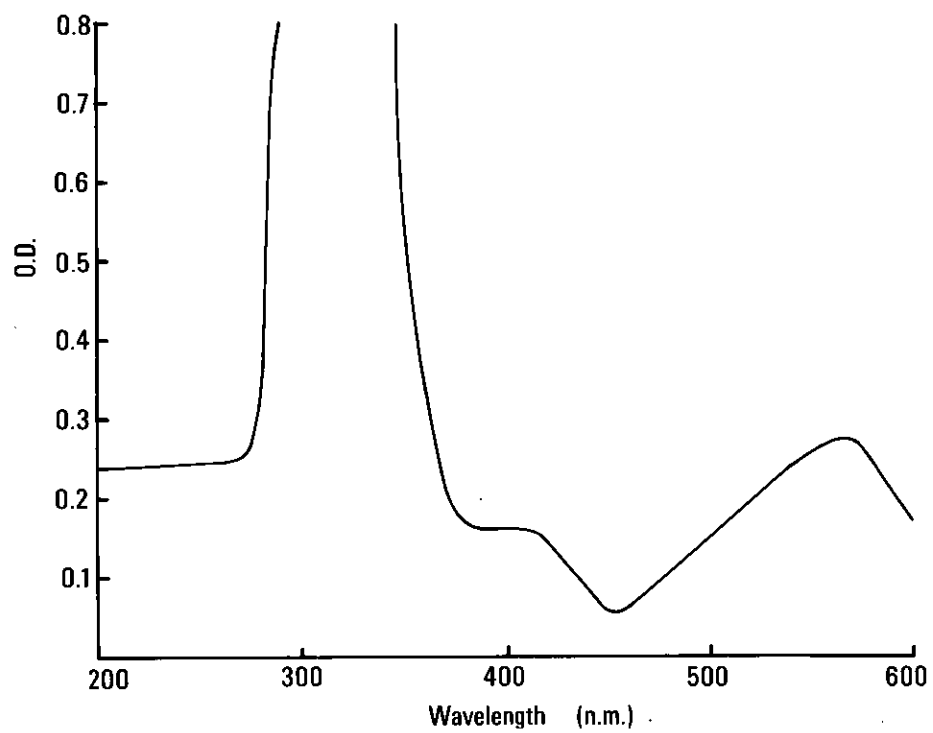
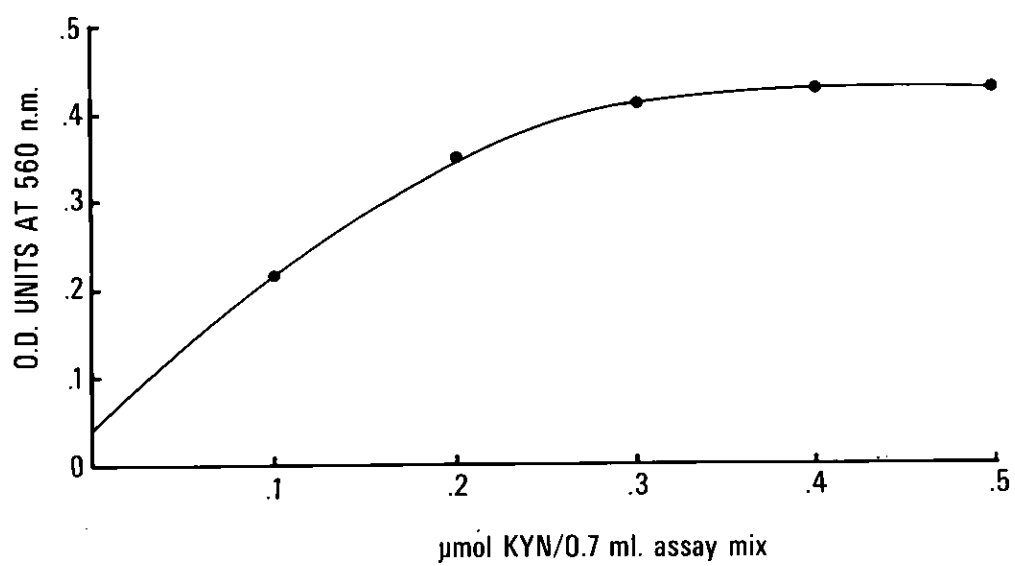


Figure 12.

Concentration curves such as this were determined using known concentrations of kynurenine each time that the kynurenine concentration of pupae was determined experimentally.

TYPICAL KYNURENINE CONCENTRATION CURVE



pigments present in the sample. 400nm was checked and found to be the wavelength of maximum absorbance (Figure 13). Again, the curve of absorbance is non-linear with concentration (Figure 14). All values were converted to absolute quantities of 3-hydroxykynurenine using the standard curve obtained for each experiment.

Time of Measurement of Intermediates

It was originally hoped that it would be possible to use a single time point to compare the levels of the three intermediates present in the various strains of flies under consideration. In order to choose a suitable time, the three intermediates were assayed in the Oregon K line at twelve hour time points throughout development. The results of this study are shown in Figure 15. It is apparent that the levels of all three intermediates change throughout the pupal period. Since it is possible that mutants in eye pigment synthesis may affect the pattern of intermediate pools over time as well as the absolute quantities present at a given time, it was decided to measure the three intermediate pools at each of the last three time points on Figure 15. This period (72 - 96 hours post pupation) covers approximately the second half of xanthommatin synthesis in the pupa.

Absorption Spectrum of 3-Hydroxykynurenine.

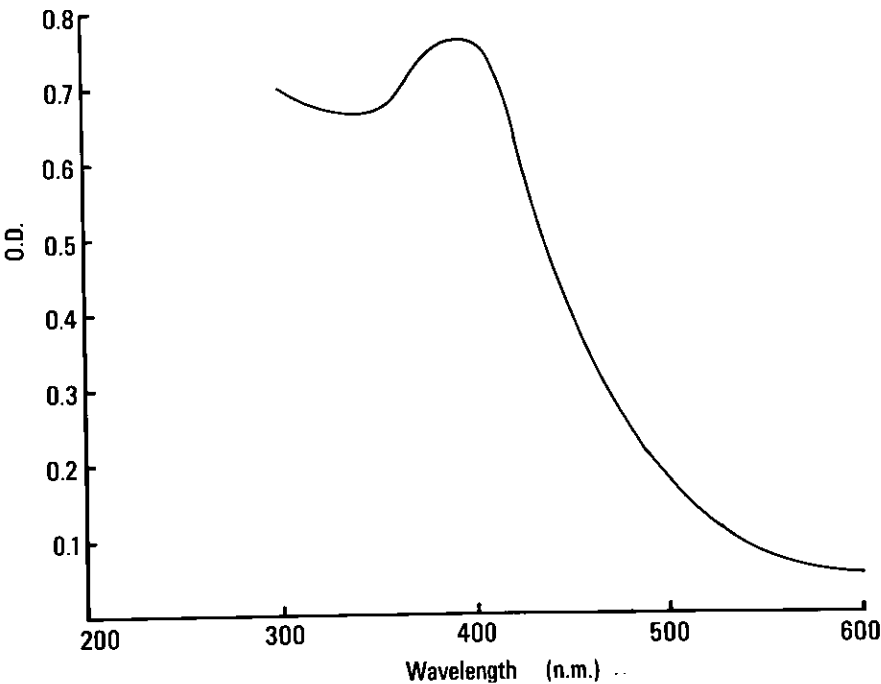


Figure 14.

Concentration curves such as this were determined using known concentrations of 3-hydroxykynurenine each time that the 3-OH kynurenine concentration of pupae was determined experimentally.

TYPICAL 3-HYDROXYKYNURENINE CONCENTRATION CURVE

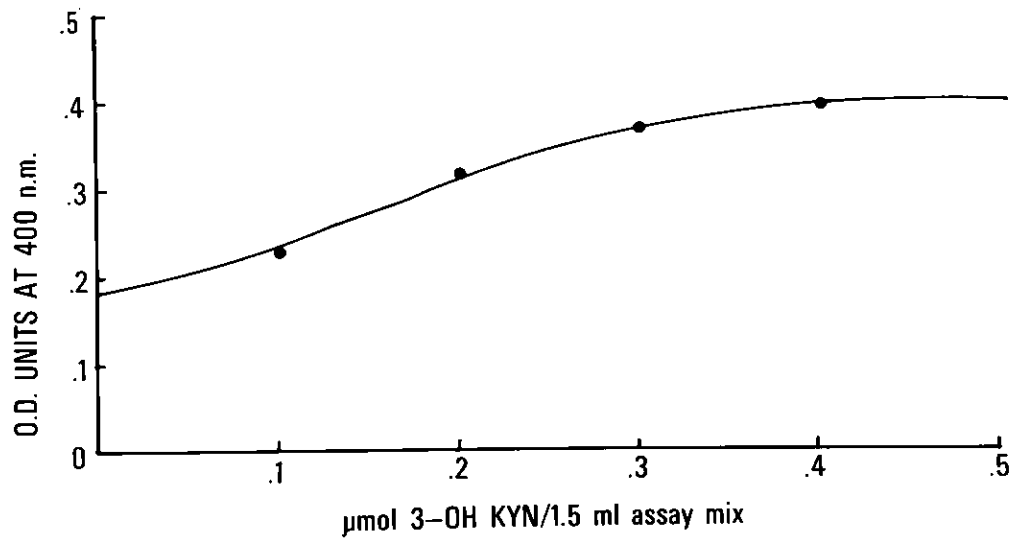
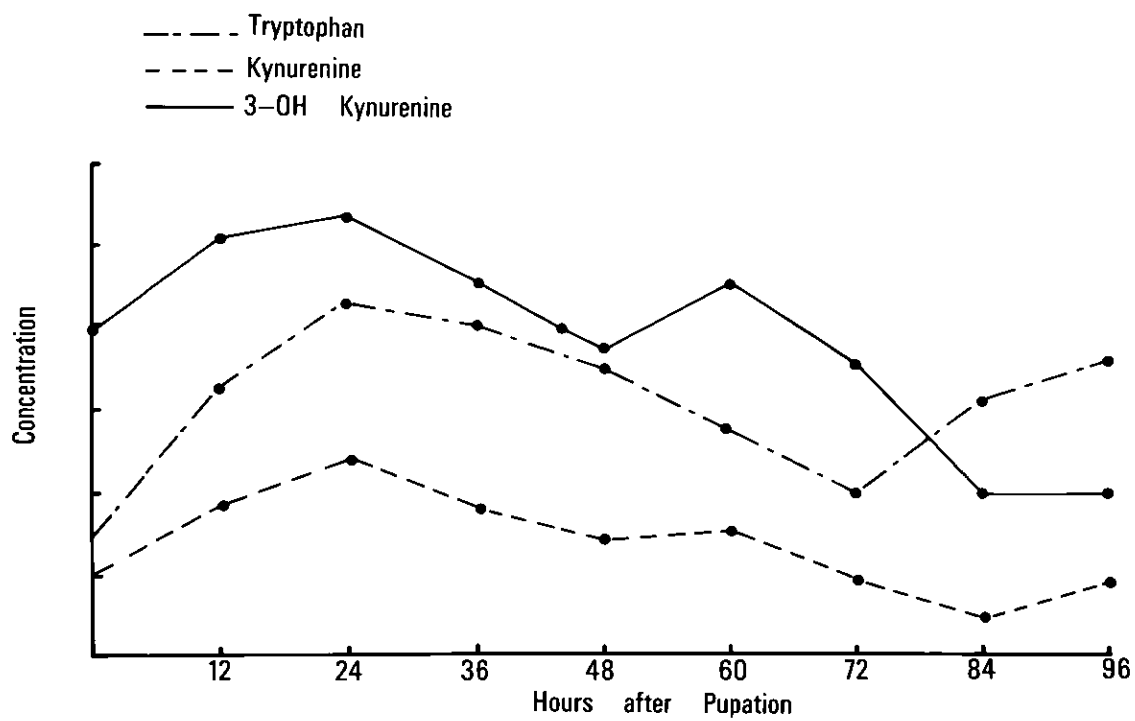


Figure 15.

Time course of intermediate pool levels throughout pupation. Each division on the concentration axis represents 0.05 $\mu\text{mol/gram}$ live weight for tryptophan and kynurenine and 0.2 $\mu\text{mol/gram}$ live weight for 3-hydroxy-kynurenine.



Quantitative Variation of the Brown Eye Pigment

As stated in the Introduction, there is a great deal of information available on the biosynthesis of the brown eye pigment of Drosophila melanogaster. Apart from Nolte's report in 1955 of the apparent polygenic control of xanthommatin synthesis in wild type populations, there is no evidence as to its behaviour as a quantitative genetic character. If brown eye pigment content may be regarded as a "classical" quantitative character, then the system offers the possibility of investigating how metabolism acts to produce the variation observed in "wild type" populations.

The first problem is therefore to establish whether or not the brown eye pigment content does behave as a quantitative genetic character. If this were the case, then it would be possible to investigate, at the level of the pathway, the nature of the genetic variability present in wild populations affecting a reasonably well-established aspect of metabolism.

A. Preliminary Experiments

The first set of experiments designed to investigate the quantitative genetic aspect of the brown eye pigment was not entirely successful. However, the lessons learned at this stage were valuable when further experiments had to be designed. Because of this, some brief discussion of the preliminary experiments is warranted.

The aims were to investigate the relationship between the red and brown pigments and to calculate the heritability from parent-offspring regression for a number of laboratory cage populations.

Design of the Experiments

Large samples (in excess of 200) of flies were drawn from nine population cages and set up in single pair culture in vials containing the molasses medium routinely used in the laboratory. The parents were discarded after three days and the offspring were raised at 25°C under constant light. Virgin flies were collected and aged for four days. Forty random single pair matings were set up for each population. After three days the parents were removed and their eye pigments extracted, measured and recorded, using a Beckman DB-GT spectrophotometer. The offspring were reared under standard conditions. Seven days after eclosion, two males and two females were sacrificed for pigment extraction from each vial.

Results and Discussion

The results of this experiment are summarized in Table 2. From this, it appears that non-zero heritabilities may be obtained for both the red and brown eye pigments. However, the standard errors obtained for the brown eye pigment content, and reflected in the errors attached to the heritability estimates for brown eye pigment content, were disappointingly high. These error terms were probably more a reflection of technical problems than due to real variation in pigment content. This opinion stemmed from the fact that the optical density of the brown pigment from a single fly extracted into the smallest volume of solvent that could be measured in the Beckman DB-GT spectrophotometer was around 1/100th of the full scale deflection of the instrument. Discrimination of the reading to two significant figures was extremely difficult and not

TABLE 2

Mean Red and Brown Eye Pigment Contents and their Heritabilities

POPULATION	RED PIGMENT CONTENT		BROWN PIGMENT CONTENT	
	MEAN	HERITABILITY	MEAN	HERITABILITY
Pacific	91 $\pm$ 29*	0.00 $\pm$ 0.10	17 $\pm$ 7	0.10 $\pm$ 0.14
Kaduna	119 $\pm$ 24	-0.25 $\pm$ 0.20	18 $\pm$ 10	0.37 $\pm$ 0.20
Malawi	94 $\pm$ 19	0.36 $\pm$ 0.10	12 $\pm$ 6	0.11 $\pm$ 0.13
Dahomey	89 $\pm$ 22	0.42 $\pm$ 0.10	13 $\pm$ 8	0.12 $\pm$ 0.17
Canberra	108 $\pm$ 34	-0.11 $\pm$ 0.14	12 $\pm$ 5	-0.45 $\pm$ 0.42
Sexi	99 $\pm$ 35	0.27 $\pm$ 0.04	14 $\pm$ 10	0.06 $\pm$ 0.06
Mancha	80 $\pm$ 24	0.25 $\pm$ 0.01	8 $\pm$ 5	1.01 $\pm$ 0.32
Riudevella	87 $\pm$ 26	0.07 $\pm$ 0.14	11 $\pm$ 6	0.18 $\pm$ 0.21
Barreira	127 $\pm$ 25	-0.01 $\pm$ 0.17	33 $\pm$ 13	0.03 $\pm$ 0.09

* Pigment contents are expressed as O.D. units $\times 10^{-3}$ per head per 0.5ml. of solvent.

very accurate. For this reason, a more sensitive method of measuring the optical density of pigment solutions had to be developed, and the flow system described in the Materials and Methods section was adopted for all subsequent work with the brown eye pigment. In spite of the large errors attached to the heritability estimates for brown pigment, an attempt was made at selection for increased and decreased brown eye pigment content.

Selection for brown eye pigment content

A short-term, unreplicated two way selection experiment for brown eye pigment content was carried out using the Dahomey population. Dahomey was chosen as the base population on the grounds that it shows high levels of genetic variation both for bristle numbers (Briscoe, personal communication) and also in terms of enzyme polymorphism.

Selection Procedure

A hundred pairs of flies drawn from the cage population were set up in single pair cultures in vials containing the molasses medium. After two days the parents were discarded, and the cultures were maintained at 25°C. One male and one female were collected from each vial at eclosion. Males and females were kept separately at 25°C for five days, then random single pair matings were set up using those individuals in vials containing molasses medium. After two days, the parents were removed and sacrificed for extraction and measurement of both eye pigments. Selection was made on the basis of the mean parental brown pigment content. In the first generation, five males and five females were taken from the highest ten families and the same from the lowest ten families to set up high

and low selection lines. Ten pairs of flies were taken at random from among all the vials to provide a control line.

This gave fifty single pair cultures in each of the high and low lines, and ten in the control. In subsequent generations five males and five females were taken from each of the top ten families in the high line and each of the bottom ten families in the low line, giving fifty families within each line every generation, and a selection intensity of 20% . Selection throughout was on the basis of mean parental brown pigment. In each generation flies were allowed to age for five days before the matings were set up and then given a further two days before the pigment contents were assayed.

In the control line, one male and one female were drawn from each of the ten vials. They, too, were allowed to age for five days before being set up in single pair culture. Two days later the parents were removed and their pigment contents assayed to give control values of both brown and red pigments.

Results and Discussion

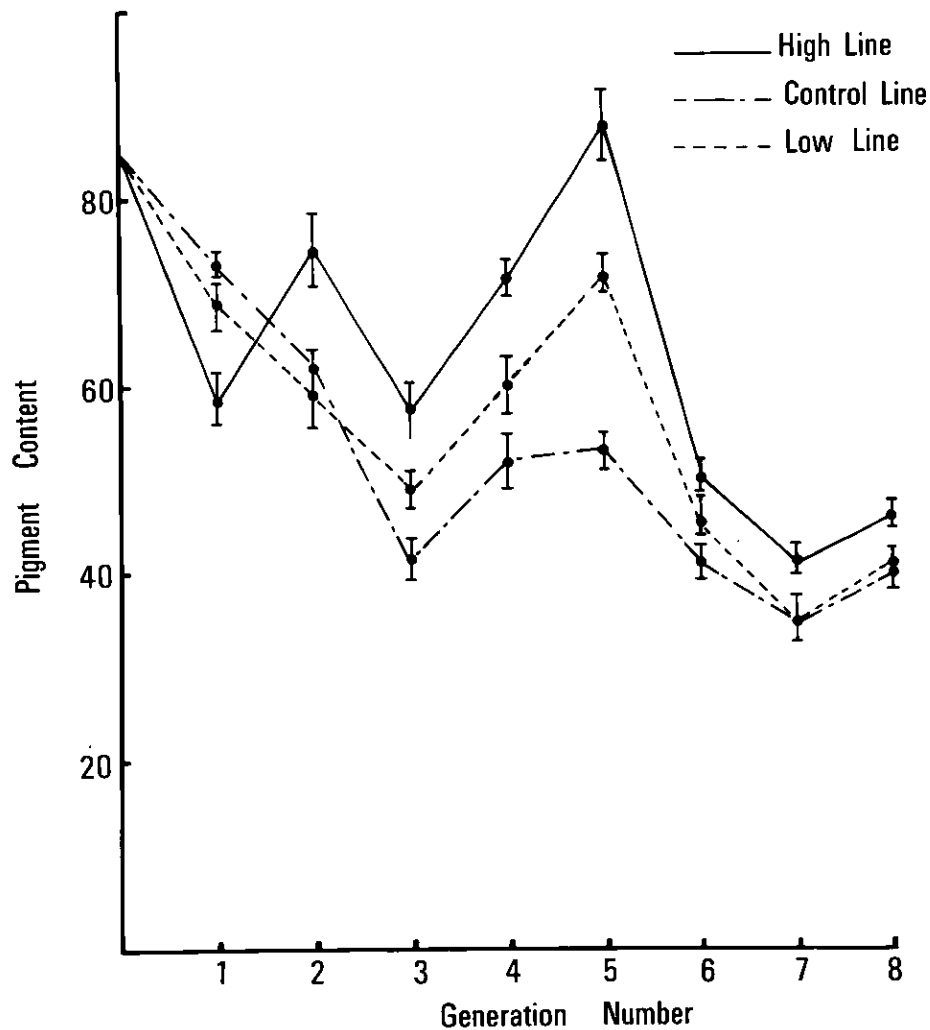
The results of eight generations of selection are shown in Figure 16. Some divergence was obtained between the high line and the control and low lines. Although from generation 3 the low line lies below the control line value, success in downward selection is far less marked than in the upward direction.

Apart from showing that it is possible to select for, and maintain under selection, a divergence between lines of *melanogaster* in terms of brown eye pigment content, the variation between generation means was interesting. The parallel movements of the three lines

Figure 16.

Results of eight generations of selection for brown eye pigment content in the Dahomey population. Pigment content is given in arbitrary units.

DAHOMY LINES SELECTED FOR BROWN PIGMENT CONTENT



from generation three onwards, suggested some sort of environmental influence on the brown eye pigment. Enquiries as to the source of environmental variation ruled out temperature and light as the culprits, as these had both been held constant. However, certain components of the food medium, notably the maize meal and the treacle, showed considerable variation between deliveries. This variation could account for some of the between generation differences in pigment content.

From this experiment it is possible to conclude that brown eye pigment content is a character capable of response to selection, a quantitative character. Moreover, as a great deal of information on the synthesis of brown pigment is available, it is a likely system to investigate how polygenes affect metabolism to produce the variation seen in the end product.

However, it does appear that brown eye pigment content may be susceptible to environmental variation. This could have serious consequences on comparison between, and evaluation of, experiments where observations are not all carried out in the same generation. In order to counter this, a standardised medium recommended by Professor J.H. Sang (and referred to as "Sang's Yeast Medium") was used throughout the remainder of the project. Also, an Oregon K inbred line was checked on this medium every generation to monitor gross changes in pigment content due to environmental changes.

In view of the inaccuracy of the method originally used to measure the brown pigment extracted from the individual flies and the possibility that environmental variation may have influenced the parent-offspring regressions in the first experiment, a re-examination

of the brown eye pigment content in laboratory cage flies was desirable. Further experiments to investigate this question were therefore carried out.

B. The Nature and Extent of Variation in Brown Eye Pigment Content in Laboratory Cage Populations

Making use of the improved system for measuring the brown eye pigment content, an investigation into the relationships between body size, eye size, and the content of each of the two eye pigments was carried out. Heritability estimates for each parameter were also obtained. This approach was adopted because it was necessary to show that, if the brown eye pigment content does behave as a quantitative character this is not merely due to a simple correlation between pigment content and eye or body size. If variation in pigment content were to be simply due to variation in the size of the flies, then there would be little point in trying to examine such variation in terms of changes in the metabolic pathway leading to pigment synthesis.

Eight of the laboratory populations available in Edinburgh (see Table 3) were chosen for investigation into the relationships between the brown eye pigment and four other traits. These traits were:

1. Thorax length (as a measure of body size)
2. Eye width)
) giving an estimate of eye size
3. Eye length)
4. Red eye pigment content.

Two questions were under consideration:

1. Can non-zero heritabilities be obtained for the brown pigment content?

2. Is there any genetic correlation between the brown pigment content and any of the other traits examined?

Design of the Experiment

A hundred males and a hundred females were drawn at random from each of eight population cages and placed in half-pint milk bottles containing Sang's yeast medium, one per population. After two days the flies were discarded and the cultures maintained under standard conditions. Twenty virgin females and twenty males were collected from each bottle. These were mated randomly within each population, single pair matings being set up in vials containing Sang's yeast medium. The flies were allowed two days of egg laying before being transferred to fresh vials. After a further two days, the flies were discarded. All cultures were maintained under standard conditions ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and constant light). Two males and two females were collected from each vial at eclosion, placed in fresh vials and kept under the standard conditions for seven days to allow for red pigment deposition.

At seven days, thorax length and eye width and length were measured using a graticule before the flies were sacrificed for pigment extraction.

To recapitulate the structure of the experiment:

```

      eight populations
          |
      twenty families from each population
          |
      two vials for each family
          |
      two males and two females from each
      vial scored for five traits.
  
```

In all, one hundred and sixty flies were scored for each population.

Results and Discussion

Table 3 shows the means and standard deviations of all five traits for each of the eight populations, treating the sexes separately. Sexual dimorphism is apparent for all five traits, the males being shorter in thorax length and having smaller eyes and less pigment than the females. This agrees well with Andrew's (1970) finding that males have on the average around 70% of the number of ommatidia of females of the same strain.

The data were reanalysed by Dr George Clayton, using a computer statistical package which corrected for the sex differences. This analysis revealed a flaw in the design of the experiment which renders most of the data useless for the purposes of estimating heritabilities or genetic correlations between the five parameters measured. It will be recalled that two males and two females were scored from each of two vials for each family. The reason for representing each family by two vials was, in retrospect, naive. Heritability estimates obtained from the intra-class correlation of full sibs are generally overestimates, as the environmental variance is usually less within full sib families than in the population at large. By spreading the family, as it were, between two vials, it was hoped that the environmental variance within families might be increased. Although it would not be possible to calculate by how much heritability estimates might be improved by this design, it was felt that a better estimate of environmental variance could be obtained by spreading each family over two vials.

TABLE 3

Means and standard deviations of all five traits -

SEXES SEPARATE

POPULATION	SEX	THORAX LENGTH	EYE WIDTH	EYE LENGTH	RED PIGMENT	BROWN PIGMENT
RIVERSIDE	Female	48.75+ 2.32	18.34+ 1.02	24.76+ 1.92	129.63+ 23.23	34.29+ 8.89
	Male	43.38+ 2.67	16.85+ 1.32	21.88+ 1.82	114.94+ 119.10	28.90+ 8.04
PREVOSTI	Female	48.10+ 2.91	17.65+ 1.95	24.52+ 1.64	127.00+ 17.69	36.6+ 7.90
	Male	41.11+ 2.83	16.45+ 1.09	21.77+ 1.49	106.86+ 21.29	32.71+ 8.03
BARREIRA	Female	47.73+ 2.73	17.93+ 1.11	24.74+ 1.76	114.93+ 15.04	33.46+ 7.75
	Male	41.67+ 2.87	16.51+ 2.10	21.85+ 1.52	99.25+ 12.51	30.74+ 7.20
MANCHA	Female	48.59+ 2.38	17.52+ 1.17	23.94+ 1.40	104.23+ 14.82	29.85+ 9.50
	Male	42.69+ 2.52	16.77+ 0.99	21.28+ 1.65	95.56+ 15.72	26.85+ 9.18
RIUDEVELLA	Female	46.84+ 2.01	18.48+ 1.16	24.54+ 1.58	116.20+ 20.35	36.49+ 9.36
	Male	40.09+ 1.74	17.13+ 1.40	21.40+ 1.39	106.77+ 18.38	33.41+ 9.17
CANBERRA	Female	47.44+ 0.39	18.24+ 0.20	23.98+ 0.23	114.25+ 22.90	30.43+ 7.89
	Male	40.89+ 0.35	16.91+ 0.20	21.21+ 0.24	103.19+ 26.30	28.10+ 7.32
KADUNA	Female	46.55+ 2.31	17.89+ 0.93	24.30+ 1.52	120.83+ 17.97	38.16+ 10.52
	Male	40.20+ 1.67	16.97+ 1.06	21.20+ 1.32	108.23+ 19.23	34.73+ 9.94
DAHOMAY	Female	46.84+ 2.01	18.48+ 1.16	24.54+ 1.58	116.20+ 20.35	36.49+ 9.36
	Male	40.09+ 1.74	17.13+ 1.40	21.40+ 1.39	106.77+ 18.38	33.41+ 9.17

Table 4 shows the means and standard deviations of the five characters, for both sexes combined. The values obtained for eye length, eye width, thorax length and red pigment content are within the ranges recorded by other workers (e.g. Chauham, 1966). This suggests that the development of the flies on Sang's yeast medium is normal.

The analysis of variance carried out on the data revealed the existence of a large family x vial component of variance which swamped the between family component for most practical purposes. This can clearly be seen in Table 5, where the data on each of the five characters have been summarised over the eight populations.

Of the five, a reasonable average estimate of heritability within vials was obtained only for the brown eye pigment content. The family x vial interaction component for each of the four other characters was excessive and rendered the heritability estimates meaningless. No attempt was made to investigate source of the vial effect. It is most unlikely that the variation between vials within families is a consequence of variation in the food medium or in culture conditions. Both of these environmental factors were rigidly controlled and no systematic trends in the differences between first and second vials means. Some sort of maternal age effect may explain the difference. Flies taken from the second vial hatched from eggs laid up to four days after those from the first vial. This effect could be tested by following the means of each of the five characters in the offspring of a number of females, taking offspring from different stages in the mother's egg laying life. (cf. Beardmore, Lints & Al-Baldawi, 1975).

TABLE 4

Means and standard deviations of all Five Traits Measured - Sexes Combined

POPULATION	THORAX LENGTH	EYE WIDTH	EYE LENGTH	RED PIGMENT	BROWN PIGMENT
RIVERSIDE	46.04 $\pm$ 3.69	17.64 $\pm$ 1.42	23.37 $\pm$ 2.37	121.99 $\pm$ 22.53	31.81 $\pm$ 9.20
PREVOSTI	44.54 $\pm$ 4.52	17.16 $\pm$ 1.25	23.12 $\pm$ 2.09	119.44 $\pm$ 16.50	34.65 $\pm$ 6.26
BARREIRA	44.66 $\pm$ 4.20	17.31 $\pm$ 1.25	23.29 $\pm$ 2.29	107.03 $\pm$ 14.93	31.96 $\pm$ 5.58
MANCHA	45.53 $\pm$ 3.78	17.14 $\pm$ 1.14	22.59 $\pm$ 1.95	100.18 $\pm$ 12.77	28.21 $\pm$ 7.12
DAHOMY	43.46 $\pm$ 3.87	17.80 $\pm$ 1.45	22.97 $\pm$ 2.16	110.83 $\pm$ 20.48	35.03 $\pm$ 0.32
RIUDEVILLA	44.50 $\pm$ 4.35	17.54 $\pm$ 1.28	21.62 $\pm$ 1.86	99.27 $\pm$ 24.42	37.49 $\pm$ 9.52
CANBERRA	44.16 $\pm$ 4.19	17.58 $\pm$ 1.47	22.60 $\pm$ 2.05	108.72 $\pm$ 18.66	29.49 $\pm$ 6.90
KADUNA	43.39 $\pm$ 3.95	17.42 $\pm$ 1.13	22.79 $\pm$ 2.18	114.49 $\pm$ 19.18	36.57 $\pm$ 7.97

As the analysis of variance on data pooled over all eight populations gave a reasonable estimate of heritability for brown eye pigment content (Table 5), a further analysis was carried out on this character using the data from each population separately (Table 5A). Five of the populations gave very high estimates of heritability (namely, Riverside, Prevosti, Barreira, Kaduna and Mancha), while three, (Riodevella, Dahomey and Canberra) gave very low estimates. The errors attached to these estimates are all high. However, it is apparent that in at least some of the Edinburgh cage populations there is a considerable degree of genetic variation in brown eye pigment content. The very low heritability estimate obtained for the Dahomey population (approximately zero) is consistent with the low divergence after eight generations of two-way selection for eye pigment content in this population.

The two classes into which the populations fall according to their heritabilities for brown eye pigment correspond approximately to the "young" (Laboratory age of less than 4 years), and "old" (Laboratory age of greater than 10 years) cage populations, with the "young" populations showing a high genetic variation for this character which has been lost in the "old" populations. Only Kaduna, which had the lowest heritability of the high group, is anomalous, as it has been in the laboratory for approximately the same time as Dahomey and Canberra.

Because of the family x vial interaction effect on the other four characters, it was impossible to derive any meaningful correlations between any of these and the brown eye pigment content. It does seem unlikely on the basis of this data that much of the genetic variation in brown pigment content can be ascribed to variation in

TABLE 5.

Analyses of variance and heritability estimates for all five traits, data pooled over populations.

Source	E.M.S.			
Family	$V_W + 4V_I + 8V_F$		$V_G = \frac{MS_F - MS_{F \times V}}{8}$	
Family x vial	$V_W + 4V_I$		$\frac{2 \times MS_F - MS_{F \times V}}{8}$	
Within vial	V_W		$h^2 = \frac{MS_F - MS_{F \times V}}{MS_W + \frac{MS_F - MS_{F \times V}}{8}}$	
<u>Thorax length</u>				
	d.f.	M.S.	$\hat{h}^2$	se ($\hat{h}^2$)
family	152	69.79		
family x vial	152	84.84	-0.19	0.096
within vial	952	34.63		
<u>Eye Width</u>				
	d.f.	M.S.	$\hat{h}^2$	se ($\hat{h}^2$)
family	152	14.29		
family x vial	152	16.65	-0.095	0.075
within vial	952	8.53		
<u>Eye Length</u>				
	d.f.	M.S.	$\hat{h}^2$	se ($\hat{h}^2$)
family	152	34.38		
family x vial	152	30.50	0.06	0.084
within vial	952	15.53		
<u>Red Pigment Content</u>				
	d.f.	M.S.	$\hat{h}^2$	se ($\hat{h}^2$)
family	152	5941		
family x vial	152	4801	0.14	0.081
within vial	952	1954		

TABLE 5 (continued)

Brown Pigment Content

	d.f.	M.S.	$\hat{h}^2$	se ($\hat{h}^2$)
family	152	2068		
family x vial	152	845	0.69	0.144
within vial	952	290		

TABLE 5A.

Analyses of variance and heritability estimates for brown eye pigment content, for each population.

	<u>d.f.</u>	<u>M.S.</u>	<u>h²</u>	<u>se (h²)</u>
<u>Riverside</u>				
family	19	348		
family x vial	19	77	0.97	0.16
within vial	118	36		
<u>Prevosti</u>				
family	19	270		
family x vial	19	112	0.95	0.29
within vial	118	22		
<u>Barreira</u>				
family	19	269		
family x vial	19	60	1.06	0.19
within vial	118	23		
<u>Kaduna</u>				
family	19	426		
family x vial	19	127	0.86	0.17
within vial	118	49		
<u>Mancha</u>				
family	19	374		
family x vial	19	100	1.01	0.24
within vial	118	27		
<u>Riudevella</u>				
family	19	148		
family x vial	19	141	0.05	0.11
within vial	118	46		
<u>Dahomey</u>				
family	19	130		
family x vial	19	143	-0.06	0.10
within vial	118	49		

TABLE 5A (continued)

	<u>d.f.</u>	<u>M.S.</u>	<u>h²</u>	<u>se (h²)</u>
<u>Canberra</u>				
family	19	103		
family x vial	19	85	0.11	0.66
within vial	118	38		

in body or eye size. It is likely, therefore, that the genetic variation observed in brown eye pigment represents intrinsic variation in xanthommatin metabolism.

Selection for Brown Eye Pigment Content in the Prevosti Population

Another selection experiment was set up, following the protocol given for the Dahomey experiments, with two differences. First, Sang's yeast medium was used throughout as the food source; second, the base population was drawn at random from the Prevosti population cage instead of from the Dahomey cage.

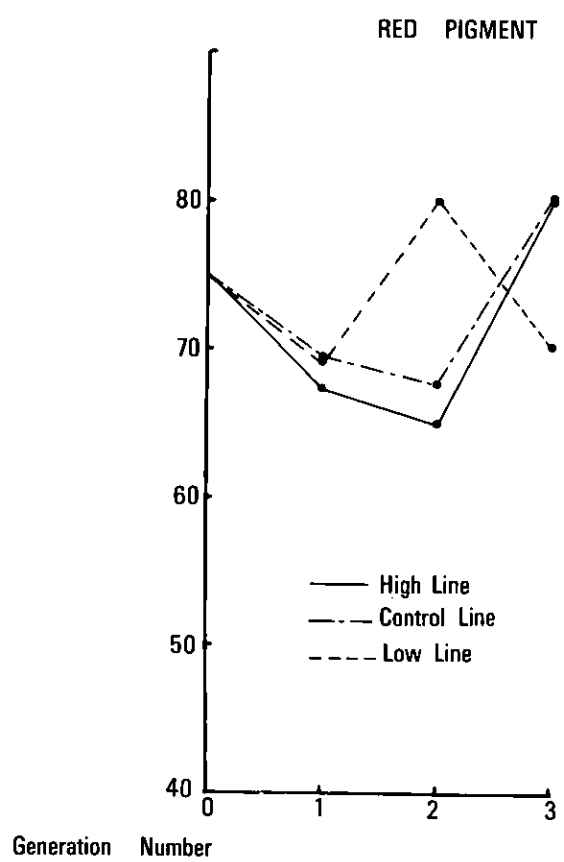
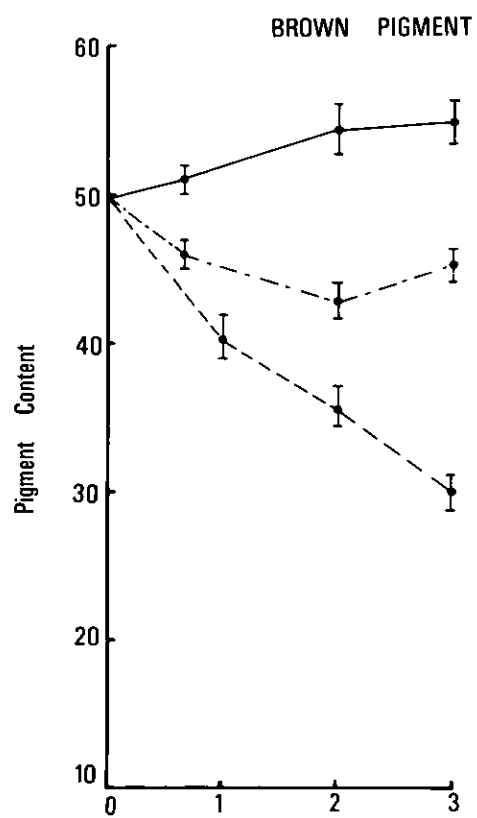
Unfortunately, there was only time to carry out three generations of selection. The results are shown in Figure 17. The divergence obtained between the high and low lines after only three generations suggests that selection for brown eye pigment content can readily generate between line differences. The lack of any correlated response in red pigment is further evidence that the two eye pigments are the products of independent metabolic systems.

Effects of the Second Chromosome on "Wild Type" Eye Pigment Contents

Another approach to studying variation in natural populations is to examine the effects of substituting blocks of genetic material from wild type individuals in a standard background. Drosophila melanogaster is particularly suited to this approach, since by manipulation of appropriate stocks it is fairly easy to obtain strains of flies differing only in a single chromosome or defined chromosome segment. J.C. McKay kindly donated samples of a number of strains which differed only in their second chromosomes. These strains had

Figure 17.

Results of three generations of selection for brown eye pigment content in the Prevosti population. The corresponding mean red pigment contents are also shown.



the general composition:

$$\frac{\text{Ork}}{\text{Ork}} ; \quad \frac{\text{Prev}^*}{\text{Prev}^*} ; \quad \frac{\text{Pr}}{\text{Tm}^3} ; \quad \frac{\text{Ork}}{\text{Ork}} .$$

That is, first and fourth chromosomes had been derived from an inbred Oregon K strain, while the third chromosomes carried different in-versions. The second chromosomes were homozygous derivatives of single second chromosomes isolated from males of a freshly trapped population provided by Professor Prevosti (the females from this sample were used to establish the Prevosti cage population which has previously been mentioned).

Of the 78 lines obtained from this sample, one (designated P55) had a "mutant" eye colour phenotype. This line had bright red eyes reminiscent of the "bright-eyed" mutants which lack brown pigment (see later). The eye colour of all other lines appeared wild type. The bright red eye colour phenotype segregated in a 3:1 ratio among the progeny of the F_1 of a cross between P55 and Oregon K and 1:1 in a test-cross of the F_1 to P55. In other words, the phenotype agrees with the action of a Mendelian recessive mutation. Crosses between P55 and lightoid, light or cinnabar (the three bright-eyed mutants on the second chromosome) yielded only wild type progeny. It, therefore, seems unlikely that the phenotype of P55 is due to a mutation at any of these loci.

As there are at least fourteen loci on the second chromosome at which mutation can cause changes in eye colour, and since only one visible mutant was found in a sample of 78 chromosomes, the frequency of visible recessive mutants of the 2nd chromosome affecting

eye colour in the Prevosti population appears to be low.

The mean red and brown eye pigment contents of a number of second chromosomes were determined and compared with the pigment contents of a sample of the cage population.

Experimental Details

Four single pair matings of each of a number of the Prevosti second chromosomes and of a "standard" stock were set up in vials containing Sang's yeast medium. The standard stock was of similar constitution to the second chromosome lines, but carried second chromosomes derived from the Oregon K strain. After two days the parents were discarded. The cultures were maintained at 25°C. At eclosion, females were placed in fresh vials and aged for seven days at 25°C. Eye pigments were extracted and measured from five females from each vial.

In order to assess the variation in eye pigments in the cage population, a sample of fifty inseminated flies was taken from the cage. These were placed separately in vials containing Sang's yeast medium. Two males and two females were collected from each vial, and a hundred randomly-mated pairs were set up on Sang's yeast medium in vials. After two days, the parents were discarded and the cultures maintained at 25°C. One female from each vial was sacrificed for pigment determination seven days after eclosion.

Results and Discussion

The distribution of the mean pigment contents of the second chromosome lines and of the "cage" females is compared in Figure 17A. The range and distribution of eye pigments in both groups are essentially similar. This indicates that the second chromosome lines represent a reasonable sample of the population. The pigment contents and their standard errors of the second chromosome lines are presented in Table 6. It is possible to estimate the proportion of the phenotypic variance observed in these lines which is due to genetic effects of the second chromosome. The variance within lines gives an estimate of the environmental variance, while the variance between lines gives an estimate of the environmental variance plus ten times the genetic variance (each line being represented by ten individuals). Table 6A, shows the analysis of variance for both red¹ and brown eye pigment contents.

0.34 of the variation between lines in red eye pigment content may be ascribed to genetic variation in the second chromosome, whereas 0.71 of the variation between lines in brown eye pigment content may be ascribed to this source. The red eye pigment may be more susceptible to environmental variation than the brown eye pigment. Alternatively, there may be less variation in loci affecting red pigment content among the second chromosomes sampled than in loci affecting brown eye pigment content.

As there is almost a four-fold difference between the brown eye pigment contents of the highest and lowest lines in this sample, and a large proportion of the between line variation is attributable to genetic variation in the second chromosome, this sample of lines

TABLE 6

Pigment Contents of Prevosti Second Chromosome Lines

LINE NUMBER	RED PIGMENT CONTENT	BROWN PIGMENT CONTENT	% RED	% BROWN
Standard	94.2 $\pm$ 0.74	46.6 $\pm$ 0.60	100	100
2/50	90.2 $\pm$ 0.94	47.3 $\pm$ 0.68	95.75	100.85
1/5	76.0 $\pm$ 1.16	39.6 $\pm$ 0.15	80.85	84.98
1/44	99.3 $\pm$ 1.20	35.0 $\pm$ 0.54	84.04	75.11
1/16	85.5 $\pm$ 1.00	45.2 $\pm$ 0.36	90.43	96.99
1/24	81.4 $\pm$ 1.02	46.2 $\pm$ 0.44	86.17	99.14
1/146	87.6 $\pm$ 0.92	52.1 $\pm$ 1.50	92.55	111.59
1/20	79.1 $\pm$ 1.02	51.0 $\pm$ 0.38	84.04	109.44
2/77	89.0 $\pm$ 0.38	35.4 $\pm$ 0.19	94.68	75.11
2/110	81.0 $\pm$ 1.02	50.8 $\pm$ 0.40	86.17	109.01
1/141	82.0 $\pm$ 1.21	31.2 $\pm$ 0.37	87.23	66.52
1/139	86.2 $\pm$ 0.37	33.2 $\pm$ 0.40	90.43	70.82
2/97	90.1 $\pm$ 1.10	50.0 $\pm$ 0.41	95.75	107.30
2/65	79.2 $\pm$ 0.86	56.3 $\pm$ 0.37	84.04	120.17
1/57	81.3 $\pm$ 0.92	44.4 $\pm$ 0.70	86.17	95.28
1/104	91.30 $\pm$ 0.95	44.5 $\pm$ 0.50	97.13	95.49
2/72	81.0 $\pm$ 0.73	37.4 $\pm$ 0.30	86.17	80.26
1/57	84.4 $\pm$ 0.91	39.7 $\pm$ 0.60	89.60	85.19
2/108	93.2 $\pm$ 0.51	36.1 $\pm$ 0.38	98.94	77.25
1/35	78.1 $\pm$ 1.35	42.6 $\pm$ 0.44	82.98	91.42
2/53	72.1 $\pm$ 0.74	41.6 $\pm$ 0.42	76.60	89.27
1/59	100.0 $\pm$ 0.71	44.1 $\pm$ 0.38	106.38	94.42
1/108	82.2 $\pm$ 0.88	33.0 $\pm$ 0.24	87.23	70.82
1/29	70.2 $\pm$ 1.26	48.4 $\pm$ 0.56	74.46	103.86
2/103	90.4 $\pm$ 0.55	35.6 $\pm$ 0.34	95.75	76.39
2/102	83.7 $\pm$ 1.16	46.4 $\pm$ 0.62	88.30	99.57
2/15	84.7 $\pm$ 0.58	40.0 $\pm$ 0.70	89.36	85.84
2/89	74.2 $\pm$ 1.20	41.6 $\pm$ 0.28	78.72	89.27
2/	76.6 $\pm$ 0.92	38.2 $\pm$ 0.18	80.85	81.97
1/105	94.2 $\pm$ 0.74	38.4 $\pm$ 0.56	100	82.40
1/141	87.51 $\pm$ 0.64	36.3 $\pm$ 0.26	93.09	78.54
P55	76.3 $\pm$ 0.50	13.2 $\pm$ 0.13	80.85	28.33

Table 6A.

Red Pigment

<u>Source</u>	<u>d.f.</u>	<u>S.S.</u>	<u>M.S.</u>	<u>E.M.S.</u>
between lines	31	1524.89	49.19	$\sigma_W^2 + 10\sigma_B^2$
within lines	288	2426.68	8.43	σ_W^2

$$\sigma_B^2 = 4.07$$

Proportion of variance due to genetic effects of

$$\text{2nd chromosome} = \frac{\sigma_B^2}{\sigma_B^2 + \sigma_W^2} = \frac{4.07}{4.07 + 8.43} = 0.34$$

standard error of estimate = 0.09

Brown Pigment

<u>Source</u>	<u>d.f.</u>	<u>S.S.</u>	<u>M.S.</u>	<u>E.M.S.</u>
between lines	31	2028.64	65.44	$\sigma_W^2 + 10\sigma_B^2$
within lines	288	753.36	2.62	σ_W^2

$$\sigma_B^2 = 6.28$$

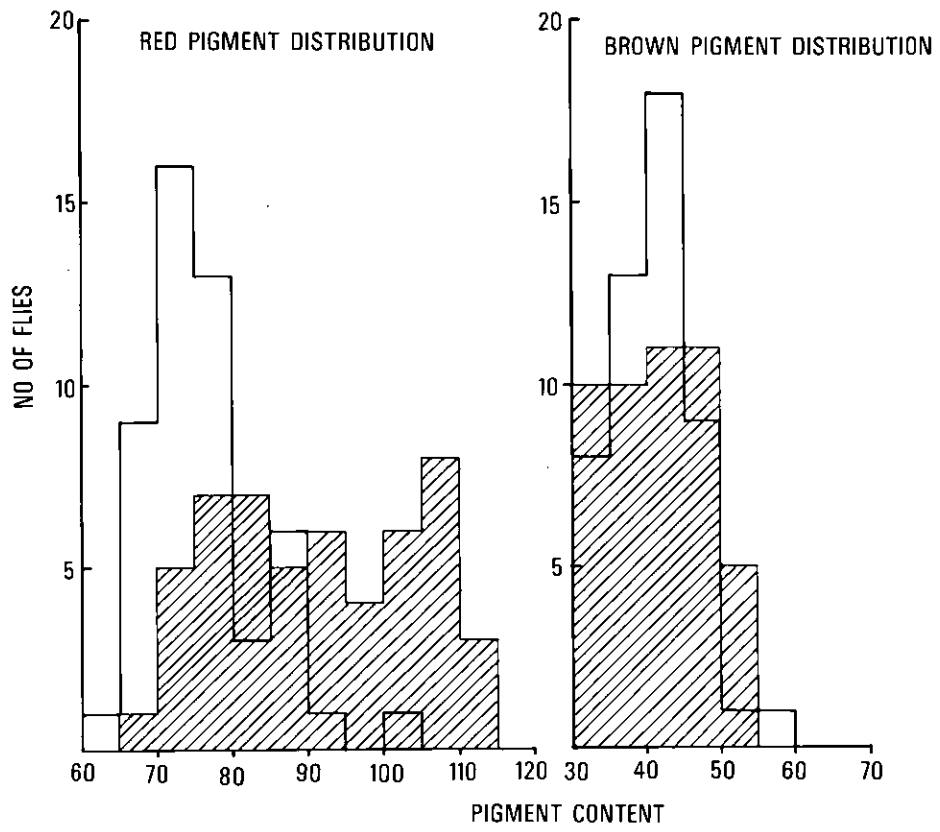
Proportion of variance due to genetic effects of

$$\text{2nd chromosome} = \frac{\sigma_B^2}{\sigma_B^2 + \sigma_W^2} = \frac{6.28}{6.28 + 2.62} = 0.71$$

standard error of estimate = 0.18

Figure 17A.

Eye pigment content distributions of the Prevosti cage population and of the Prevosti second chromosome lines. The hatched areas represent the cage population distributions; the plain areas the second chromosome lines distributions.



seems a hopeful candidate for further investigation into the nature of the metabolic variation resulting in the observed variation in brown eye pigment content.

The Red and Brown Eye Pigment Contents of Mutant Stocks

The brown eye pigment content of Drosophila melanogaster was shown in the last chapter to behave as a quantitative character in cage populations. The evidence suggests that the genetic variation in this character is more or less independent of eye or body size. What is the mode of action whereby the polygenes controlling this character act? A simple hypothesis is that the polygenes affecting brown eye pigment content are alleles at loci known by the effect of major mutants on eye colour. Polygenes may simply be alleles which affect the pathway, the deposition or the retention of the brown pigment to a degree which can be detected by extracting and measuring the quantity of pigment but not by the simple eyeball test.

The vast majority of mutants affecting eye colour are recessive. This suggests that, as a general rule, one "normal" gene at any eye colour locus is sufficient to produce an eye which looks wild type. It would be interesting to find out what sort of range of eye pigment contents can be found among mutant stocks and their heterozygotes.

Drosophila mutants are rated according to their ease of recognition. The RK numbers (rank or valuation numbers) run from 1 to 5 in decreasing order of ease of recognition as mutants and our knowledge of their location on the chromosomes. Eye colour mutants which have an RK3 rating, such as bright are often extremely difficult to distinguish from wild type. It seems highly likely that mutants of this type may easily be misclassified as wild type, and thus fall into the class of polygenes affecting brown eye pigment content.

Two approaches are possible in order to test whether or not eye pigment polygenes are mutants of "major" loci. One, the simplest, is to test for the presence of alleles at major loci. The second method is to compare the variations produced in brown pigment metabolism

by major loci and by polygenes.

A simple outline of the brown pigment pathway has already been given in Figure 2. A more detailed version is given in Figure 18, showing the mutants associated with each step in the pathway. Ryall and Howells (1974) have shown that over the period from pupation to eclosion, the enzyme activities and the intermediate pool levels change fairly rapidly, and that at no point does the system approximate a steady state. (For a time course of tryptophan, kynurenine and 3-hydroxykynurenine in the Oregon K line over this period see Figure 15). Brown pigment is first detected about half-way through pupation.

No information is available on the relationships between enzyme activities, intermediate pool levels, and final pigment content. Relationships between enzyme activities and intermediate pool concentrations have been studied in steady state systems. For example, in the arginine pathway of exponentially growing Neurospora crassa, the reductions in enzyme activity may be reflected in changes in intermediate accumulations which are quickly buffered out, resulting in little change in the end product (Barthelmess, Curtis and Kacser, 1974). On the other hand, heterozygotes for some of the aminoacidurias in mouse and man may exhibit no clinical symptoms or metabolic differences, but nevertheless have 50% of the respective enzyme. There is no information available on the effects of reduction in enzyme activity on the intermediate pools in the xanthommatin pathway of Drosophila melanogaster. However, such information may be obtained by studying strains homozygous and heterozygous for the mutants known to affect enzyme activity, namely vermillion (tryptophan oxygenase), cinnabar (kynurenine hydroxylase) and cardinal (phenoxazinone synthetase).

Figure 18.

The conversion of tryptophan to xanthomattin.

(The brown pigment pathway).

- (1) Vermilion (Baglioni, 1960).
- (2) cinnabar (Gosh & Forrest, 1967).
- (3) cardinal (Phillips et al., 1974).

No mutant forms of kynurenine formamidase have been identified. The two forms of this enzyme have been described by Moore and Sullivan (1978) and are apparently coded for by separate genes.

TRYPTOPHAN

N-FORMYL
KYNURENINE

KYNURENINE

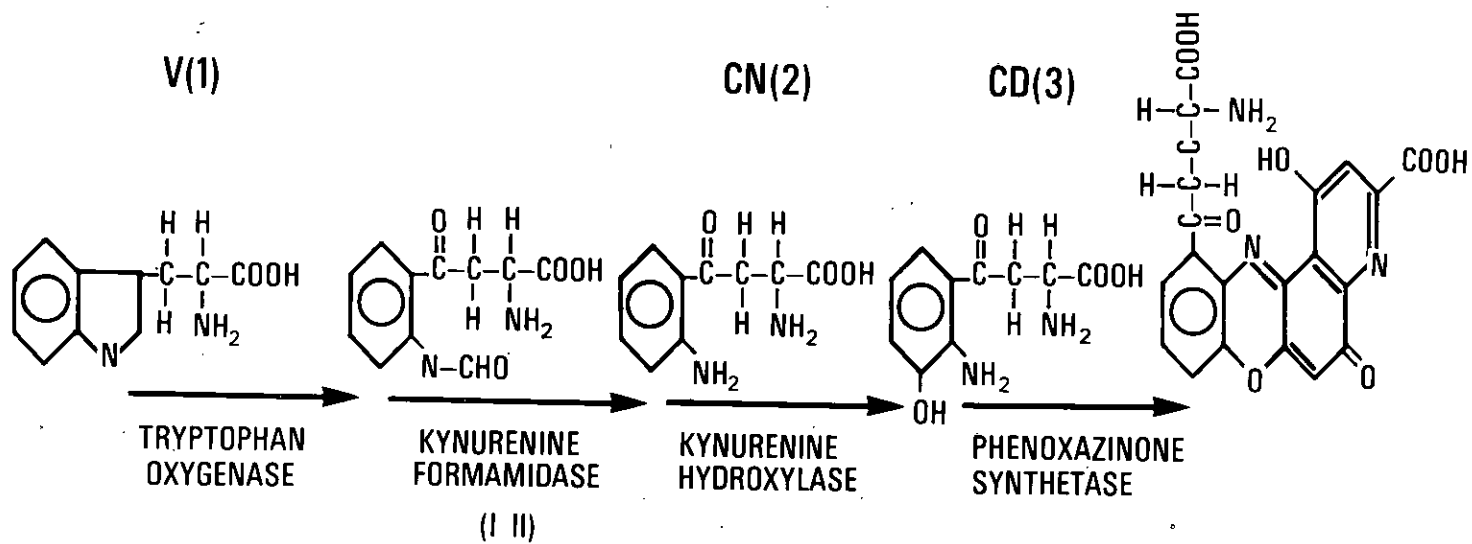
3-HYDROXY
KYNURENINE

XANTHOMMATIN

V(1)

CN(2)

CD(3)



Howells, Summers and Ryall (1978) have shown that the mutant scarlet has an abnormal pool level profile. This mutant has been shown to be deficient in the transport of 3-hydroxykynurenine into the pigment granules (Sullivan and Sullivan, 1975; Howells and Ryall, 1976). Similarly, mutants of the white locus which appears to affect the structure of the pigment granules (Ziegler, 1960) also produce alterations in the developmental pattern of intermediate pool levels (Howells, Summers and Ryall, 1978).

Three other mutants which suppress xanthommatin accumulation are known, namely karmoisin, light and lightoid. So far it is not known how any of these produce their effect on the eye pigment. However, in view of the evidence available on the mutants scarlet and white, it seems likely that these will also affect the intermediate accumulations.

A detailed study of how these mutants affect the synthesis and accumulation of brown eye pigment should ideally involve time courses of enzyme activities and intermediate pool levels. In view of the technical difficulties involved in assaying the enzyme activities associated with this pathway, the present study was confined to the effect of mutants on the intermediate pool levels.

Given that eye colour mutants may produce changes in intermediate pool profiles, is there any resemblance between the pool profiles of different alleles of the same locus? Presumably if this is the case it may be possible to identify the functions affected by polygenes by comparing their effects on the pool levels with those of known mutants. The white locus provides a useful system for examining this question. A large number of mutant alleles are known at this locus. The vast majority of these show a complete lack of both red

and brown eye pigments, but a number of alleles are known at this locus which show different degrees of colour in the eye ranging from pale ivory to dark coffee. Comparison of the intermediate profiles in a number of these alleles should indicate whether or not it is likely that alleles at a locus may be recognised by similarity of these profiles.

The mode of action of two mutants affecting the red pathway, namely rosy (xanthine dehydrogenase) and maroonlike (XDH cofactor) is well established (Glassman and Mitchell, 1956). Henna recessive may be the structural locus for sepiapterin reductase (Taira, 1960), and sepia also affects sepiapterin metabolism.

Of the remaining fifty or so loci which affect eye colour, it is reasonable to suppose that there are probably some at which mutation may affect the net brown pigment content. In attempting to increase the background of information on the pathway before investigating the mode of action of the brown pigment polygenes, mutants of such loci cannot be ignored. The simplest criterion for choosing which mutants may be profitably included in a study of brown pigment metabolism is whether or not the mutants available affect the adult brown eye pigment content. Thus mutants which either significantly increase or significantly decrease brown eye pigment content would be worthy of investigation.

Lindsley and Grell (1967) give the levels of both red and brown eye pigments as percentages of wild type found in a number of eye colour mutants. Unfortunately, there are some flaws in these data. The percentages are derived from data found in a series of papers by Nolte (1950; 1954a,b; 1955; 1959). No mention is made in these papers of any attempt to standardise the genetic background so it is

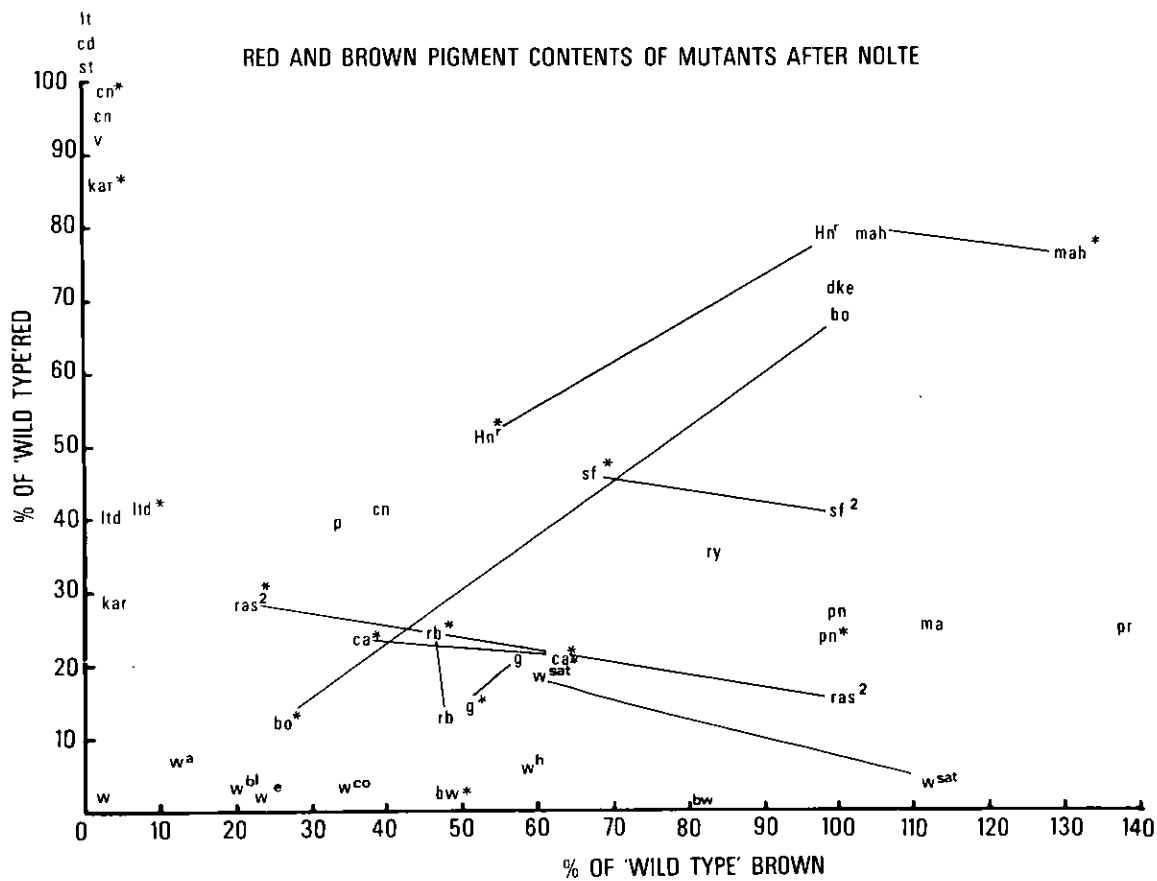
impossible to decide what proportion of the difference between mutant and wild type strains can reasonably be ascribed to the locus under study. Early in the series of papers (1954) Nolte rejects the Florida strain originally used as the wild type on the grounds of "insufficient vigour". The red and brown eye pigment contents of the Canton S strain, which he adopts as the new wild type, are as different from those of the Florida strain as are many of the mutant values (see Nolte, 1954). The data used by Lindsley and Grell as the basis for their percentage of wild type calculations are the Canton S values given in the 1954 paper. This is in spite of the fact that very different data for this strain appear in a later paper (1957). The percentages of "wild type" red and brown pigment contents taken from Lindsley and Grell are shown in Figure 19.

As more rigorous data were required before any choice could be made on the basis of which mutants have a real effect on brown eye pigment content, the pigment contents of a number of eye colour mutants were determined. The mutants sepia, clot and Henna^{recessive} are known to accumulate the yellow pteridine, sepiapterin, in their eyes (Gregg and Smucker, 1966). In order to check that other possible pigmented compounds are not eluted from the eyes of any of the mutants by the solvents used to extract either the red or brown pigment, the absorption spectra of both solutions were obtained for each mutant.

Male and female pigment contents were determined for first (sex) chromosome mutants, in order to check whether or not dosage compensation is complete in sex-linked eye colour mutant strains.

Figure 19.

Red and brown pigments taken from Lindsley and Grell (1967), as described in the text. Points marked with a star are values obtained in the course of experiments detailed later, for comparison.



1. Red and Brown Pigment Contents of a Sample of Eye Colour Mutants.

Experimental Details:

A sample of forty-four different mutants was obtained, representing twenty-eight loci. The sources of these stocks are given in Table 1. As described in the Materials and Methods section, each mutant was backcrossed^{six times} to an inbred Oregon K strain to give some degree of standardisation of the genetic background.

Four single pair matings of each of the back-crossed mutant stocks and of the inbred Oregon K strain were set up in vials containing Sang's Yeast Medium. The cultures were raised at 25°C, the parents having been discarded after two days of egg laying. The offspring were placed in fresh vials at eclosion and allowed to age for 7 days at 25°C. By this time, pigment deposition in the Oregon K line is complete (see Figure 8).

Five females from each vial were sacrificed for pigment extraction and measurement.

Results and Discussion:

The means and standard errors of the means were calculated for both pigments for each mutant strain. Means were also expressed as a percentage of the Oregon K mean. These data are presented in Table 7 and Figure 20. The Student's t test was used to compare mutant and Oregon K values. With the exception of cinnabar and dark red brown, all mutants showed significant departures from the Oregon K content of both pigments. The red pigment of cinnabar was not significantly different from that of Oregon K, nor was the brown pigment of dark red brown.

The results are shown graphically in Figure 20, depicting the

TABLE 7

Red and Brown Pigment Contents of Mutants

GENOTYPE	RED PIGMENT	BROWN PIGMENT	% RED	% BROWN
Oregon K	113.01 $\pm$ 1.79	36.20 $\pm$ 0.95	100	100
prune	25.00 $\pm$ 0.94	33.71 $\pm$ 1.30	22.12	93.12
white	0	4.4 $\pm$ 1.28	0	+
white ^{apricot}	12.75 $\pm$ 0.68	15.60 $\pm$ 0.54	11.28	43.09
white ^{buff} ²	12.53 $\pm$ 0.48	13.68 $\pm$ 1.18	11.09	37.79
white ^{satsuma}	21.25 $\pm$ 0.92	21.45 $\pm$ 1.04	18.80	59.25
white ^{ecru}	10.15 $\pm$ 0.70	10.60 $\pm$ 0.50	8.98	29.28
white ^{honey}	8.58 $\pm$ 0.51	18.05 $\pm$ 0.55	7.59	49.86
white ^{coffee}	45.50 $\pm$ 1.77	30.35 $\pm$ 1.46	40.27	83.84
white ^{blood}	8.65 $\pm$ 0.51	20.15 $\pm$ 0.72	7.65	55.66
white ^{eosin}	11.65 $\pm$ 0.48	15.50 $\pm$ 0.73	10.31	42.81
white ^{Brownex}	10.75 $\pm$ 0.68	20.95 $\pm$ 0.71	9.51	57.87
white ^{copper}	11.93 $\pm$ 0.58	17.30 $\pm$ 0.91	10.56	47.79
white ^{coral}	11.43 $\pm$ 0.38	21.05 $\pm$ 0.80	10.12	58.15
white ^{cherry}	9.29 $\pm$ 2.39	15.33 $\pm$ 0.54	8.22	42.34
chocolate	14.25 $\pm$ 0.91	28.35 $\pm$ 0.89	12.61	78.31
ruby	25.00 $\pm$ 1.15	16.89 $\pm$ 0.68	22.12	46.65
bordeaux	14.50 $\pm$ 0.95	9.60 $\pm$ 0.73	12.83	26.52
carmine	22.50 $\pm$ 1.56	18.65 $\pm$ 1.31	19.92	51.52
raspberry ²	21.45 $\pm$ 0.67	28.05 $\pm$ 0.92	18.98	77.49
vermillion	80.23 $\pm$ 1.45	4.50 $\pm$ 0.94	71.00	*
vermillion ^k	83.06 $\pm$ 1.63	3.62 $\pm$ 1.00	73.50	
vermillion ^{36f}	91.98 $\pm$ 0.98	2.13 $\pm$ 0.72	81.43	
vermillion ^{48a}	67.60 $\pm$ 1.78	3.12 $\pm$ 0.83	59.82	
vermillion ^{57c}	70.63 $\pm$ 0.95	2.42 $\pm$ 0.54	62.50	
garnet	14.50 $\pm$ 0.61	17.50 $\pm$ 0.99	12.83	48.34
carnation	34.85 $\pm$ 0.94	15.51 $\pm$ 0.53	30.84	42.84
maroonlike ^{bronzy}	34.85 $\pm$ 0.92	22.40 $\pm$ 0.73	30.84	61.87
clot	62.50 $\pm$ 1.33	42.50 $\pm$ 1.34	55.30	117.40
light	18.00 $\pm$ 0.92	5.2 $\pm$ 0.47	15.92	*
lightoid	42.25 $\pm$ 1.43	2.2 $\pm$ 0.46	40.04	
cinnabar	114.75 $\pm$ 2.34	0.85 $\pm$ 0.33	101.54	
safranin ²	54.90 $\pm$ 1.24	24.50 $\pm$ 0.88	45.58	67.67
brown	0.65 $\pm$ 0.34	15.15 $\pm$ 0.62	0.58	42.80
purpleoid/				

TABLE 7 (Cont/d....)

purpleoid	23.57 ± 0.92	11.30 ± 0.84	20.85	31.22
orange	26.00 ± 1.18	28.20 ± 1.37	23.01	77.90
Henna ^{recessive}	55.75 ± 1.46	20.53 ± 0.92	49.34	56.22
sepia	19.50 ± 0.72	24.90 ± 0.90	17.26	68.78
scarlet	102.75 ± 1.53	2.4 ± 0.55	90.93	*
dark red brown	32.50 ± 1.56	35.85 ± 0.88	28.76	99.00
pink	25.75 ± 1.16	6.45 ± 0.82	22.78	17.80
karmoisin	97.75 ± 1.17	0.67 ± 0.52	86.50	*
red Malpighian tubules	32.60 ± 1.72	38.90 ± 1.16	28.85	107.46
cardinal	102.89 ± 3.09	3.85 ± 0.93	91.05	*
mahogany	87.00 ± 1.68	46.75 ± 1.88	76.99	129.14
claret	26.00 ± 0.86	12.55 ± 0.96	23.00	33.84

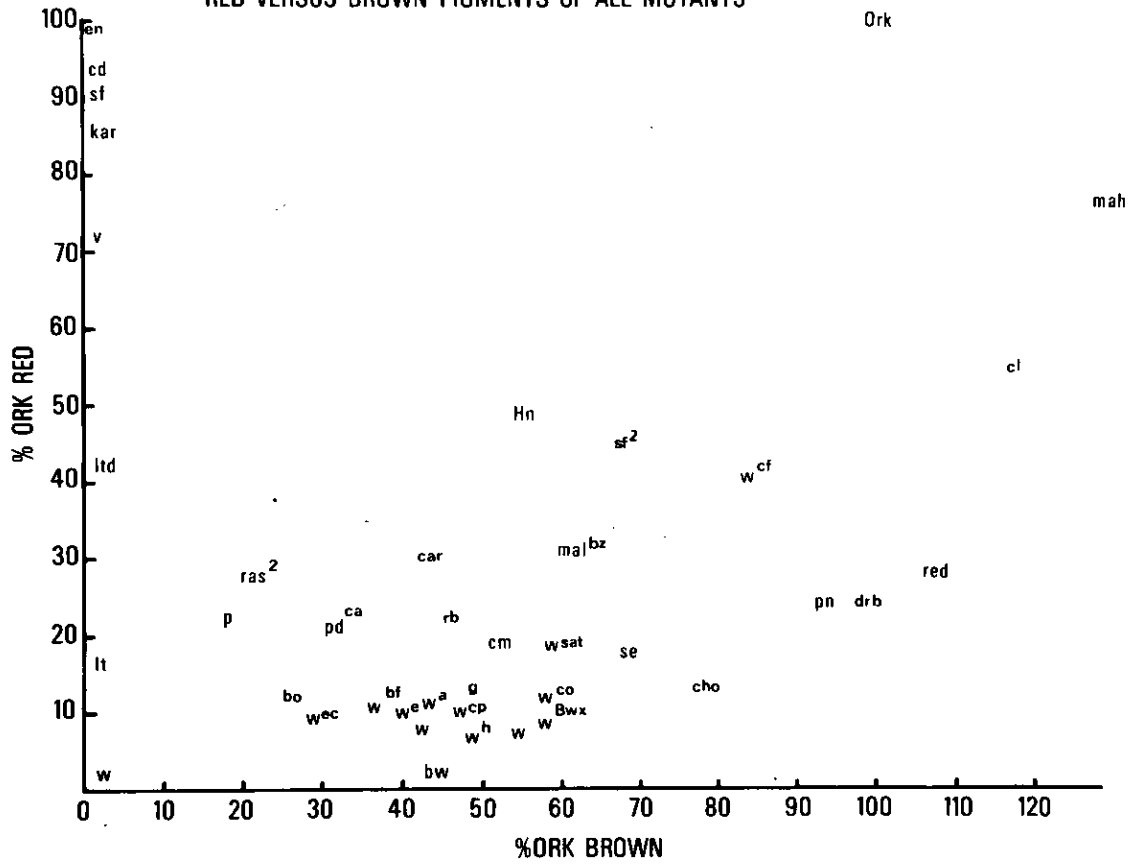
+ the value obtained for the brown pigment content of white was taken as the base line value and subtracted from the Oregon K and other mutant values.

* the observed brown pigment contents of those mutants which are said to prevent brown pigment synthesis are given without correcting for the base line obtained from the white mutant.

Figure 20.

Red and brown pigments of a number of mutants expressed as percentages of Oregon K pigment contents. Each mutant has been backcrossed into the Oregon K strain used as a standard wild type.

RED VERSUS BROWN PIGMENTS OF ALL MUTANTS



relationship between the percentages of the Oregon K red and brown pigment contents found in the mutants. There is very little agreement with the data given by Lindsley and Grell (1967). This is clearly seen in Figure 19, where the percentages of wild type pigment taken from Nolte's data are compared with those obtained here. In particular, mutants such as raspberry and bordeaux, which were reported as having little or no effect on brown pigment caused significant decreases in both pigments in this study.

These discrepancies can be explained by the differences in the means of ascertainment of the two sets of data. Nolte's mutant strains presumably had a variety of different genetic backgrounds. The wild type values used as the basis of percentage calculations are more or less arbitrary. In the present study an attempt has been made to control the genetic background, so that as far as possible any effects on pigment content may be reasonably ascribed to the eye colour mutant carried in each strain. The wild type values are obtained from the strain which was used as the standard background. Efforts were made to reduce environmental variation as much as possible, by the use of a standard medium, and controlled temperature.

Five of the bright eyed mutants and two very dark eyed mutants had red eye pigment contents greater than 50%. Cinnabar, cardinal, scarlet, karmoisin and vermilion have insignificant brown eye pigment contents, while mahogany and clot have high brown eye pigment contents. A large area of Figure 20 is devoid of mutants owing to a deficiency of strains having red pigment contents in the range of 50 - 100% of the Oregon K value. The apparent deficit of red pigment values greater than 50% of the wild type level may simply be due to the fact

that mutants at less than half of the eye colour loci have been included in this study. There may be some real biological significance attached to this observation. For example, "Mutants" which possess some brown pigment and more than 50% of red may appear wild type to the eye. On the other hand, there may be some peculiarity of the red pathway such that mutation at any step in the pathway leads to a gross reduction in pigment synthesis. More data on the pigment contents of other mutants, combinations of mutants and wild type flies must be considered to obtain further information on this point.

It appears that almost all of the eye colour mutants sampled have statistically significant effects on the brown eye pigment content, and therefore presumably affect some aspect of pigment synthesis or deposition. On this basis, all are worthy of inclusion in a study of intermediate pool levels in the brown pigment pathway, with the possible exception of dark red brown, which apparently only affects the red pigment.

2. Absorption Spectra of Pigment Extracts

Experimental Details

Ten females from each line were reserved from the previous experiment (determination of pigment contents), and allowed to age at 25°C for 7 days. The flies were beheaded, the heads were split longitudinally, and soaked together in 1 ml of acidified ethanol (see Materials and Methods) for twenty four hours. This provided a concentrated "red pigment" solution. Making use of the scanning facility on the Beckman DB-GT spectrophotometer, the absorption spectrum over the range 200 - 600 nm was determined for each strain,

using a solvent blank as reference. The heads were washed with methanol, and soaked for twenty four hours in 1 ml of acidified methanol. The absorption spectrum of this concentrated "brown pigment" solution over the range 200 - 600 nm was also obtained.

The absorption spectra of the mutants, scarlet, brown and white are shown in the Materials and Methods section (Figures 4 and 6). In common with scarlet, the bright-eyed mutants (vermilion, cinnabar, cardinal, karmoisin, light and lightoid) lack the broad peak in the visible region in "brown pigment" extracts.

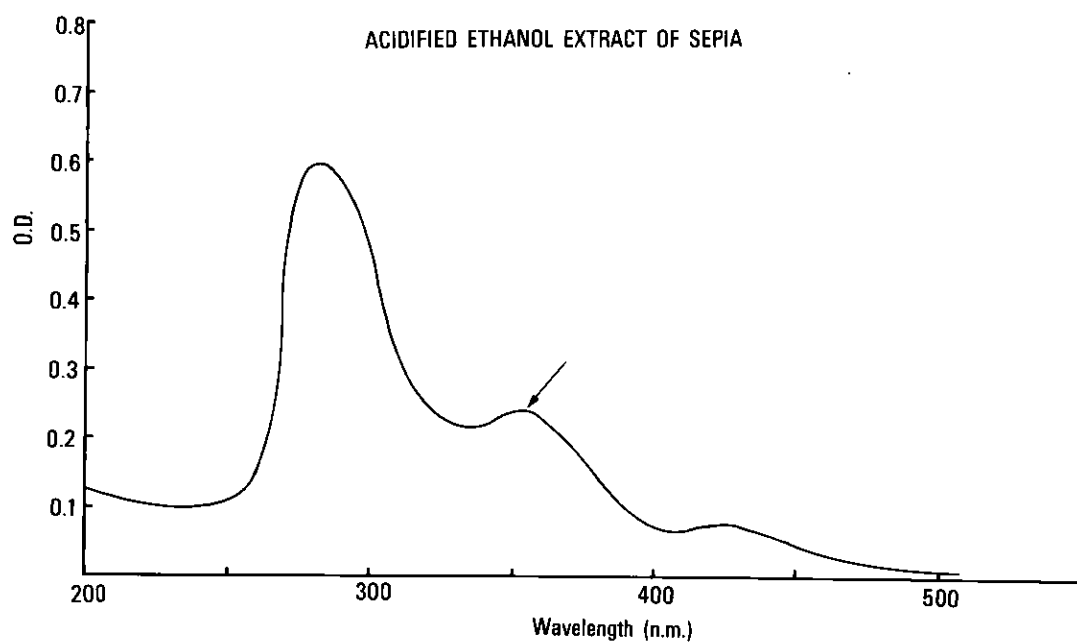
The "red" extracts of the mutants sepia, clot and Henna^{recessive} showed abnormal spectra. In each case the same atypical peak was present, apparently due to the presence of sepiapterin in these extracts. Each of these mutants accumulates this compound (Gregg & Smucker, 1966). Figure 21 shows the spectrum of a "red" extract of sepia, with the presumptive sepiapterin peak arrowed. The other mutants only showed quantitative rather than qualitative changes in the absorption spectra of both extracts. With the exception of se, cl and Hn^r there was no evidence for the presence of any unusual compound in either extract in any of the mutants. This, of course, does not exclude the possibility that compounds which absorb around the same wavelengths as the red and brown pigments may be accumulated.

3. Dosage Compensation in Eye Pigment Mutants

The data on the cage populations show that on the average males have considerably less eye pigment than females of the wild type strain. This agrees well with Andrews' (1970) observation that males have, on the average, around 70% of the number of ommatidia (which contain the pigment granules) of females from the same strain.

Figure 21.

Absorption spectrum of an acid ethanol extract of sepia flies. The shoulder marked with an arrow is caused by the presence of the yellow pigment, sepiapterin. Accumulations of this compound can be found in clot and Henna^{recessive} also.



One might expect that if there is full dosage compensation in the X-linked eye colour mutants (i.e. if one gene dose in the males produce equivalent "activity" to two genes at the same locus in the females), that the sexual dimorphism for pigment content might well be maintained. Thus full dosage compensation may be obtained when the males have around 70 - 80% of the female eye pigment content.

Experimental Details

Five males as well as five females were collected from each vial containing first chromosome mutant strains and the Oregon strain, in the experiment described earlier (pigment content of mutants). These were sacrificed for pigment extraction at seven days after eclosion, enabling mean pigment contents of males and females to be determined.

Results and Discussion

The results are presented in Table 8, which gives the means and standard errors of the male and female eye pigment contents of twenty one first (X) chromosome mutants, representing ten loci.

Inspection of these data reveals a close correspondence between male and female pigment contents in all of the mutant stocks, in spite of the fact that the males were consistently smaller. This contrasts with the marked dimorphism in the pigment contents of the males and females of the Oregon K line. A possible explanation of this observation is that in "wild type" flies, the pigment content is limited by the size of the eye and/or the number of ommatidia. The differences observed between males and females may simply be dictated by the processes of sexual differentiation rather than affected by the gene dose difference for X-linked genes between males and females. The wild type eye may be envisaged as containing a

TABLE 8

Mean Male and Female Pigment Contents of X-linked Mutants

GENOTYPE	FEMALE RED	MALE RED	FEMALE BROWN	MALE BROWN
Oregon K	113.01 $\pm$ 1.79	84.00 $\pm$ 2.47	36.20 $\pm$ 0.95	26.70 $\pm$ 0.87
carnation	34.85 $\pm$ 0.94	34.25 $\pm$ 1.51	15.51 $\pm$ 0.53	23.30 $\pm$ 0.38
prune	25.00 $\pm$ 0.94	24.75 $\pm$ 0.92	33.71 $\pm$ 1.30	31.65 $\pm$ 1.07
white ^{apricot}	12.75 $\pm$ 0.68	12.75 $\pm$ 0.66	15.60 $\pm$ 0.54	14.65 $\pm$ 0.50
white ^{ecru}	10.15 $\pm$ 0.70	9.30 $\pm$ 0.66	10.60 $\pm$ 0.50	11.55 $\pm$ 0.73
white ^{eosin}	11.65 $\pm$ 0.48	11.10 $\pm$ 0.50	15.50 $\pm$ 0.73	15.35 $\pm$ 0.80
white ^{buff}	12.53 $\pm$ 0.48	13.15 $\pm$ 0.55	13.68 $\pm$ 1.18	14.40 $\pm$ 0.42
white ^{blood}	8.65 $\pm$ 0.51	8.50 $\pm$ 0.55	20.15 $\pm$ 0.72	21.40 $\pm$ 0.53
white ^{Brownex}	10.75 $\pm$ 0.68	10.80 $\pm$ 0.79	20.95 $\pm$ 0.71	19.40 $\pm$ 0.83
white ^{cherry}	9.29 $\pm$ 2.39	9.75 $\pm$ 0.80	15.33 $\pm$ 0.54	15.75 $\pm$ 0.78
white ^{coral}	11.43 $\pm$ 0.38	11.05 $\pm$ 0.62	21.05 $\pm$ 0.80	20.90 $\pm$ 0.53
white ^{copper}	11.93 $\pm$ 0.58	12.25 $\pm$ 0.85	17.30 $\pm$ 0.91	16.90 $\pm$ 0.46
white ^{coffee}	45.50 $\pm$ 1.77	44.50 $\pm$ 1.18	30.35 $\pm$ 1.46	28.40 $\pm$ 1.62
white ^{satsuma}	21.25 $\pm$ 0.92	19.10 $\pm$ 1.67	21.45 $\pm$ 1.06	20.80 $\pm$ 0.79
white ^{honey}	8.58 $\pm$ 0.51	8.30 $\pm$ 0.55	18.05 $\pm$ 0.55	19.20 $\pm$ 0.75
chocolate	14.25 $\pm$ 0.91	13.50 $\pm$ 0.73	28.35 $\pm$ 0.89	25.90 $\pm$ 0.87
ruby	25.00 $\pm$ 1.15	25.50 $\pm$ 0.72	16.89 $\pm$ 0.68	17.20 $\pm$ 0.66
bordeaux	14.50 $\pm$ 0.95	14.75 $\pm$ 0.85	9.60 $\pm$ 0.73	9.50 $\pm$ 0.72
carmine	22.50 $\pm$ 1.56	18.75 $\pm$ 0.62	18.65 $\pm$ 1.31	19.75 $\pm$ 0.55
raspberry ²	21.45 $\pm$ 0.61	17.40 $\pm$ 0.67	28.05 $\pm$ 0.92	25.45 $\pm$ 0.50
garnet	14.50 $\pm$ 0.61	13.75 $\pm$ 0.75	17.50 $\pm$ 0.99	24.25 $\pm$ 0.73
maroonlike ^{bronzy}	34.85 $\pm$ 0.92	36.25 $\pm$ 1.02	22.60 $\pm$ 0.73	21.25 $\pm$ 0.72

maximum number or maximum volume of pigment containing granules, each being "saturated" with pigment. The difference in the eye pigment contents between wild type males and females could then be due merely to the physical limits on the available "pigment storage space" which reflect the difference in overall size between the two sexes.

In the case of the sex-linked mutants, however, the absolute pigment content is greatly reduced compared with that of the wild type. The constraints here can be seen as being imposed by the biosynthetic mechanisms leading to pigment deposition rather than the "storage space" available. In the absence of any dosage compensation mechanisms, we might therefore expect that for X-linked mutants, the female:male pigment ratio might be close to 2:1. The observation that this ratio is not significantly different from 1:1 argues that dosage compensation at the level of the red and brown pigment biosynthetic pathways is complete. It would also appear that none of the sex-linked mutants is capable of synthesising more pigment than can be stored in the male eye, thus giving the deviation from the female:male pigment content ratio seen in wild type populations (see Table 3).

4. Interactions between Red and Brown Pigment Pathways

Figure 20 shows clearly the nature of some of the data which has been used to argue the existence of interactions between the biosynthetic pathways leading to the two pigments. If we choose to ignore mutants which totally abolish synthesis of either pigment, a correlation coefficient of 0.36 between the two pigments is obtained. The fact that mutants which are associated with enzyme lesions in

one pathway, such as vermilion or maroonlike^{bronzy} can apparently have a significant effect on the other pathway supports an interaction hypothesis. The lack of genetic correlation between the red and the brown pigment contents in the experiments with laboratory cage populations argues against inter-pathway interactions as an important source of variation in brown pigment content in wild populations. Any detailed enquiries into the nature of inter-pathway interactions are likely to be fraught with difficulties in view of our comparative ignorance of the red pathway. A study of the pigmented alleles of the white locus, which affect both pigments, may clarify the relationship between the two pathways at the level of the white product.

5. Summary

The red and brown eye pigment contents of a number of eye colour mutants were determined. All except one (dark red brown) significantly altered the brown pigment content from that of the background Oregon K strain. All the mutants examined are, therefore, worthy of inclusion in a more detailed study of the synthetic pathway leading to xanthommatin, as an increased understanding of the changes which major mutants can cause in the pathway is desirable as a background to the study of the biochemical nature of the "wild type" variation in the pigment.

An asymmetry in the graph of red versus brown pigment values was noted. The lack of mutants having red pigment values greater than 50% of the standard may have some biological significance, but it is impossible to rule out chance sampling of only half of the available

eye colour mutants as giving rise to this phenomenon.

Apparent dosage over compensation for pigment content was observed in the case of sex-linked mutants. A possible explanation for this phenomenon was offered.

While there is some evidence for interaction between the pathways leading to red and to brown pigment synthesis, the problems of carrying out a detailed investigation of the mechanisms of any such interactions are too great to be tackled here in any great depth.

The Bright-Eyed Mutants

Before attempting to relate differences in pigment content to alterations in the xanthommatin pathway which may be fairly subtle, it is reasonable to ask what degree of alterations may be detected by the techniques available. The experiments described in the next few pages were carried out on mutants whose effects on brown pigment synthesis are sufficient to prevent pigment synthesis.

Three of these mutants (vermillion, cinnabar and cardinal) lack enzyme activity at one or other of the four steps between tryptophan and xanthommatin. By investigating the effects of these mutants, on the pathway in the heterozygous as well as the homozygous state, some idea of the effect of reducing enzyme activity of tryptophan oxygenase (v), kynurenine hydroxylase (cn) or phenoxazinone synthetase (cd) by approximately half, may also be ascertained. Scarlet is of interest as a mutant of the granule transport system. Although the functions affected in the mutants karmoisin, light and lightoid remain a matter of speculation, their lack of brown pigment argues that they should be included with the other four xanthommatin-lacking mutants. Of this group of seven mutants, only light is distinguishable by eye from the other six, having somewhat paler, reddish eyes. The other mutants (vermillion, cinnabar, cardinal, scarlet, karmoisin and lightoid) cannot be separated on the basis of eye colour. All six have eyes of a bright, almost fluorescent, red. Collectively, the adjective 'bright-eyed' describes the group very well.

1. The Pigments of the Bright-Eyed Mutants

Two simple questions can be asked:

- (1) How complete is dominance of the wild type allele in heterozygotes?
- (2) What is the effect on the net flow through the pathway of reducing more than one step by approximately 50%?

Experimental Details

Four single pair matings of each type in Table 9 were set up in vials on Sang's yeast medium at 25°C. The parents were allowed two days for egg laying before being discarded. The cultures were maintained at 25°C, and females were collected and placed in fresh vials at eclosion. After a further 7 days at 25°C, five females from each vial were sacrificed for pigment extraction and measurement.

Results and Discussion

The mean contents and standard errors, together with percentages of the Oregon K means, for both red and brown pigments are shown in Table 9. Figure 22 shows the red and brown values as percentages of the Oregon K value. None of the heterozygotes were distinguishable microscopically from the Oregon K flies. That is, they all appeared "wild type".

The first point to note is that none of the heterozygotes have pigment contents within the range observed in the study of mutant homozygotes summarised in Figure 20. The means of both the red and brown pigments are in all cases in excess of 70% of the Oregon K mean (except lt x Ork where the red content is only 66.5% of the Oregon value). In some cases, the mean content of one or both pigments actually exceeds that of Oregon K. As the genetic background of each

TABLE 9

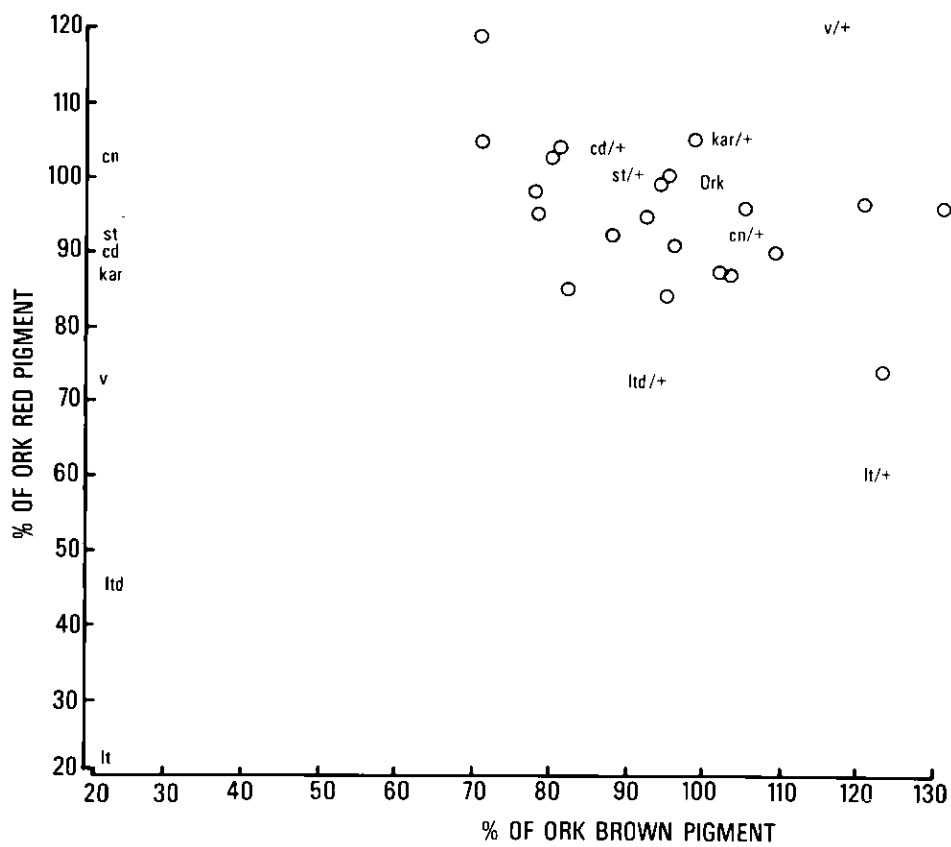
Pigment Contents of v, cn, cd, st, kar, lt, ltd and their Heterozygotes

F ₁ of CROSS	RED PIGMENT CONTENT	BROWN PIGMENT CONTENT	% RED	% BROWN
Oregon K	110.01 $\pm$ 1.71	37.08 $\pm$ 2.17	100	100
v x Ork	134.20 $\pm$ 1.90	42.50 $\pm$ 1.19	122.1	115.0
cn x Ork	101.53 $\pm$ 1.74	39.48 $\pm$ 1.82	92.3	106.7
lt x Ork	73.15 $\pm$ 1.62	44.81 $\pm$ 1.31	66.5	121.1
ltd x Ork	87.40 $\pm$ 0.95	34.34 $\pm$ 1.38	73.4	92.8
kar x Ork	115.72 $\pm$ 1.35	37.85 $\pm$ 1.05	105.2	102.3
st x Ork	113.85 $\pm$ 1.78	32.26 $\pm$ 1.72	103.5	87.2
cd x Ork	103.62 $\pm$ 1.72	29.60 $\pm$ 1.25	94.2	80.0
v x v	80.08 $\pm$ 0.82	3.75 $\pm$ 0.92	72.8	*
v x cn	104.06 $\pm$ 1.59	31.67 $\pm$ 0.77	94.6	85.6
v x lt	94.49 $\pm$ 1.38	30.41 $\pm$ 0.76	85.9	82.2
v x ltd	116.49 $\pm$ 1.58	36.41 $\pm$ 1.13	105.9	98.4
v x kar	116.50 $\pm$ 1.45	26.42 $\pm$ 0.76	105.9	71.4
v x st	96.03 $\pm$ 0.92	37.78 $\pm$ 1.18	87.3	102.1
v x cd	101.75 $\pm$ 1.56	32.78 $\pm$ 1.36	92.5	88.6
cn x cn	112.20 $\pm$ 2.13	1.07 $\pm$ 0.82	102.0	*
cn x lt	106.69 $\pm$ 1.93	45.07 $\pm$ 0.89	96.9	121.8
cn x ltd	130.68 $\pm$ 1.65	25.97 $\pm$ 0.99	118.8	70.2
cn x kar	99.11 $\pm$ 1.44	40.37 $\pm$ 1.39	90.1	109.1
cn x st	114.84 $\pm$ 1.52	30.04 $\pm$ 0.81	104.1	81.2
cn x cd	104.61 $\pm$ 1.53	29.16 $\pm$ 0.80	95.1	78.8
lt x lt	19.58 $\pm$ 1.10	3.10 $\pm$ 1.22	17.8	*
lt x ltd	106.15 $\pm$ 1.81	48.54 $\pm$ 0.65	96.5	131.2
lt x kar	96.29 $\pm$ 1.59	38.18 $\pm$ 2.37	87.5	103.2
lt x st	111.43 $\pm$ 1.83	35.41 $\pm$ 1.46	101.3	95.7
lt x cd	81.73 $\pm$ 1.44	45.58 $\pm$ 1.38	74.3	123.2
ltd x ltd	49.67 $\pm$ 1.53	1.85 $\pm$ 0.92	45.15	0*
ltd x kar	120.78 $\pm$ 1.73	35.08 $\pm$ 0.89	109.8	94.8
ltd x st	113.3 $\pm$ 1.18	29.80 $\pm$ 1.09	103.1	80.8
ltd x cd	108.52 $\pm$ 2.41	28.79 $\pm$ 0.67	98.7	77.8
kar x kar	97.59 $\pm$ 2.77	0.67 $\pm$ 0.52	88.7	*
kar x st	100.44 $\pm$ 1.75	35.73 $\pm$ 1.03	91.3	96.6
kar x cd	106.69 $\pm$ 1.67	38.96 $\pm$ 1.67	96.9	105.2
st x st	100.27 $\pm$ 1.30	2.4 $\pm$ 0.55	91.1	*
st x cd	104.83 $\pm$ 1.42	34.19 $\pm$ 1.42	95.3	92.4
cd x cd	98.45 $\pm$ 1.86	2.25 $\pm$ 0.73	89.5	*

The other genotypes had already had the w baseline brown content subtracted.

Figure 22.

Red and brown pigment contents of the "bright-eyed" mutants, their heterozygotes, and double heterozygotes expressed as percentages of the Oregon K pigment contents.



of the parental strains should be mainly derived from Oregon K (as a result of backcrossing) it is unlikely that this increase in pigment content could be explained by invoking heterosis for pigment content in the F_1 's of crosses such as that between vermilion and Oregon K, or light and karmoisin. In the absence of information on the enzyme activities and pool levels in these strains, it is impossible to offer a plausible explanation for this observation.

The double heterozygotes (those lines heterozygous at two loci) all have mean red pigment contents well within the range observed for the single heterozygotes. With regard to the mean brown pigment contents, a wider range is found among the double heterozygotes than for the single heterozygotes.

v/+; cn/+ has a mean brown pigment content of 31.67 units, compared with 42.50 units for v/+; +/+ or 39.48 units for +/+; cn/+. As the step affected by cinnabar (kynurenine hydroxylase) follows closely on that affected by vermilion (tryptophan oxygenase) it is tempting to explain the brown pigment content of v/+; cn/+ in terms of the reduction of the kynurenine pool available to the (also reduced activity of) kynurenine hydroxylase due to the reduction in tryptophan oxygenase activity. This, however, is another case where data on enzyme activities and intermediate pool levels is necessary before plausible mechanisms can be suggested.

If the mean pigment contents listed in Table 9 are compared with the data shown in Table 4 for cage populations, it is evident that the pigment contents of single and double heterozygotes fall well within the range obtained by sampling 'wild type' populations, although in homozygous form these mutants show drastic effects on brown pigment content at least.

2. Intermediate Profiles

As shown in Figure 15 in the Materials and Methods section, the brown pigment pathway is not a steady state system. Consequently, the three intermediates studied, tryptophan, kynurenine and 3-hydroxy-kynurenine were assayed at three time points: 72, 84 and 96 hours after pupation. The 96 hour time point is close to eclosion in the Oregon K strain. However, the duration of pupation is extended in some of the mutant strains. The three time points chosen cover approximately the second half of pupation.

Only mutant homozygotes and single heterozygotes, that is, progeny of crosses between mutant strains and Oregon K, were studied.

Experimental details

Large batches (over 200) of fertilised females were allowed to lay for one hour in half pint milk bottles containing Sang's Yeast Medium. The cultures were maintained at 25°C and the larvae grew more or less synchronously.

At 72, 84 or 96 hours after pupation, the pupae were gently removed from the sides of the bottles using a spatula. The intermediates were assayed as described in Materials and Methods. Three repeat determinations were carried out on each of three independent samples of pupae for each time point.

For the heterozygote cultures, mixtures of mutant and Oregon K females which had been mated to the appropriate males prior to egg-laying were used. That is, mutant females were mass-mated with Oregon K males, which were discarded before egg-laying. Oregon females were mated with mutant males, and these were also discarded. Mutant and Oregon females were then allowed to lay in the same bottles.



Results and Discussion

The results of the intermediate determinations are shown graphically in Figures 23 and 24. The data are given in Table A1 in the Appendix. Figure 23 shows the "intermediate profiles" of Oregon K, vermilion, cinnabar, cardinal, scarlet, karmoisin, light and lightoid.

Vermilion, which lacks tryptophan oxygenase, shows an increased tryptophan pool at each time point. However, the almost two-fold increase in the pool which is observed when the tryptophan oxygenase activity is reduced to zero suggests that it is unlikely that smaller reductions in enzyme activity will be reflected in an increased tryptophan pool. Both kynurenine and 3-hydroxykynurenine are barely detectable. This is to be expected if tryptophan oxygenase activity is necessary for the formation of xanthommatin intermediates at subsequent steps in the pathway.

Cinnabar, representing a loss of kynurenine hydroxylase activity lacks 3-hydroxykynurenine. There is no evidence of any real increase in the pool sizes of kynurenine or tryptophan. Again, as far as the prospect of detecting small changes (of less than 50%) in enzyme activity by investigating intermediate pools goes, this is a disappointing result.

The absence of a massive elevation in precursor pools when either tryptophan oxygenase or kynurenine hydroxylase activity is lacking is not necessarily surprising. Tryptophan, after all, is an amino acid involved in protein synthesis and other aspects of metabolism. The proportion of total tryptophan in the pupae which is diverted to xanthommatin synthesis may be quite small. It may be

Figure 23.

Pool levels of tryptophan, kynurenine and 3-hydroxy-kynurenine at 72, 84 and 96 hours post pupation in the "bright-eyed" mutants. 1 unit on the concentration axis represents 0.1 μ moles/gram live weight for tryptophan and kynurenine and 0.4 μ moles/gram live weight for 3-hydroxy-kynurenine.

INTERMEDIATE PROFILES OF 'BRIGHT' MUTANTS

▲ TRY ● KYN ■ 3-OH KYN

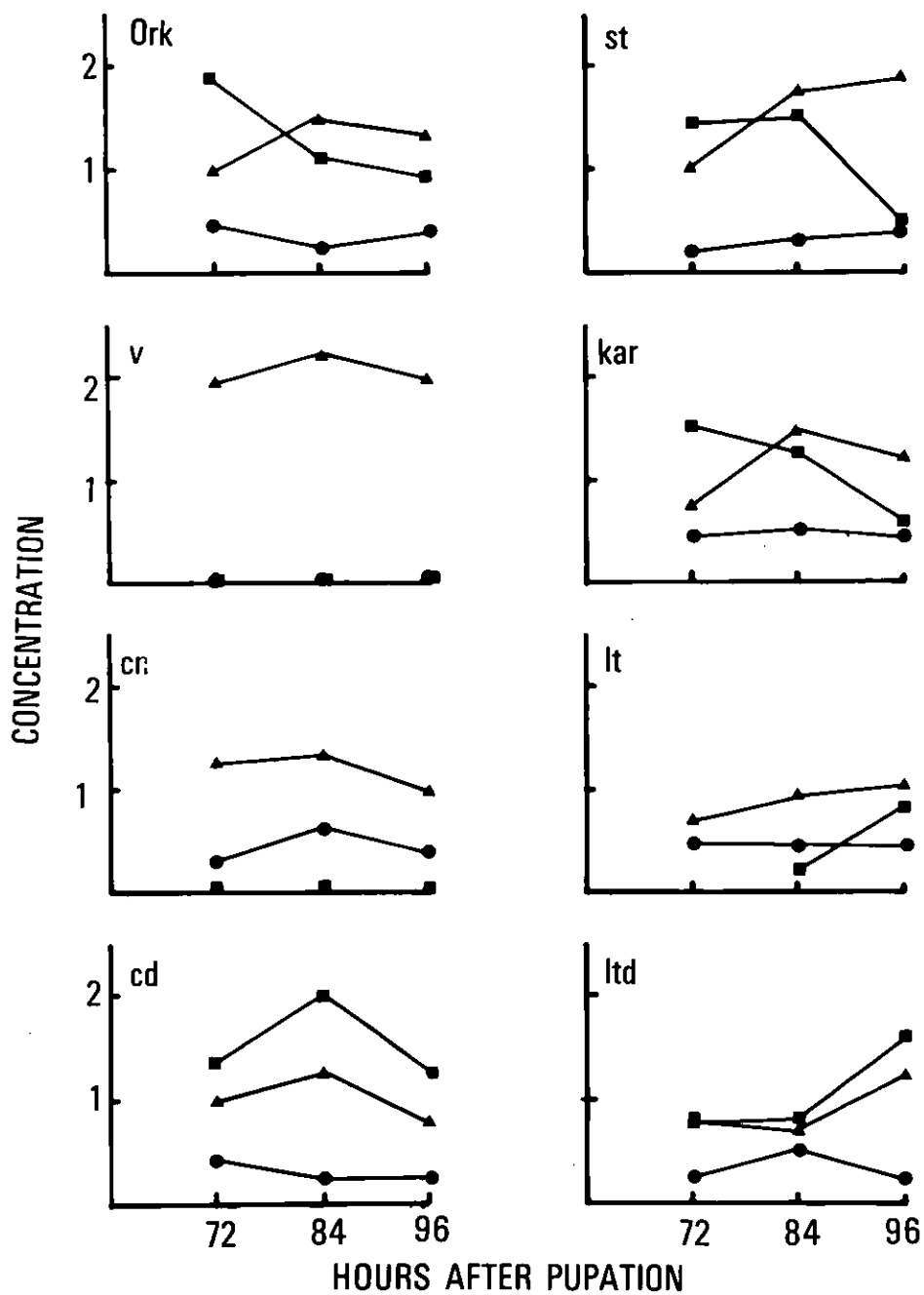
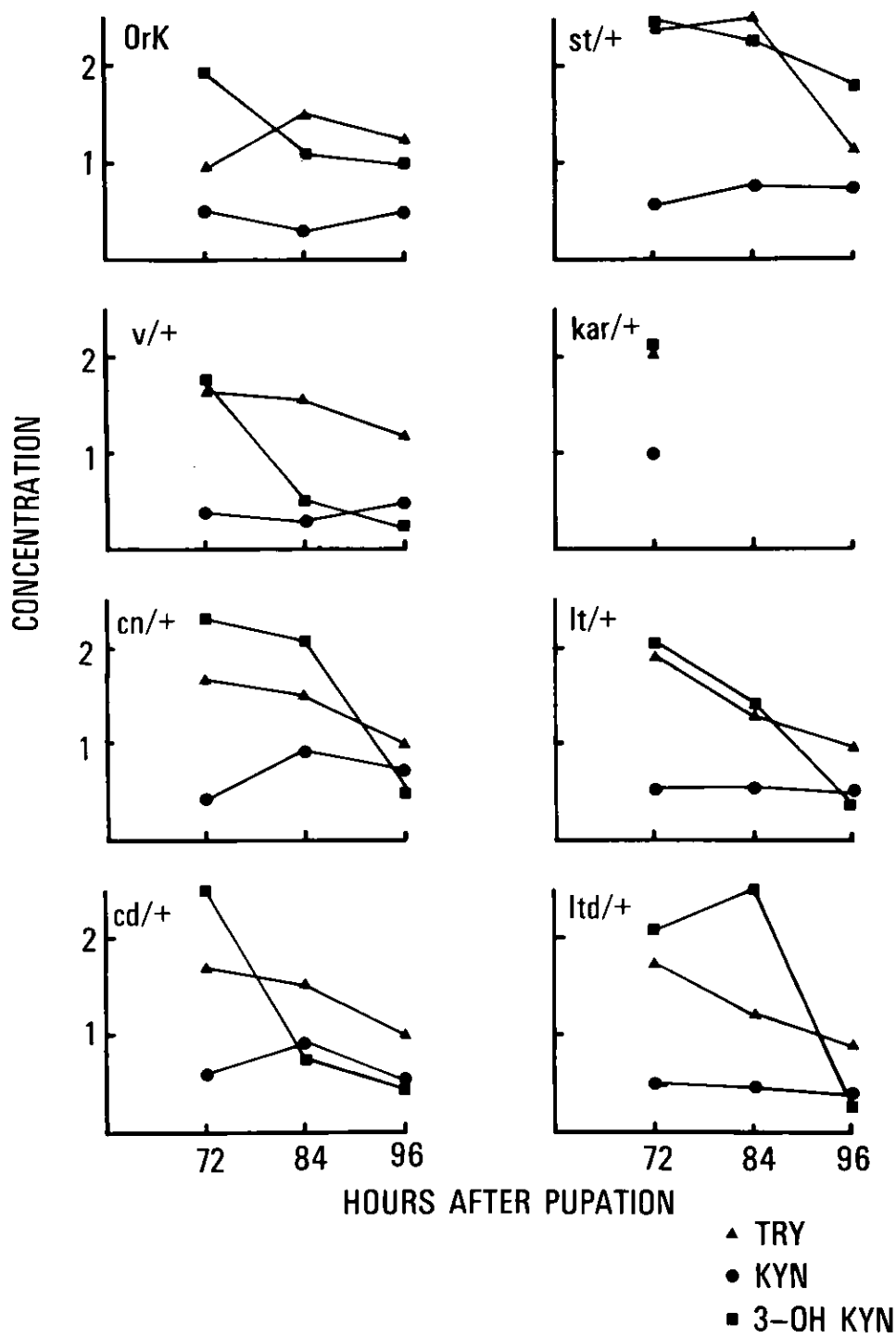


Figure 24.

Pool levels of tryptophan, kynurenine and 3-hydroxy-kynurenine at 72, 84 and 96 hours post pupation in heterozygotes of the "bright-eyed" mutants. Concentration scale as for Figure 23.

INTERMEDIATE PROFILES OF 'BRIGHT HETEROZYGOTES'



totally unrealistic to expect a dramatic rise in the total tryptophan pool simply because xanthommatin synthesis is blocked at an early stage. Any surplus in the tryptophan content caused by a block at tryptophan oxygenase may be readily diverted to some other aspect of tryptophan metabolism.

Similarly, any increase in the kynurenine pool occasioned by a block in kynurenine hydroxylase may be "mopped up" by a reverse reaction producing tryptophan which may then be used in other metabolic pathways.

Cardinal, which lacks the granule-bound phenoxazinane synthetase activity associated with xanthommatin synthesis, shows no dramatic increase in any of the precursors measured. At 84 hours after pupation there is a doubling of the 3-hydroxy kynurenine pool, but this increase has disappeared at 96 hours.

Scarlet, which appears to be deficient in the transport of 3-hydroxy kynurenine into the pigment granule, shows a steep drop in the pool of this compound between 84 and 96 hours after pupation. This agrees with the observation of Howells, Summers and Ryall (1977) that there is a dramatic fall in the 3-hydroxy kynurenine pool around the time of eclosion in the scarlet mutant.

Karmoisin appears to have little effect on any of the three intermediates, while light and lightoid appear to have greater effects on the 3-hydroxy kynurenine pool than on either the kynurenine or tryptophan pools.

Figure 24 shows the intermediate profiles of the heterozygotes. One point which must be remembered when considering the results of the vermillion x Oregon K F_1 . As vermillion is an X-linked gene, the

pupae which were assayed consisted of a mixture of heterozygous females, vermilion males and wild type males. The main point to note about the profile of the vermilion "heterozygotes" is that there is still a detectable increase in the tryptophan pool at 72 and 84 hours after pupation, although the pool has returned to the Oregon K level by 96 hours. Kynurenine and 3-hydroxy kynurenine are both present in appreciable quantities, although there is an exaggerated fall in the 3-hydroxy kynurenine pool.

The tryptophan and kynurenine pool levels are somewhat higher in cinnabar heterozygotes than in the cinnabar homozygote. 3-hydroxy kynurenine is present in reasonable abundance.

The levels of the tryptophan and 3-hydroxy kynurenine pools in the heterozygotes of cardinal, scarlet, light and lightoid show little resemblance to either the wild type or mutant pool levels. The kynurenine pool appears to be least affected. In general, the heterozygotes of these mutants show increases in the tryptophan pool at the earliest time points examined, although by 96 hours this pool is more or less at the wild type level in each case. There is less regularity in the behaviour of the 3-hydroxy kynurenine pool.

Two general conclusions can be drawn from the study of intermediate pool profiles in the bright eyed mutants.

The first is that it is unlikely that a study of intermediate pool levels could reveal any changes in enzyme activity less severe than a complete loss of activity. Even in this special case, the absence of metabolites subsequent to the particular step in the pathway is the only criterion that could be applied in this kind of study. There is no evidence of any dramatic increases in precursor

pools consequent on complete loss of enzyme activity.

The second conclusion is that there is no resemblance between the intermediate profiles of mutant homozygotes and their heterozygotes. This is not due simply to a complete dominance of the wild type allele; the intermediate profiles of the heterozygotes show little resemblance to the intermediate profile of the wild type.

The White Locus

Most students of genetics have heard the tale of how one day in 1910, a Drosophila with white eyes was spotted in Morgan's laboratory. The fly somehow escaped. The laboratory was sealed off, and everyone in the neighbourhood was set to tracking down this diminutive-and elusive -game. The fly, a male, was eventually caught and persuaded to breed. Thus the first recorded Drosophila mutant was preserved for posterity. Since 1910, the white locus has provided material for many researchers all over the world, but no one has yet shown definitely what function this locus controls in the fly.

Morgan showed that the gene causing white eyes is sex-linked. It was later mapped to 1 - 1.5. Over a hundred and fifty alleles have been reported at this locus, a number of them exhibiting varying degrees of pigmentation (Lindsley & Grell, 1967). These pigmented alleles have been used in extensive fine structure mapping studies by Judd (1964) and Green (1959). The results of this work are summarised in Figure 25. The locus appears to consist of at least five pseudoallelic sites, within which only unequal crossing over has been observed to occur. No complementation has been reported between alleles of this locus (except in the case of spotted-white, which shows other anomalies). That is, heterozygotes between the various alleles do not appear wild type.

Crossing over between coloured alleles of the same recombination group has occasionally been observed, giving rise to progeny having a "true" white phenotype. Judd (1964) showed that such recombinants behave like small deficiencies, arising from unequal crossing over. It has been suggested (Lewis, 1954) that the pseudoalleles of the white locus have arisen by duplication. Although there has been divergence

Figure 25.

Map of the white locus, from the work of Judd (1964)
and Green (1959).

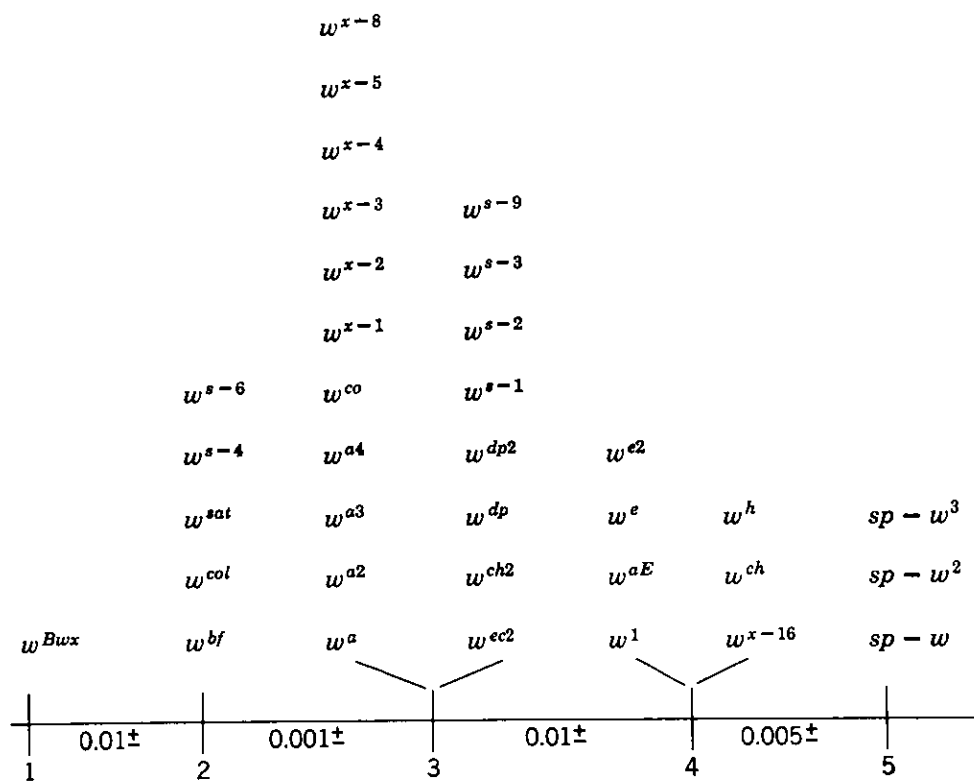


Figure 8.9. The white pseudoallelic loci.

between the duplicates over evolutionary time, the duplicates may still be sufficiently similar to pair, thus allowing for unequal crossing over to take place with a relatively high frequency. Some functional differences between the alleles have been reported. For example, the lemon-yellow eyed phenotype of zeste homozygotes is suppressed in flies heterozygous for mutants of sites four or five of the white region. Zeste is another first chromosome eye colour mutant whose expression requires the presence of two w^+ or one w^+ and a mutant allele of sites 1, 2 or 3 of the white locus for expression. Zeste is expressed in males only when they carry an extra copy of white. Interactions with various enhancer or diluter loci also discriminate between different pseudoallelic sites of the white locus.

Precursors of pteridine synthesis are excreted in the meconium of white flies early in adult life (Ziegler, 1961). Intermediates of the brown pathway can be detected in the pupae, although these also are lost in early adulthood (Howells, Summers & Ryall, 1977, and following). Shoup (1966) reports the presence of granules of unusual and variable morphology, often containing glycogen, ribosomes and showing acid phosphatase activity coupled with a complete lack of the ommochrome or drospterin types of pigment granules in the eyes of white flies.

These observations reduce the probability of white mutants affecting an early common step in both pathways, as suggested by Nolte (1953). They do not rule out the possibility that the "white product" is an essential component of both types of granules.

Gosh and Forrest (1967) suggested that the product of the white

locus is an enzyme in the drosopterin synthesis pathway. They had shown an in vitro inhibition of tryptophan oxygenase by pteridines of the biopterin type, and found reduced levels of kynurenine 3-hydroxylase in white mutants. This they hypothesised to be due to lack of a pteridine cofactor. If white mutants are completely blocked at an enzyme step subsequent to biopterin synthesis, the resulting accumulation of biopterin would almost completely inhibit tryptophan oxygenase. Failure to synthesise the supposed pteridine cofactor would render the kynurenine 3-hydroxylase ineffective, and by a combination of these two effects, brown pigment synthesis would be prevented. However, there is evidence that white pupae synthesise kynurenine and 3-hydrokynurenine in a manner which disagrees with Gosh and Forrest's expectations (Howells, Summers & Ryall, 1977, and following).

A summary of additional information on the white locus which does not appear to be relevant to the present investigation is given in the Appendix (A.1.4).

The pigmented alleles of the white locus have been used in this study to investigate two separate questions. The first of these concerns the interactions between the two pigment pathways at the level of the white locus. This was pursued by studying the relationship between the two pigments in heterozygous combinations of a selection of fourteen alleles at the white locus.

The second question, important to the interpretation of the study of the "wild type" variation in the brown pigment pathway, is whether or not it is possible to detect alleles at a locus by examining the profiles of metabolic intermediates. By comparing the profiles of a number of white locus alleles which had been backcrossed at least six times to a common genetic background (i.e. the inbred Oregon K strain),

it is possible to make predictions about the likelihood of detecting "minor alleles" at other loci for which intermediate profiles of major mutants may be obtained.

Interactions between Red and Brown Pigments of White Locus Alleles

1. Experimental Details

Crosses were set up to obtain all possible heterozygous combinations between the thirteen alleles which had been backcrossed to Oregon K, and between each allele and the Oregon K "wild type". The resulting heterozygotes are listed in Table 11. Six single pair matings for each cross were set up on Sang's Yeast Medium. After two days, the parents were discarded, and the cultures were maintained under standard conditions. At eclosion, five females were collected from each of four vials for each cross, and allowed to age for seven days. At the end of this time, an attempt was made to score the heterozygotes visually according to their resemblance to the parental phenotypes. In order to do this, the thirteen mutant alleles were split into the seven groups between which it was easy to distinguish on visual scoring, and the heterozygotes were assigned to one of these groups on the basis of microscopic examination. The red and brown pigments were then measured by the double extraction technique on each fly. Means and standard errors were obtained for both pigments for each of the 105 genotypes. Means were also expressed as percentages of the Oregon K mean.

The dominance coefficient for each pigment for each combination of alleles was calculated according to Wright's formula:

$$\text{dominance coefficient (D.C.)} = \frac{\text{homozygote}_1 - \text{heterozygote}}{\text{homozygote}_1 - \text{homozygote}_2}$$

Homozygote₁ in this case was always taken as the higher parental value.

Results and Discussion

The results of the subjective scoring are shown in Table 10. The three alleles which fall into the two most darkly pigmented classes (cf, sat and Bwx) are dominant over all others. The eyes of Brownex flies resemble those of the brown mutant, being dull brown in colour, while the eyes of coffee and satsuma flies are considerably darker. Groups 3 and 4 have eyes of reddish-orange. Coral, in class 4, is dominant over the very light alleles, shows intermediacy in combination with apricot and honey is recessive to the darker alleles and unpredictable in its behaviour in combination with the slightly lighter group 3 alleles. Among the lighter alleles, there is little consistency in the dominance relationships in crosses with other members of groups 0, 1, 2 or 3, while all tend to be recessive in combination with darker alleles. The wild type is not included in this scale. It is fully dominant at this level of scoring over all the white alleles examined. The wild type colour is not part of the series of progressively darker shades exhibited by the white alleles, which makes it difficult to assign a "class" of phenotype to it.

The data on the extracted pigments are given in Table 11, and the percentage values of red and brown are shown in Figure 26 and Table 12.

Where the higher parental type is completely dominant, the

TABLE 10

SUBJECTIVE SCORES OF WHITE LOCUS HETEROZYGOTES

	w	bf	ec	a	h	e	bl	cp	ch	co	Bwx	cf	sat
w	0	0	1	1	1	1	2	1	1	4	5	6	6
	bf	1	1	1	1	1	2	2	2	4	6	6	6
		ec	1	1	1	2	2	2	3	4	5	6	6
			a	2	1	2	2	2	3	3	6	6	6
				h	2	1	2	3	2	3	5	6	6
					e	3	2	3	3	3	5	6	6
						bl	3	3	3	4	5	6	6
							cp	3	3	3	5	6	6
								ch	3	4	5	6	6
									co	4	5	6	6
										Bwx	5	6	6
											cf	6	6
												sat	6

Homozygotes were classified according to eye colour on a scale 0-6.

Double heterozygotes were then assessed using the homozygote scale as a basis for classification.

TABLE 11

Red and Brown Pigment Contents and Dominance Coefficients of White
Locus Heterozygotes.

w genotype	red pigment	% red	D.C. red	brown pigment	% brown	D.C. brown
+/+	112.51 $\pm$ 5.18	100	-	39.03 $\pm$ 3.08	100	-
+/w	100.80 $\pm$ 5.70	89.6	0.10	37.25 $\pm$ 1.58	95.5	0.45
+/bf	59.36 $\pm$ 2.54	53.0	0.53	26.09 $\pm$ 0.5	66.9	0.54
+/ec	101.25 $\pm$ 4.40	90.4	0.11	53.39 $\pm$ 2.55	136.9	-1.91
+/ce	111.33 $\pm$ 1.60	99.4	0.12	28.16 $\pm$ 3.93	72.2	0.49
+/h	76.27 $\pm$ 2.32	68.1	0.35	28.55 $\pm$ 1.22	73.2	0.54
+/e	87.58 $\pm$ 3.04	78.2	0.25	60.53 $\pm$ 1.58	155.2	-9.50
+/bl	92.64 $\pm$ 4.75	82.7	0.19	44.46 $\pm$ 2.11	114.0	-0.32
+/cp	71.90 $\pm$ 2.00	64.2	0.41	60.84 $\pm$ 1.87	156.1	-1.09
+/ch	61.72 $\pm$ 3.45	55.4	0.50	31.32 $\pm$ 1.78	80.3	0.38
+/co	120.40 $\pm$ 6.63	107.5	-0.08	36.50 $\pm$ 1.96	93.6	0.15
+/Bwx	101.92 $\pm$ 3.32	91.0	0.10	50.93 $\pm$ 1.87	130.6	0.73
+/cf	96.99 $\pm$ 5.61	86.6	0.23	48.44 $\pm$ 2.92	124.2	-1.45
+/sat	106.62 $\pm$ 2.25	95.2	0.07	31.36 $\pm$ 1.89	80.4	0.48
sat/sat	21.50 $\pm$ 1.59	19.2	-	23.11 $\pm$ 0.80	59.3	-
sat/w	14.67 $\pm$ 1.20	13.1	0.32	18.89 $\pm$ 0.49	48.4	0.18
sat/bf	15.34 $\pm$ 1.11	13.7	0.66	20.12 $\pm$ 2.33	51.6	0.38
sat/ec	23.74 $\pm$ 1.24	21.2	-0.17	24.84 $\pm$ 1.58	63.7	-1.42
sat/a	11.98 $\pm$ 0.97	10.7	1.29	19.27 $\pm$ 2.47	49.4	0.61
sat/h	15.01 $\pm$ 0.32	13.4	0.50	13.42 $\pm$ 1.02	34.4	2.74
sat/e	17.02 $\pm$ 0.37	15.2	0.42	10.18 $\pm$ 2.46	26.1	1.91
sat/bl	17.02 $\pm$ 0.02	15.2	0.35	16.38 $\pm$ 0.97	42.0	5.40
sat/cp	22.06 $\pm$ 1.46	19.7	-0.06	13.18 $\pm$ 1.16	33.8	2.40
sat/ch	19.71 $\pm$ 1.32	17.6	0.16	17.63 $\pm$ 1.80	45.2	0.88
sat/co	12.66 $\pm$ 1.08	11.3	0.86	37.75 $\pm$ 1.75	96.8	-46.88
sat/Bwx	23.07 $\pm$ 0.73	20.6	-0.14	33.54 $\pm$ 4.64	86.0	-22.25
sat/cf	27.10 $\pm$ 2.33	24.2	0.75	22.85 $\pm$ 1.57	58.6	1.01
cf/cf	44.33 $\pm$ 5.61	39.6	-	32.70 $\pm$ 1.83	83.8	-
cf/w	34.72 $\pm$ 2.57	31.0	0.22	40.99 $\pm$ 8.05	105.1	-0.25
cf/bf	24.64 $\pm$ 2.04	22.0	0.61	59.63 $\pm$ 3.74	152.9	-1.54
cf/ec	48.50 $\pm$ 2.92	43.3	1.17	52.81 $\pm$ 2.50	135.4	-0.93
cf/a	37.74 $\pm$ 2.18	33.7	0.22	37.25 $\pm$ 5.40	95.5	-0.29

Table 11 (Contd....)

cf/h	35.06 $\pm$ 1.00	31.3	0.26	38.49 $\pm$ 1.00	98.7	-0.47
cf/e	43.12 $\pm$ 0.58	38.5	0.04	22.62 $\pm$ 0.20	58.0	0.62
cf/bl	41.78 $\pm$ 3.16	37.3	0.08	34.79 $\pm$ 2.75	89.2	-0.19
cf/cp	12.32 $\pm$ 1.03	11.0	1.01	32.80 $\pm$ 1.81	84.1	-0.01
cf/ch	15.68 $\pm$ 0.25	14.0	0.83	33.54 $\pm$ 1.22	86.0	-0.05
cf/co	28.45 $\pm$ 1.22	25.4	0.48	16.89 $\pm$ 1.56	43.3	1.60
cf/Bwx	28.45 $\pm$ 1.22	25.4	0.47	35.53 $\pm$ 3.31	91.1	-0.31
Bwx/Bwx	10.08 $\pm$ 1.12	9.0	-	22.66 $\pm$ 1.18	58.1	-
Bwx/w	18.37 $\pm$ 1.00	16.4	-0.82	30.30 $\pm$ 2.04	77.7	-0.34
Bwx/bf	18.03 $\pm$ 2.08	16.1	-2.73	45.47 $\pm$ 1.89	116.6	-3.03
Bwx/ec	19.04 $\pm$ 0.98	17.0	-7.28	51.40 $\pm$ 3.39	131.8	-2.47
Bwx/a	13.33 $\pm$ 1.22	11.9	0.19	38.77 $\pm$ 1.59	99.4	-2.75
Bwx/h	27.78 $\pm$ 1.63	24.8	-11.8	35.76 $\pm$ 0.49	91.7	-4.27
Bwx/e	17.36 $\pm$ 0.51	15.5	-8.33	44.46 $\pm$ 0.37	114.0	-3.45
Bwx/bl	19.71 $\pm$ 0.92	17.6	-8.82	36.78 $\pm$ 1.60	94.3	-18.1
Bwx/cp	14.34 $\pm$ 0.93	12.8	-0.72	23.60 $\pm$ 1.06	60.5	-0.15
Bwx/ch	5.04 $\pm$ 1.76	4.5	49.6	44.46 $\pm$ 3.01	114.0	-3.78
Bwx/co	22.96 $\pm$ 2.17	20.5	10.82	27.03 $\pm$ 1.18	69.3	-27.00
co/co	11.11 $\pm$ 0.98	9.9	-	22.83 $\pm$ 1.96	58.5	-
co/w	8.4 $\pm$ 1.58	7.5	0.24	26.83 $\pm$ 1.44	68.8	-0.18
co/bf	5.38 $\pm$ 1.11	4.8	6.20	23.09 $\pm$ 2.91	59.2	-0.04
co/ec	5.71 $\pm$ 0.98	5.1	2.35	30.54 $\pm$ 2.04	78.3	-0.66
co/a	9.63 $\pm$ 1.53	8.6	1.49	20.12 $\pm$ 1.74	51.6	0.45
co/h	6.38 $\pm$ 1.46	5.7	1.82	25.08 $\pm$ 2.03	64.3	-0.70
co/e	1.34 $\pm$ 0.37	1.2	39.08	26.09 $\pm$ 1.60	66.9	-0.83
co/bf	8.06 $\pm$ 1.11	7.2	1.35	33.77 $\pm$ 1.02	86.6	-11.71
co/cp	12.99 $\pm$ 1.15	11.6	-11.56	18.64 $\pm$ 0.84	47.8	1.09
co/ch	16.85 $\pm$ 0.37	14.6	-4.60	20.63 $\pm$ 1.03	52.9	0.37
ch/ch	9.97 $\pm$ 0.92	8.9	-	38.12 $\pm$ 1.87	42.31	-
ch/w	8.06 $\pm$ 1.11	7.2	0.19	22.62 $\pm$ 1.59	58.0	-0.34
ch/bf	9.41 $\pm$ 0.92	8.4	1.25	18.37 $\pm$ 1.50	47.1	-0.84
ch/ec	13.66 $\pm$ 0.86	12.2	-3.24	18.14 $\pm$ 1.44	46.5	-0.21
ch/a	2.02 $\pm$ 0.97	1.8	2.92	21.10 $\pm$ 2.00	54.1	-54.00
ch/h	7.06 $\pm$ 0.80	6.3	1.99	20.67 $\pm$ 3.84	50.3	-0.14
ch/e	21.06 $\pm$ 1.03	18.8	11.46	28.31 $\pm$ 1.53	72.6	-20.96
ch/bl	-	-	-	-	-	-

Table 11 (Contd....)

ch/cp	0.67 $\pm$ 0.25	0.6	4.62	23.60 $\pm$ 1.87	60.5	-2.19
cp/cp	12.54 $\pm$ 0.20	11.2	-	18.99 $\pm$ 1.87	48.7	-
cp/w	4.03 $\pm$ 1.08	3.6	0.68	23.83 $\pm$ 1.66	61.1	-0.25
cp/bf	13.22 $\pm$ 1.16	11.8	-2.06	14.90 $\pm$ 1.22	38.2	1.06
cp/ec	10.98 $\pm$ 1.03	9.8	0.44	22.35 $\pm$ 1.84	57.3	-0.42
cp/a	7.06 $\pm$ 0.80	6.3	4.49	24.84 $\pm$ 3.16	63.7	-2.68
cp/h	16.02 $\pm$ 0.25	14.3	-8.64	24.57 $\pm$ 0.20	63.0	-8.53
cp/e	1.34 $\pm$ 0.20	1.2	6.67	22.62 $\pm$ 1.53	58.0	-1.37
cp/bl	21.73 $\pm$ 2.00	19.4	-2.49	13.65 $\pm$ 1.83	35.0	2.85
bl/bl	8.85 $\pm$ 0.73	7.9	-	21.38 $\pm$ 2.20	56.1	-
bl/w	16.35 $\pm$ 0.20	14.6	-0.85	20.63 $\pm$ 1.03	52.9	0.06
bl/bf	32.82 $\pm$ 1.67	29.3	-6.14	9.67 $\pm$ 0.80	24.8	1.00
bl/ec	55.78 $\pm$ 1.89	49.8	- ∞	27.96 $\pm$ 3.79	71.7	-0.56
bl/a	18.37 $\pm$ 1.00	16.4	-0.81	22.35 $\pm$ 2.00	57.3	-0.09
bl/h	8.74 $\pm$ 1.42	7.8	0.68	27.96 $\pm$ 1.44	71.7	-2.66
bl/e	21.73 $\pm$ 2.00	19.4	-5.4	13.18 $\pm$ 1.33	33.8	1.57
e/e	10.86 $\pm$ 0.37	9.7	-	16.34 $\pm$ 1.85	41.9	-
e/w	2.69 $\pm$ 0.87	2.4	0.75	24.84 $\pm$ 1.58	63.7	-0.52
e/bf	32.48 $\pm$ 0.40	79.0	-15.01	8.46 $\pm$ 0.74	21.7	6.52
e/ec	33.60 $\pm$ 0.32	30.0	-11.31	7.68 $\pm$ 0.73	19.7	1.63
e/a	11.09 $\pm$ 1.03	9.9	0.93	17.39 $\pm$ 1.87	44.6	-1.25
e/h	7.50 $\pm$ 0.97	6.7	1.43	18.64 $\pm$ 2.04	47.8	0.29
h/h	8.51 $\pm$ 0.93	7.6	-	19.58 $\pm$ 1.83	50.2	-
h/w	0.00 $\pm$ 0.00	0.0	1	22.35 $\pm$ 1.22	57.3	-0.14
h/bf	16.35 $\pm$ 0.37	14.6	-1.12	23.28 $\pm$ 1.25	59.7	-0.83
h/ec	26.77 $\pm$ 2.66	23.9	-52.73	21.37 $\pm$ 0.96	54.8	-0.21
h/a	16.35 $\pm$ 0.66	14.6	-0.40	24.34 $\pm$ 1.29	62.4	-1.72
a/a	14.11 $\pm$ 0.49	12.6	-	16.81 $\pm$ 0.49	43.1	-
a/w	4.37 $\pm$ 1.03	13.9	0.69	26.83 $\pm$ 1.44	68.8	-0.59
a/bf	33.15 $\pm$ 0.37	29.6	-10.02	7.45 $\pm$ 1.38	19.1	5.58
a/ec	11.65 $\pm$ 1.76	10.4	0.47	36.50 $\pm$ 2.94	93.6	-3.41
ec/ec	8.85 $\pm$ 2.04	7.9	-	11.04 $\pm$ 1.44	28.3	-
ec/w	6.05 $\pm$ 1.33	5.4	0.32	19.34 $\pm$ 2.74	49.4	-0.75
ec/bf	10.42 $\pm$ 1.74	9.3	0.54	18.14 $\pm$ 1.29	46.5	-0.73
bf/bf	12.21 $\pm$ 1.37	10.9	-	15.13 $\pm$ 1.17	38.8	-
bf/w	0.34 $\pm$ 0.20	0.3	0.97	20.12 $\pm$ 0.73	51.6	-0.82
w/w						*

* the absolute values obtained for w/w flies were subtracted from all the other values, as was done for the other mutant pigment determinations.

Figure 26.

Red and brown pigments of white locus mutants and their heterozygotes expressed as percentages of the Oregon K pigment contents. Homozygotes are represented by squares, heterozygotes by circles. The dotted lines in the upper right corner enclose the "wild type" heterozygotes.

	w
	w bf
	w ec
	w a
	w h
	w e
	w bl
	w cp
	w ch
	w co
	w Bwx
	w cf
	w sat

RED AND BROWN PIGMENT CONTENTS OF WHITE LOCUS HETEROZYGOTES

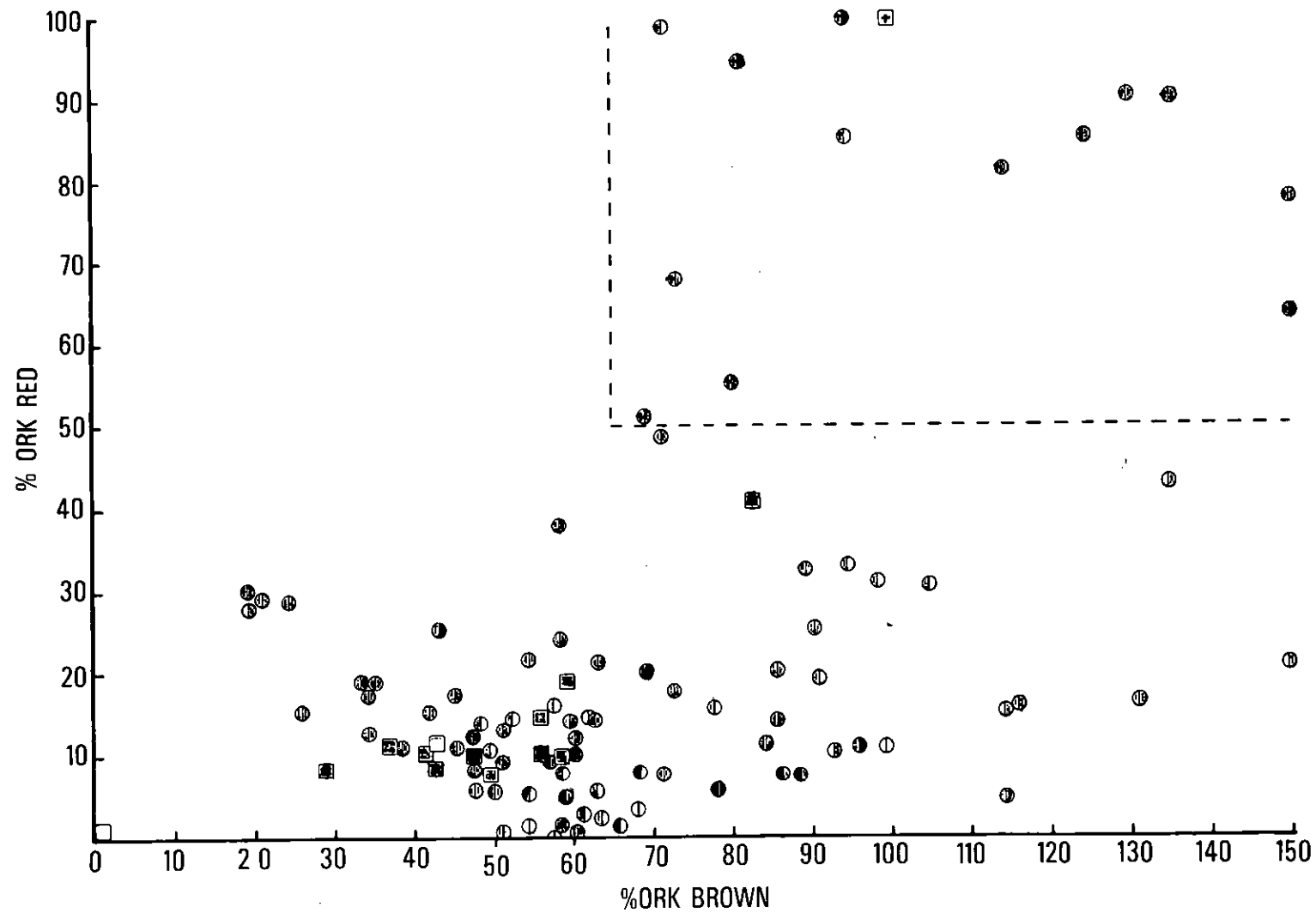


TABLE 12

Brown and Red Pigments of White Heterozygotes as % of Oregon K.brown pigment

	O	28	38	42	42	43	49	50	56	58	58	59	84
	w	ec	bf ²	e	ch	a	cp	h	bl	Bwx	co	sat	cf
w	O	<u>49</u>	<u>52</u>	<u>64</u>	<u>58</u>	<u>69</u>	<u>61</u>	<u>57</u>	<u>53</u>	<u>78</u>	<u>69</u>	48	<u>105</u>
	ec	28	<u>47</u>	20	47	<u>94</u>	<u>57</u>	55	<u>72</u>	<u>132</u>	<u>78</u>	<u>64</u>	<u>135</u>
		bf ²	38	22	47	19	38	<u>60</u>	25	<u>117</u>	59	52	<u>153</u>
			e	42	<u>73</u>	45	<u>58</u>	48	34	<u>114</u>	<u>67</u>	26	58
				ch	42	54	<u>61</u>	50		<u>114</u>	53	45	<u>86</u>
					a	43	<u>64</u>	<u>62</u>	57	<u>99</u>	52	49	<u>96</u>
						cp	49	<u>63</u>	35	61	48	34	<u>84</u>
							h	50	<u>72</u>	<u>92</u>	<u>64</u>	34	<u>99</u>
								bl	56	<u>94</u>	<u>87</u>	42	<u>89</u>
									Bwx	58	69	<u>86</u>	<u>91</u>
										co		<u>97</u>	43
											sat	59	59
												cf	84

red pigment

	O	8	11	10	8	13	11	8	7	10	10	19	40
	w	ec	bf ²	e	ch	a	cp	h	bl	Bwx	co	sat	cf
w	O	5	0	2	7	4	4	0	<u>15</u>	<u>16</u>	8	13	31
	ec	8	9	<u>30</u>	12	10	10	<u>24</u>	<u>50</u>	<u>17</u>	5	21	43
		bf ²	11	<u>29</u>	8	<u>30</u>	12	15	<u>29</u>	<u>16</u>	5	14	22
			e	10	<u>19</u>	10	1	7	<u>19</u>	<u>16</u>	1	15	39
				ch	8	2	1	6		5	<u>15</u>	18	14
					a	13	6	15	<u>16</u>	12	9	11	34
						cp	11	14	<u>19</u>	13	12	11	11
							h	8	9	<u>25</u>	6	13	31
								bl	7	<u>18</u>	7	15	37
									Bwx	10	<u>21</u>	21	25
										co	10	20	25
											sat	19	24
												cf	40

Cases of overdominance are underlined.

dominance coefficient (D.C.) = 0. Intermediacy yields a D.C. of 0.5, while complete dominance of the lower parental type is represented by D.C. = 1. Overdominance results in D.C. values of less than zero or greater than one, the sign giving the direction of overdominance.

There are difficulties in interpreting the results where the dominance coefficients exceed unity. Large dominance coefficients can arise when both homozygotes have similar values (giving rise to a small denominator in the D.C. equation). In this case, the heterozygote value need not be greatly in excess of the higher homozygote value. Large values may also be obtained when the heterozygote value is substantially greater than the higher homozygote, with a reasonable disparity between the two homozygotes. There is no intuitively obvious way of scaling the results to allow discrimination between these two extreme situations.

Consideration of the dominance coefficients shows an overall tendency for the higher content parental type to be dominant. It should be noted that the red and brown pigments appear to behave independently; that is while one allele can be dominant over another for red pigment, the dominance relationships for the brown pigment may be reversed, depending on which parental type has the higher pigment content.

Dominance coefficients are not a very useful guide when it comes to examining cases of complementation. A number of combinations show apparent overdominance for the red pigment; that is, the dominance coefficient is negative. However, when the errors attached to each pigment value are taken into account, many of the apparent cases of overdominance or complementation for the red pigment are more

properly to be considered as simply cases of complete dominance of the allele having the higher red pigment content.

On the other hand, a number of genuine cases of overdominance or complementation for the brown pigment were observed. This is illustrated by reference to Figure 26.

From this, it can be seen that white^{coffee} has a red pigment content of 40% of the Oregon K value, representing the highest value observed in white allele homozygotes. None of the heterozygotes surpass, or even equal, this value. On the other hand, a number of heterozygotes have brown pigment contents considerably higher than w^{ef}'s 83%. Some indeed have higher brown contents than the wild type.

For example:	cross	$\frac{w^{sat}}{w}$	x	$\frac{w^{co}}{w}$	& wild type brown:	96.8
	"	$\frac{w^{cf}}{w}$	x	$\frac{w}{w}$	" " "	105.1
	"	$\frac{w^{cf}}{w}$	x	$\frac{w^{ec}}{w}$	" " "	135.4
	"	$\frac{w^{bwx}}{w}$	x	$\frac{w^{bf}}{w}$	" " "	116.6
		$\frac{w^{bwx}}{w}$	x	$\frac{w^{ch}}{w}$	" " "	114.0

Although on visual scoring none of the heterozygotes appear wild type, the evidence of the extracted pigments shows that for the brown pigment, complementation between alleles of the white locus can - and does - occur. This observation adds weight to the argument that the white locus is unlikely to control a common early step in both pathways in the manner suggested by Nolte (1953). Such a suggestion appears improbable in any case in view of current knowledge of the biosynthesis of the two pigments, as the red is a product of purine metabolism and the brown is a tryptophan derivative. The apparent inability of white alleles to complement for red pigment combined with the observed complementation for brown would be hard to explain if a common step were indeed affected.

Other Hypotheses

The data also tend to contradict Gosh and Ferrest's (1967) suggestion that the white locus controls an enzyme step in the pathway subsequent to bipterin synthesis. They suggest that the lack of brown pigment in white mutants is a pleiotropic effect resulting from the inhibition of tryptophan oxygenase by bipterin, accumulating as a result of the enzyme block. On the basis of this hypothesis it could be argued that complementation in the red pathway is a necessary prerequisite for "complementation" in the brown (i.e. a pleiotropic reduction in the inhibition of tryptophan oxygenase by bipterin).

On the basis of a similarity of 3-hydroxykynurenine metabolism in white and scarlet, Howells, Summers and Ryall (1977) have suggested that the white locus mutants are defective in the transport system which allows ommochrome intermediates access to the ommatidia or to the granules within the ommatidia. A similar transport mechanism may operate for the red pigment intermediates.

Ziegler and Jaenicke (1959) isolated "core granules" from the eyes of wild type and white mutants, and, although they found the same protein: RNA ratio in the two genotypes, differences in some enzyme activities were noted. However, it is not very clear what the isolated granules actually represent. Shoup (1966) in a detailed study found four types of granules in *Drosophila* eyes. The type of granule she designated as type I contained ommochrome, and was found in primary and secondary pigment cells of the wild type and of the brown mutant. These granules appeared to originate from

the Golgi apparatus, their first appearance in the eyes coinciding with the onset of xanthommatin accumulation at 48 hours after pupation. Type II granules, found in the secondary pigment cells of the wild type and of the vermillion mutant contain drosopterin. These granules were first detected about 60 hours after pupation as 60 - 80 angstrom fibres forming bundles which develop into elliptical granules.

Type III granules, which were found in the secondary pigment cells of the brown and vermillion mutants, and occasionally in wild type appear to originate from the Golgi apparatus and contain small quantities of a pigment. These granules, often irregular in shape, were interpreted as being abnormal type I granules. The type of pigment they contain was not identified and may have been either ommochrome or drosopterin.

A fourth type of granule (type IV) was found in the eyes of white flies. These usually appeared at around 60 hours after pupation, and were of variable appearance. These granules were shown to contain ribosomes, glycogen and often to have acid phosphatase activity. Shoup describes these as "lysosome-like". Occasionally, type IV granules were found in the eyes of late brown pupae. They were consistently observed in the eyes of v;bw pupae; and were always associated only with the unpigmented ommatidia of variegated Dp(w^m) flies. The wild type ommatidia in the eyes of these flies showed both type I and type II granules. As type I and type II granules were never found in the eyes of flies which had white eyes, and type I were only found in those flies whose eyes

contained ommochrome while type II were only present in eyes containing drosopterin, Shoup suggests that the two pigments are essential to maintain the integrity of their respective granule types. The type IV granules may represent "pigment" granules in varying stages of autolysis. The fact that type IV granules are characteristic of v₁bw shows that they are associated with a lack of pigment. It does not help in discriminating whether or not these granules are present in white flies as a consequence of the inability of these flies to synthesise the pigments or that inability to produce competent granular structure with a subsequent failure to accumulate pigments is closer to the primary defect in white mutants.

Phillips, Forrest and Kulkarni (1972) proposed a model of structural relationships of the enzymes in the ommochrome pathway. In essence, this scheme suggests that "phenoxazinone synthetase (P.S.) complexes" are formed in the ommatidia prior to 48 hours post pupation. These complexes are hypothesised to all the enzymes of the pathway. Around 48 hours post pupation, synthesis of xanthommatin begins, with an "activation" of phenoxazinone synthetase. As the xanthommatin is reduced to dehydroxanthommatin, it forms an association with the PS complex, inhibiting the phenoxazinone synthetase activity in much the same way as tyrosinase is inhibited by melanin (Kotser, personal communication). The product of the white locus, according to this scheme, would be a "matrix" protein, required to hold the multi-enzyme complex in the correct spatial orientation for the pathway to function normally. It is further postulated that the enzymes of drosopterin synthesis are also held

in association in a similar fashion by the white locus product.

This model is based on observations on the mutants brown, vermillion, cinnabar, scarlet, light, lightoid, cardinal and white. The data, culled from a dozen papers by various authors, are less than a satisfactory basis for the hypothesis as most of the observations were made on adult flies, whereas Phillips et al. are concerned to explain events which occur half-way through pupation and earlier. Although they report that white flies lack 3-hydroxykynurenine, Howells, Summers and Ryall (1977) have shown this to be accumulated in white pupae and rapidly excreted on eclosion. The main justification for the model is that each of the mutants which lack xanthommatin show greatly reduced levels of phenoxazinone synthetase.

Since it has been demonstrated (Rizki and Rizki, 1966⁶) that the steps leading to 3-hydroxykynurenine at least are operating in the Malpighian tubules as early as 3rd instar larvae, and Sullivan and Sullivan (1975) and Howells and Ryall (1975) have shown that the intermediates, rather than tryptophan, are transported into the ommatidia, the multi-enzyme complex in the eye seems an unlikely entity. Further, although the phenoxazinone synthetase activity clearly seems to be associated with the pigment granule, Phillips et al. do not report any attempts to assay the extracted granules for the other enzyme activities. Their data are consistent with the induction or activation of phenoxazinone synthetase activity by some signal which requires that certain - or all - of the steps required for xanthommatin synthesis are functional up to a given point.

Their claim that dihydroxanthommatin inhibits phenoxazinone synthetase activity is based on the observation that intact ommochrome granules show no synthetase activity in the test tube, while granules from which the pigment has been extracted do. However, as the extraction process ruptures the granule, it is impossible to distinguish between enzyme inhibition or failure of the substrate to penetrate the intact granule membrane in vitro.

Beadle and Ephrussi (1936a, b) showed by transplant experiments that white is autonomous. However, a series of transplants involving wild type, v, cn, w^av or w^a;cn as either hosts or eye anlage donors, showed that w^a eyes at least are capable of taking up kynurenine and 3-hydroxykynurenine, and subsequently synthesising small quantities of brown pigment.

On the evidence currently available, it is not possible to discriminate between two models for the function of the white locus "product":

- (1) Failure of the mutant "granular structure" to provide the conditions necessary for the final stages of pigment synthesis followed by the subsequent autolysis of the granules.
- (2) A hypothesis which would place the lesion in a transport mechanism (or mechanisms) which normally ensures that the precursors of both pigments are transported into the pigment cells of the ommatidium.

From the observation on non-complementation of red associated with complementation of brown (Fig. 26, and Table 11), we must conclude that the 'white product' enters in different ways in the red

and brown pathways. If we postulate that a monomeric version of the white locus product (i.e. a single polypeptide) is required for normal function of Shoup's type II (red pigment) granules, while an oligomeric version of the white product (an association of two or more white polypeptides) is a component of the type I (brown pigment) granules, then the differences in complementation between the two pigments observed in this series of crosses may be explained. In the case of the red pathway, the heterozygote will have two alternative mutant forms of the polypeptide. In the case of the brown pathway there is the possibility that an association of the two forms of polypeptide present in the heterozygote may prove more effective than an oligomer composed entirely of one or the other. More pigment may be formed, and complementation would then be observed (see Fincham, 1978, for details of molecular mechanisms of complementation).

The "transport" hypothesis would imply that monomers of the white locus product were involved in red precursor transport, while oligomers were required in brown pigment transport. This latter suggestion receives some support from the work of Sullivan and Sullivan (1975), who found greatly reduced rates of uptake of tritiated kynurenine in the eyes and Malpighian tubules of white pupae and larvae in vitro. They do, however, admit that dissection and culture of eyes, especially of light eyed mutants is very difficult. The evidence is probably on balance in favour of the transport hypothesis.

Comparison of the visual scores and the extracted pigment contents of the heterozygotes shows that, as in the case of the series of satsuma heterozygotes, a wide range of pigment contents may fall

into the same phenotypic class. Further, similar pigment contents may be obtained from flies which fall into easily distinguishable pigment classes. For example:

<u>Cross</u>	<u>red %</u>	<u>brown %</u>	<u>Phenotypic Class</u>
$w^{co} \times w^{ch}$	14.6	52.9	4
$w^{sat} \times w^{bf}$	13.7	51.6	6
$w^a \times w^{bf}$	14.6	59.7	1

Microscopic studies of the eyes of such flies might provide some information as to how the distribution of pigment granules within the pigment cells relates to the phenotype at the level of visual scoring. A detailed study of white locus alleles along the lines of that carried out by Shoup would be very valuable in this respect. Furthermore, as the pigmented alleles of the white locus represent a series of alleles at a locus which can, in extreme ("true" white) mutants abolish normal pigment granule formation, such a study might give valuable insight on the mechanism of granule formation.

Intermediate Profiles of White Locus Mutants

As the white locus is situated on the first (X) chromosome, it is impossible to obtain data on the brown pathway intermediates since these are assayed during pupation. In view of the difference of the red and brown pigment complementation in double heterozygotes, a study of the intermediates of both pathways in the various allele combinations might well have been illuminating. At the present time, brown pathway intermediate profiles were only obtained for each of

the homozygous lines. Comparison of these profiles should test the possibility of being able to identify alleles at a locus by consideration of their intermediate profiles.

Experimental Details

Large batches (over 200) of fertilised females were allowed to lay for one hour in $\frac{1}{2}$ pint milk bottles containing Sang's Yeast Medium. The cultures were maintained under standard conditions, and the larvae grew more or less synchronously.

At 72, 84, or 96 hours after pupation, the pupae were gently removed from the side of the bottle using a spatula prior to sacrifice for intermediate determination. Three repeat determinations on each intermediate were carried out for each of three cultures for each time point.

Results and Discussion

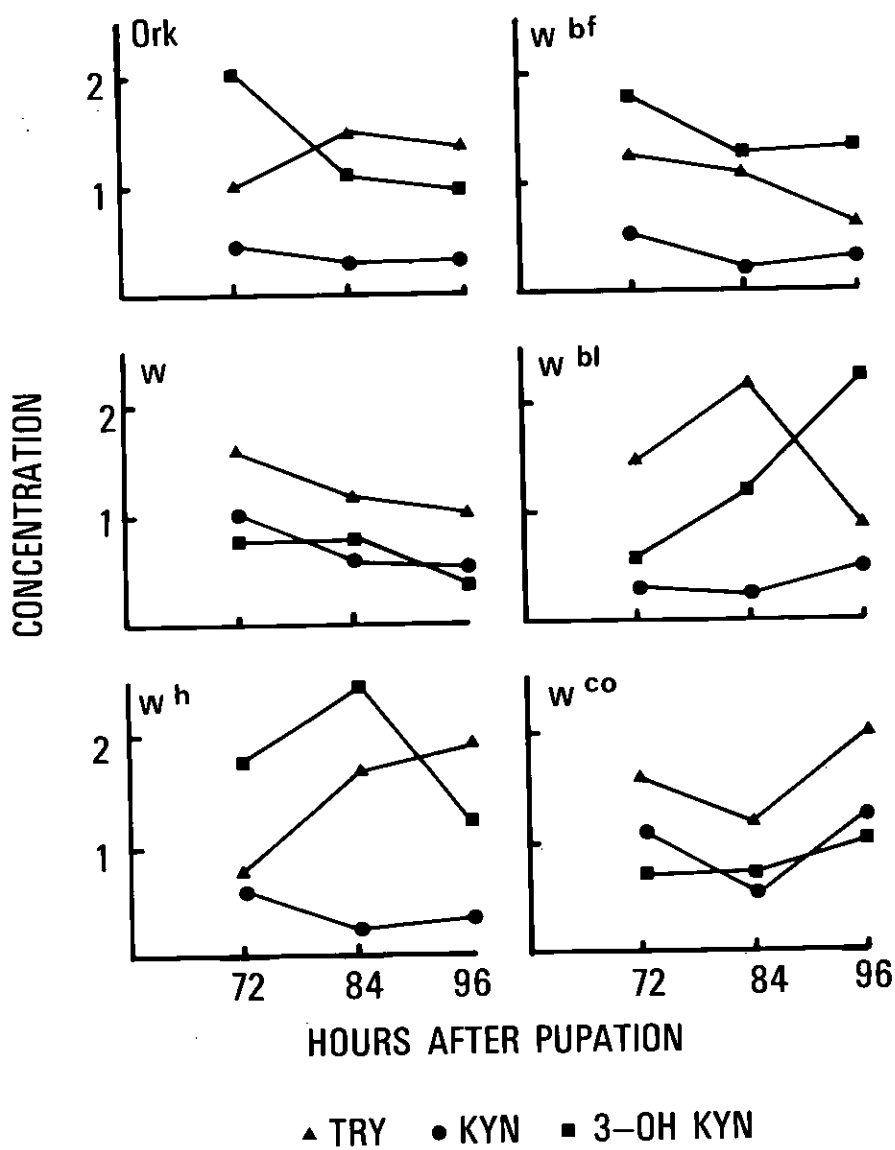
The data are given in the form of means and standard errors in Table A2 in the Appendix. Figure 27 gives a summary, illustrating the intermediate profiles of each of the white alleles over the 36 hour period.

A cursory glance at this figure is sufficient to end all hopes of being able to identify minor alleles of major loci by inspection of their intermediate profiles. Comparing profiles of alleles which give rise to very similar pigment contents, such as honey and buff emphasises the variation within the pathway which can eventually give rise to the same accumulation of end product. Breaking down the

Figure 27.

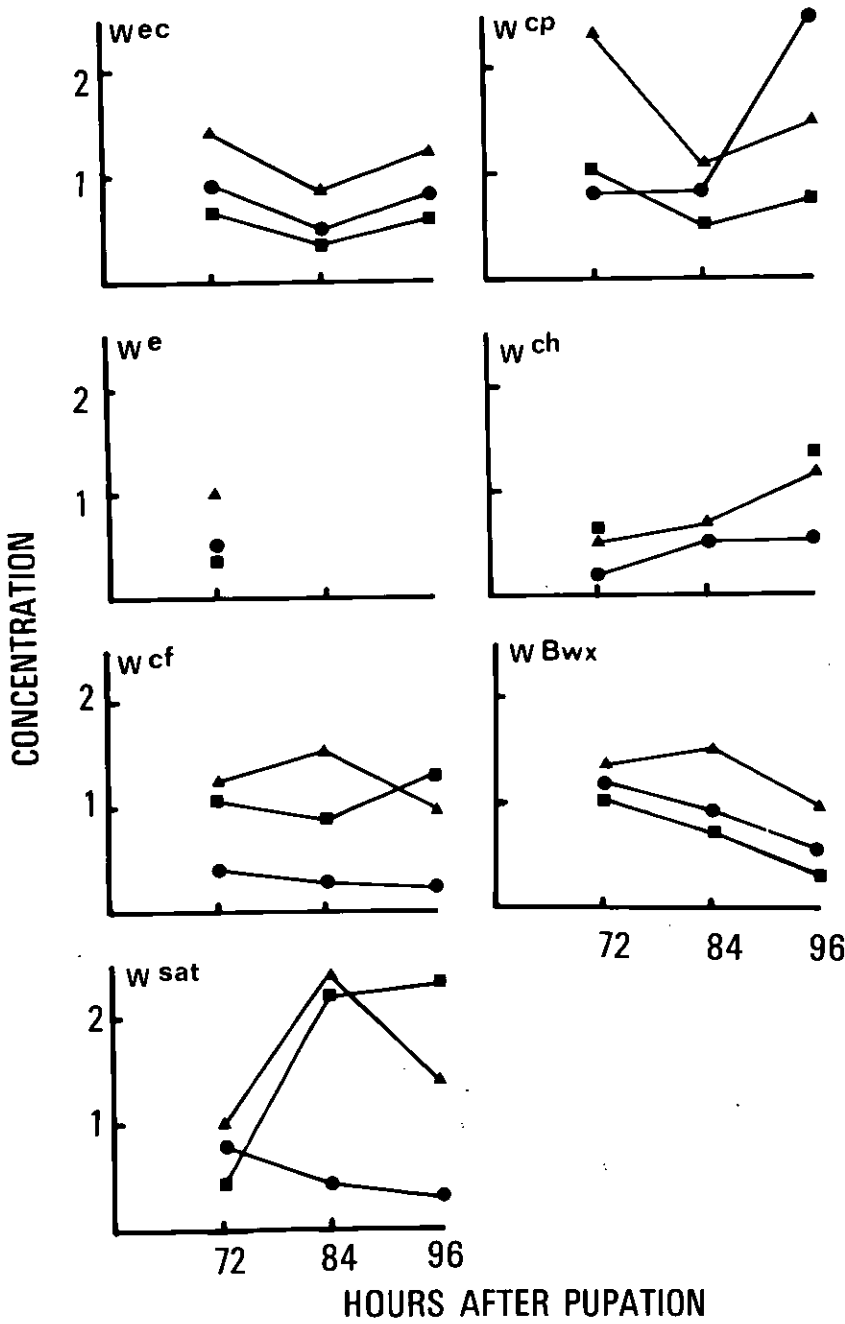
Pool levels of tryptophan, kynurenine and 3-hydroxy-kynurenine at 72, 84 and 96 hours post pupation in mutants of the white locus. Concentration scale as for Figure 23.

INTERMEDIATE PROFILES OF WHITE LOCUS MUTANTS



INTERMEDIATE PROFILES OF WHITE LOCUS MUTANTS

▲ TRY ● KYN ■ 3-OH KYN



group of thirteen pseudo-alleles into their groups defined by recombination (Fig. 25) does not give groups of alleles which in any way resemble one another in intermediate profiles. The possibility that the astounding variety of profiles exhibited by this group of alleles is a special case must not be overlooked.

Intermediate Contents of the Remaining Mutant Stocks

From the study of the "bright-eyed" mutants and the white locus alleles, it seems highly improbable that alleles of major loci which have small effects on the brown pigment content may be recognised by their effects on intermediate pool levels. It is, however, reasonable to inquire whether or not mutants at different loci may affect the pathway in similar ways. For example, the mutants Henna^{recessive} and sepia both cause accumulations of the yellow pterin, sepiapterin (Gregg & Smucker, 1966). Comparison of their brown pigment pathway intermediate profiles may reveal whether their effects on this pathway are similar. Brown and carnation have very similar brown pigment contents, close to those of white^{apricot}, white^{eosin} and white^{cherry}. Are there any parallels in the developmental profile of the pathway (represented by intermediate concentrations) among these mutants?

Twenty of the stocks for which the pigment contents had been determined remained after the "bright-eyed" and white mutants had been examined. The intermediate profiles of these mutants were determined in order to compare mutants having similar pigment contents or possibly similar modes of action.

Experimental Details

The procedure followed was identical to that for the white locus mutants.

Results and Discussion

The results are presented in graph form in Figures 28, 29 and 30, which show first, second and third chromosome mutants respectively. Tables A3, A4 and A5 give the corresponding concentrations and errors.

The first impression gained from inspection of the figures is of

Figure 28.

Pool levels of tryptophan, kynurenine and 3-hydroxy-kynurenine at 72, 84 and 96 hours post pupation in a number of first chromosome mutants. Concentration scale as for Figure 23.

INTERMEDIATE PROFILES OF FIRST CHROMOSOME MUTANTS

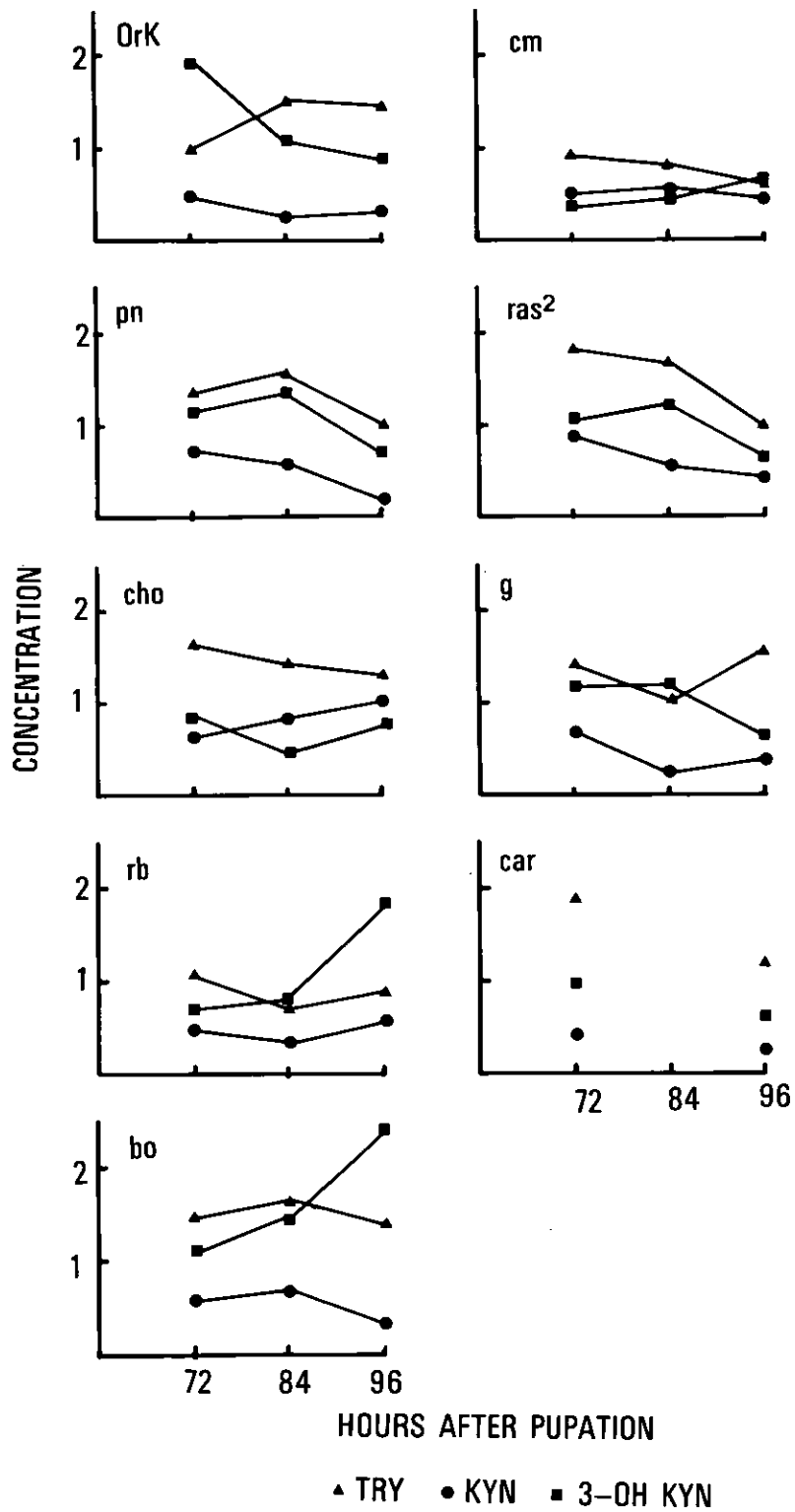


Figure 29.

Pool levels of tryptophan, kynurenine and 3-hydroxy-kynurenine at 72, 84 and 96 hours post pupation in a number of second chromosome mutants. Concentration scale as for Figure 23.

INTERMEDIATE PROFILES OF SECOND CHROMOSOME MUTANTS

▲ TRY ● KYN ■ 3-OH KYN

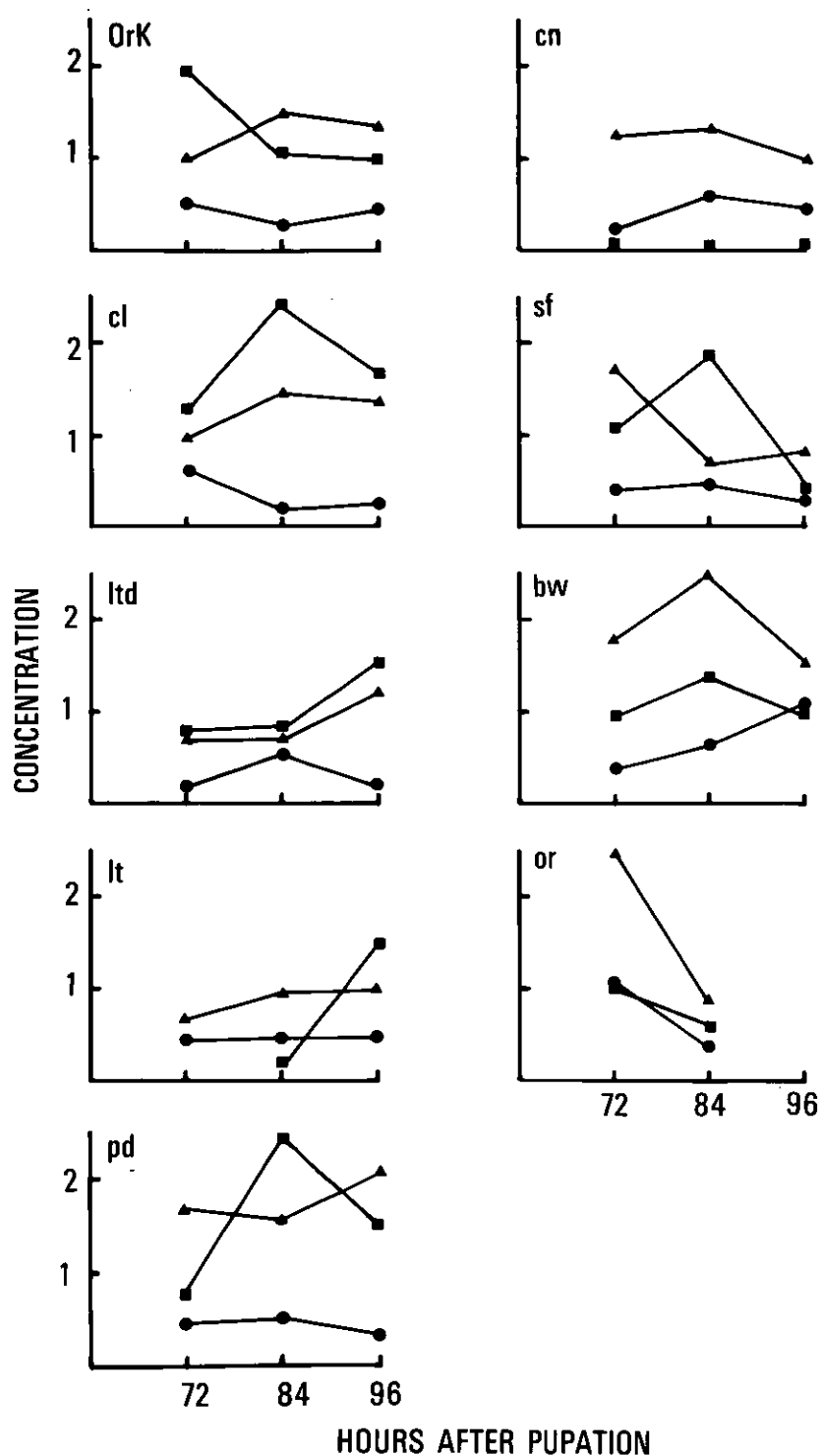
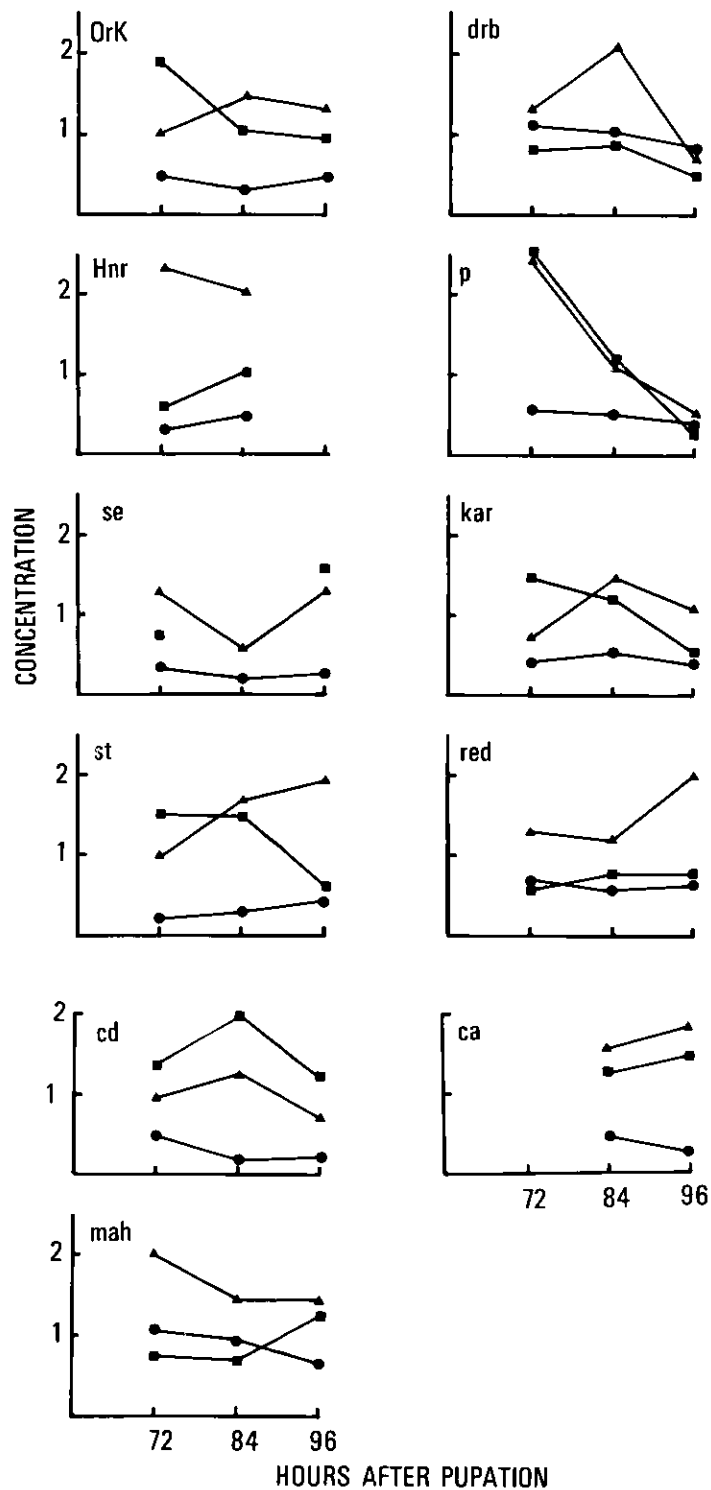


Figure 30.

Pool levels of tryptophan, kynurenine and 3-hydroxy-kynurenine at 72, 84 and 96 hours post pupation in a number of third chromosome mutants. Concentration scale as for Figure 23.

INTERMEDIATE PROFILES OF THIRD CHROMOSOME MUTANTS

▲ TRY • KYN ■ 3-OH KYN



variety - only one pair of mutants (w^h and cl) have obviously similar intermediate profiles. Note that dark red brown (Fig. 30), which did not affect the final extracted brown pigment when compared with Oregon K, shows marked differences from Oregon K in the accumulation patterns of all three intermediates.

Unfortunately, the data on Henna^{recessive} and sepia do not cover all three time points. However, differences in the tryptophan pool are evident. The tryptophan pool values for 72 and 84 hours after pupation in Hn^x are comparable with those obtained for vermilion (Fig. 23). Vermilion lacks detectable tryptophan oxygenase activity (Baglioni, 1961), and this is the enzyme which Gosh and Forrest (1967) suggested might be inhibited by biopterin. The accumulation of biopterin in Hn^x flies in conjunction with the tryptophan accumulation is in accordance with Gosh and Forrest's suggested mechanism for interaction between the two pathways. However, the absence of any increase in the tryptophan pool in sepia, which has a similar pteridine pattern to Hn^x (Gregg & Snucker, 1966) is further evidence against this particular interaction hypothesis (see "The white locus").

Brown (Fig. 29), carnation (Fig. 28), white^{eosin} and white^{cherry} (Fig. 27), all have very similar brown eye pigment contents. There is little sign of any resemblance between their intermediate pool profiles. On the other hand, white^{honey} and clot have very different pigment contents (w^h has 50% of Ork brown whereas cl has 117%), but these two mutants have the greatest similarity in intermediate profiles of any pair of mutants (Figs. 27 and 29). Crosses were set up between w^h and cl in an attempt to make a double mutant strain. This was unsuccessful. The pigment content of the double heterozygotes (w^h/+; +/cl) was ascertained, and found to lie in the same range as

the heterozygotes of the bright eyed mutants (84.7% of Ork red, 93.1% of Ork brown). As w^h is an X chromosome mutant, it was impossible to determine the intermediate profile for the double heterozygote. Occasionally, similarities in the profiles of one of the intermediates between mutants may be observed, for example, in Figure 28, the 3-hydroxy kynurenine pool levels of raspberry² and garnet show close resemblance.

There was no evidence of any correlation between any of the three intermediates at any of the time points measured and the final brown pigment content. The intermediate pool profiles can vary dramatically with little reference to the final extractable brown pigment pool.

Three sources of variation in the intermediate profiles can be considered. Mutants may act directly on the pathway by a number of ways. Enzyme structure, and hence efficiency may be altered; the rate or time of synthesis or activation of enzymes may be affected, or mutation may interfere with transport or storage systems to affect the availability of intermediates. On the other hand, the action may be less direct - mutants, which have a primary effect on another pathway may cause an excess of inhibitors or effectors of brown pathway enzymes. Finally, mutation may affect development. In this case sampling a mutant at 72 hours post pupation may be the equivalent of sampling the Oregon K strain a few hours earlier or later as far as the developmental sequence of the pathway is concerned. It is evident from figure 15 that this would result in a very different constellation of intermediate values.

Detailed studies of enzyme activities as well as intermediate

pools sampled at more frequent intervals throughout pupation would doubtless shed some light on the nature of the effect on the brown pathway of some of the eye colour mutants. This would, at least, be a step towards describing the primary lesion caused by mutants at some of the remaining eye colour loci.

Genetic Variation in Eye Pigment Content in Prevost's Second Chromosome Lines

From the preceding chapters on eye colour mutants, it is evident that studying intermediate levels at three twelve-hour intervals prior to eclosion is not likely to produce much information on the mode of action of eye pigment polygenes. Indeed, when such intermediate profiles were determined for a small group of the Prevost's second chromosome lines described earlier, these showed as much variation as almost any group of mutant stocks studied (see Figure 31). It is interesting that such variation can be ascribed solely to the action of second chromosome polygenes. This supports the conclusion that measuring each of the three intermediates at only three fixed time points is not a useful method of monitoring xanthommatin metabolism.

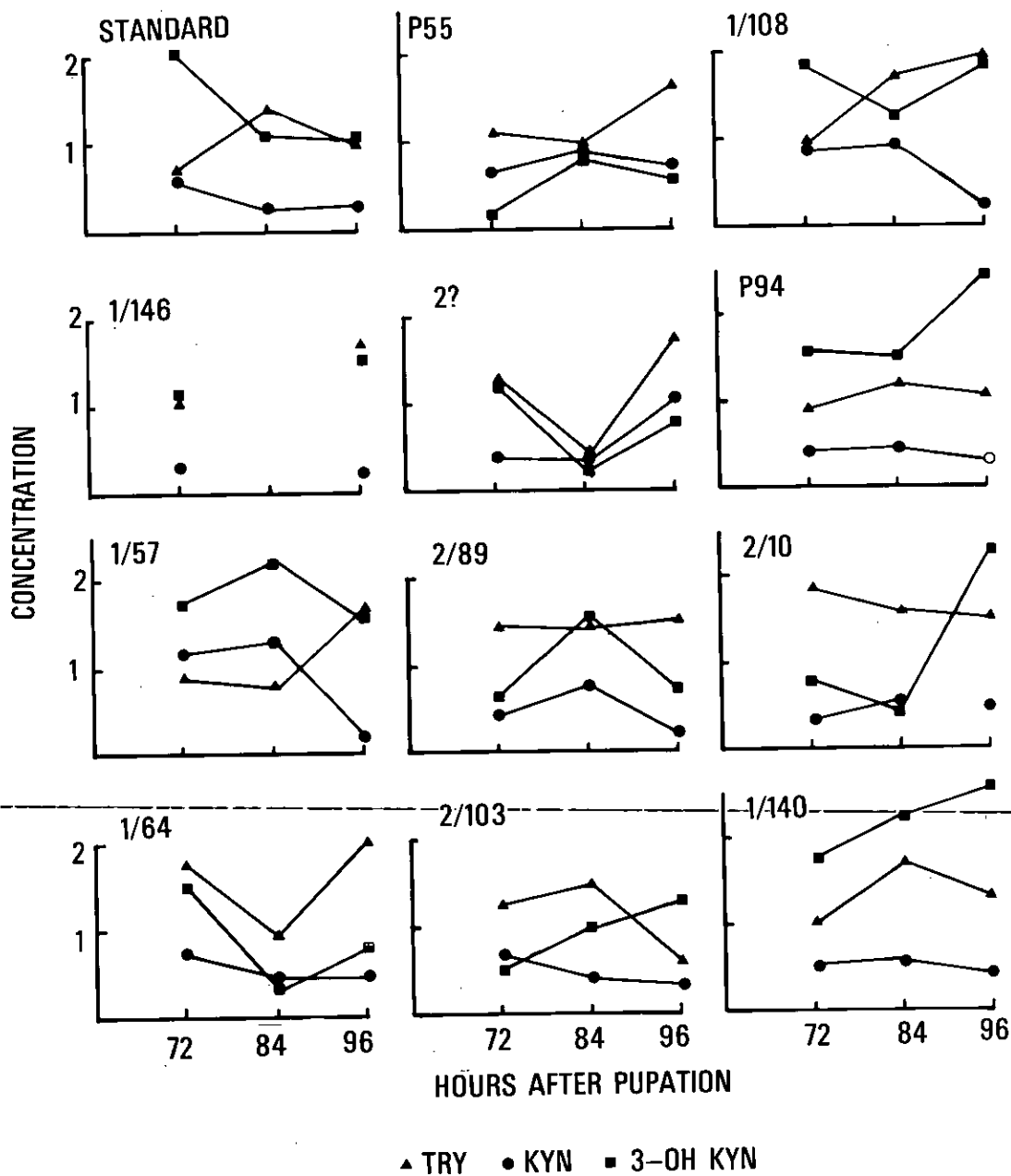
Another simple approach to the problem was available. It is possible to test the idea that at least a subset, if not all, of the second chromosome polygenes affecting eye pigment content are "minor" alleles of major loci by simple allelism tests.

It is possible that, by crossing flies taken from a number of different Prevost's second chromosome lines (see page 11) to known second chromosome mutants and extracting and measuring the pigments of F_1 flies, some minor alleles of these mutants may be identified. While this system may reveal the presence of mutant alleles at loci known to affect eye pigment content, absence of any evidence for this situation cannot be regarded as evidence of absence. The data already presented on the pigment contents of the "bright-eyed" mutants and the white locus mutants shows clearly that even reducing

Figure 31.

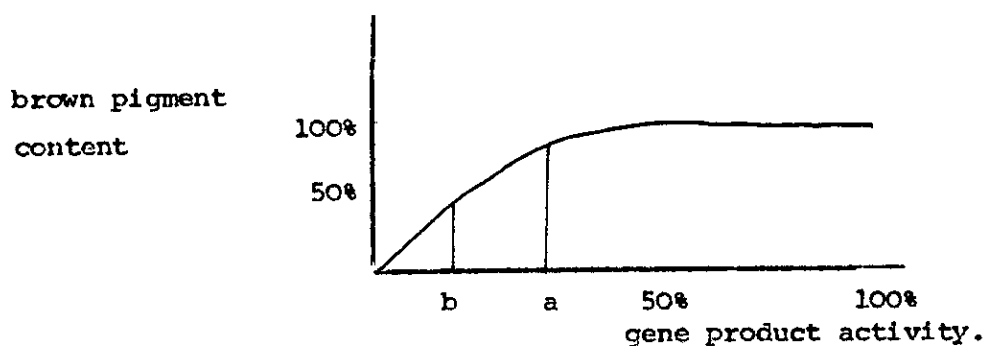
Pool levels of tryptophan, kynurenine and 3-hydroxy-kynurenine at 72, 84 and 96 hours post pupation in a number of Prevosti second chromosome lines. Concentration scale as for Figure 23.

INTERMEDIATE PROFILES OF PREVOSTI LINES



the effective gene dose by 50% at a number of steps in the pathway does not lead to a great reduction in pigment content. Kaufman (1962) gives evidence suggestive of a non-linear relationship between tryptophan oxygenase activity and xanthommatin content.

It is only reasonable to expect that a fraction of "minor" alleles may remain undetected by the proposed allelism tests. If we suppose the relationship between "gene product activity" and quantity of pigment to be of the form:



then it is easy to see that considerable reductions in gene product activity (of the order of at least 50%) may lead to no reduction in the pigment content. If the situation arises where a second chromosome line bears a "minor" allele with homozygous activity a, then the phenotype of the line will be wild type. If such a mutant is then made heterozygous with a null mutant, the activity is reduced (by around 50%) to b with a consequent detectable decrease in the pigment content. Some minor alleles of major loci may not give such reductions in gene product activity, even when heterozygous with a null mutant, as to be recognised by this system. However, in combination with alleles of similar magnitude of effect at other loci affecting eye pigment content, they may contribute to the quantitative variation observed among cage populations. A further problem is that some of the available mutants, e.g. clot, purple, purpleoid, may not be null mutants. Unfortunately,

nothing is known about the mode of action of these mutants. In spite of these reservations, allelism tests of the type proposed, may prove useful in identifying some of the second chromosome polygenes for brown eye pigment content.

Experimental Details

The second chromosome lines used in these experiments have already been described (pages 11, 30-34). With the exception of line P55, which has bright red eyes and greatly reduced brown eye pigment content, all the lines appeared wild type and had eye pigment contents within the range of the parent population represented by the Prevosti cage population. The second chromosome mutants, namely clot, purple, light, lightoid, cinnabar, safranin, brown and purpleoid had been backcrossed on to Oregon K.

Males from each of the second chromosome lines, from the Prevosti standard stock (an Oregon K derivative whose 3rd chromosome constitution was TM3/Pr) and from Oregon K were mated in single pair culture in vials containing Sang's yeast medium to females from each of the second chromosome mutant stocks. Two replicates were set up for each cross. After two days the parents were discarded, and the cultures were maintained at 25°C. Females were collected at eclosion and allowed to age for seven days. At this stage, flies were classified by third chromosome markers, and only prickly females were retained. Five females from each vial were sacrificed for pigment extraction. The reason for sampling only Pr females was to exclude variation in pigment content from third chromosome sources. Only those crosses for which five females from each replicate could be scored were included in the study.

Two sets of single pair matings were also set up for each of the mutant stocks, for Oregon K and for the Prevosti standard line. These cultures were maintained as above and five females from each vial were sacrificed for pigment extraction seven days after eclosion.

Results and Discussion

The results are presented in Table 14 and summarised in Figures 32 - 35. The red and brown pigment contents of the offspring of the Prevosti standard x Oregon K cross were taken as 100% for the purposes of the figures. Most of the crosses between second chromosome lines and mutant strains yield offspring having eye pigment contents in the range of around 80 - 120% of the standard. This is the sort of range already observed among heterozygotes of "bright eyed" mutants (see Table 9). There are some crosses which yield F_1 s having pigment contents well outside this range, notably:

<u>cross</u>	<u>red %</u>	<u>brown %</u>
cl x 1/108	68.1	119.8
cl x 27	70.6	117.6
cl x 1/57	107.4	157.1
pr x 2/10	74.2	42.5
lt x 2/10	83.8	64.8
pd x P55	75.46	35.22
sf x P55	81.6	142.9
cn x 1/108	106.9	133.2

Several interesting points emerge from this short table. The first point is that clot appears three times; in the first two cases showing a reduction in the red pigment content when heterozygous with "wild type" alleles from lines 1/108 and 27. The similarity in the pigment values obtained from the progeny of crosses between clot and each of these two lines suggests that if they do carry "minor" alleles

TABLE 14.

The pigment contents of the F₁s of Prevosti second chromosome lines x 2nd chromosome mutants are expressed as percentages of the pigment contents of the F₁ of a cross between the Oregon K strain and the Prevosti standard strain. Red pigment contents are given in the upper left corner and brown pigment contents in the lower right corner of each entry. The right hand column (*) gives the pigment contents of the Prevosti lines as percentages of the standard stock values, and the bottom row (**) gives the pigment contents of the mutant strains as percentages of Oregon K. Blanks in the Table result from insufficient flies being obtained from the cross for pigment determination. The data are given in full in the Appendix (Table A8).

TABLE 14. Pigment contents of Prevosti 2nd chromosome lines x 2nd chromosome mutants.

	clot	purple	light	light- oid	cinn- abar	safra- nin	brown	purp- loid	*
P55	83 102	/	/	/	96 84	82 143	97 95	75 53	81 28
1/108	68 120	/	83 100	90 73	107 133	94 139	/	/	87 71
1/146	85 115	105 105	/	106 104	88 94	/	98 124	/	93 112
2 ?	70 118	87 105	/	/	/	/	82 108	/	81 82
1/57	107 157	83 114	99 166	95 68	89 90	/	/	89 125	90 85
2/89	78 103	87 139	/	91 99	70 122	85 117	81 109	/	79 89
2/10	82 112	74 43	84 65	/	/	79 101	63 117	93 91	/
1/64	98 121	100 81	/	/	/	/	68 86	/	90 85
P94	/	79 126	64 91	95 110	92 91	85 104	79 91	/	/
2/103	/	/	82 119	112 91	/	69 106	/	/	96 76
2/102	/	/	71 107	/	/	102 98	/	/	88 100
Oregon K	98 121	91 95	97 92	107 88	100 84	/	95 118	82 103	/
Prevosti S	101 77	90 117	91 106	/	98 87	85 113	89 109	/	/
*	55 16	/	15	40	102	46 68	1 43	21 31	/

Figure 32.

Red and brown pigment contents of the F_1 s of crosses between a number of Prevosti second chromosome lines and the mutants clot and purple

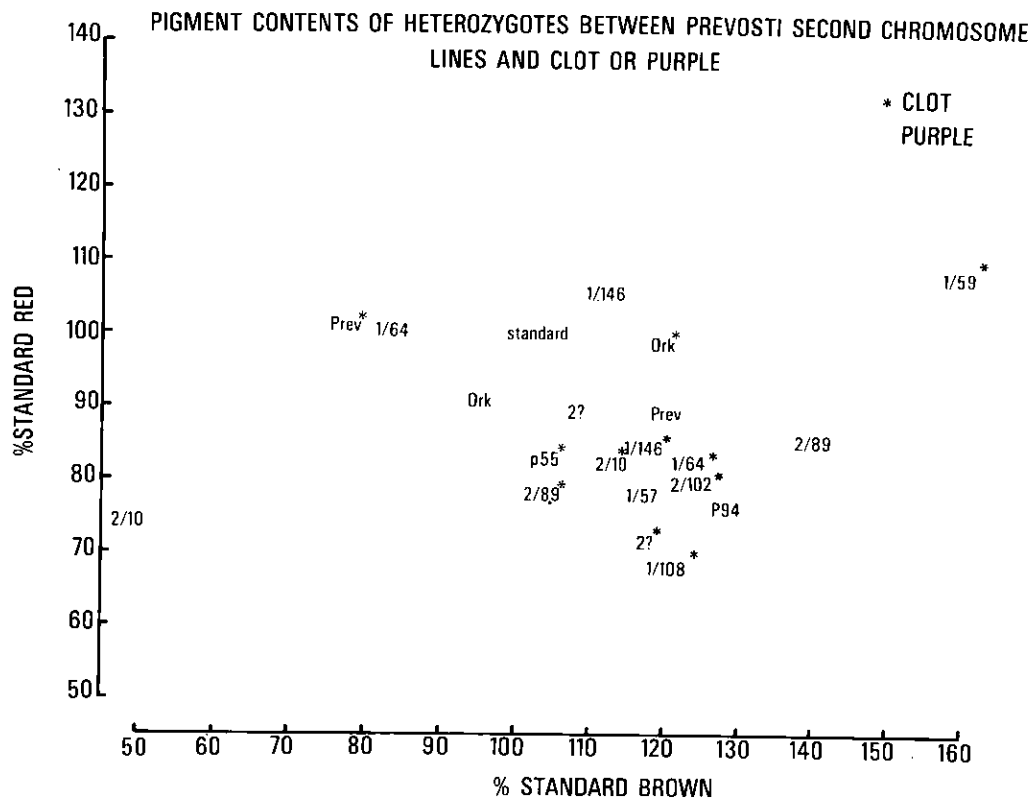


Figure 33.

Red and brown pigment contents of the F_1 s of crosses between a number of Prevost's second chromosome lines and the mutants. light and lightoid.

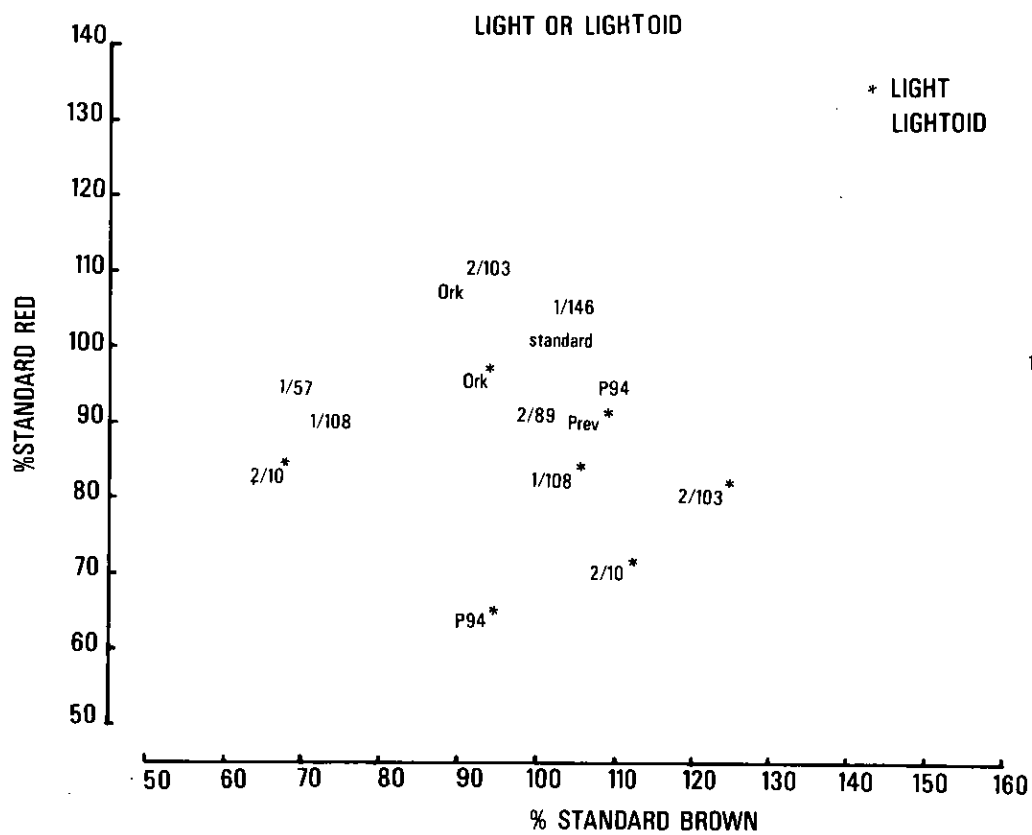


Figure 34.

Red and brown pigment contents of the F_1 s of crosses between a number of Prevost's second chromosome lines and the mutants. cinnabar and safranine

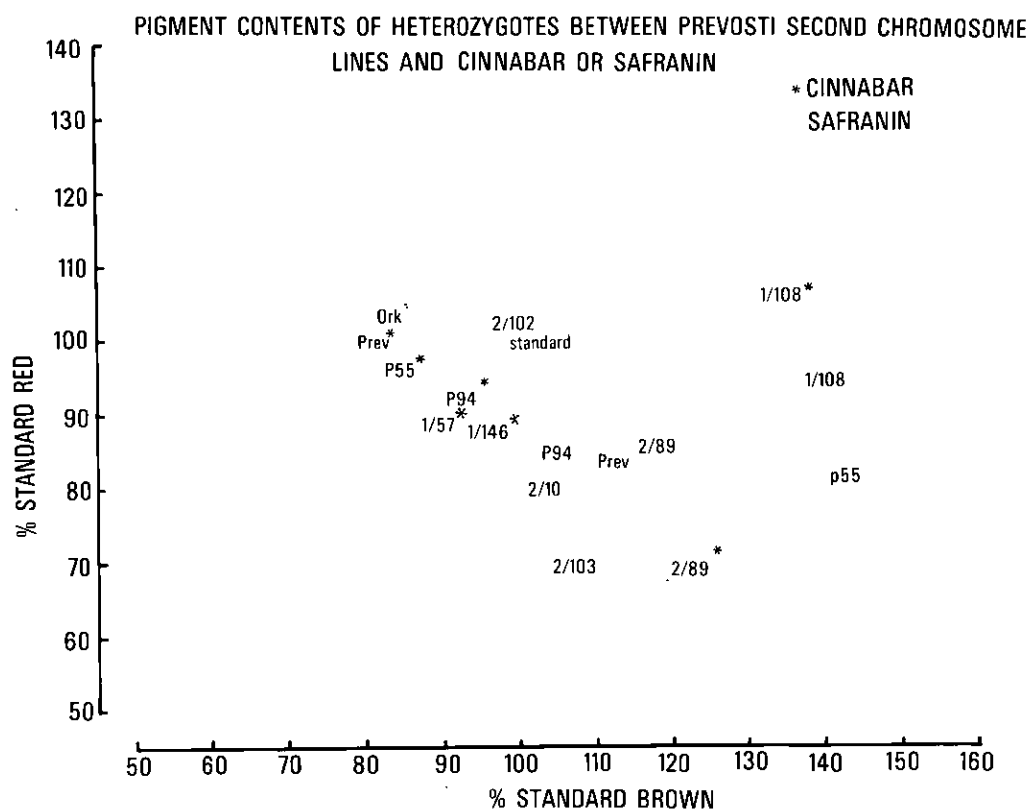
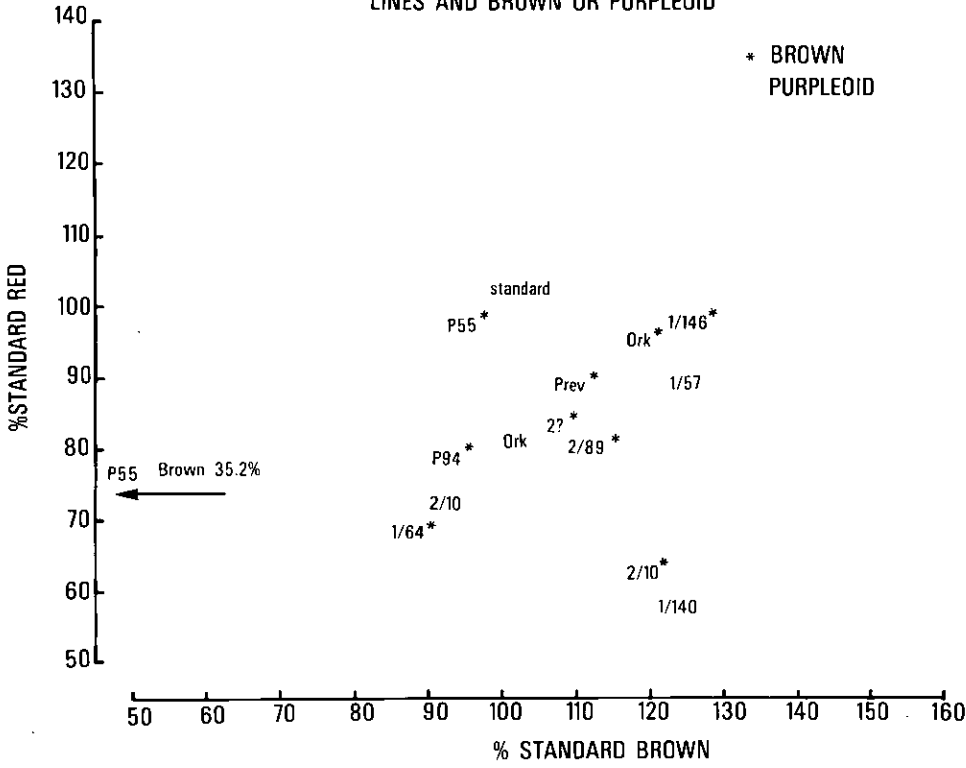


Figure 35.

Red and brown pigment contents of the F_1 s of crosses between a number of Prevosti second chromosome lines and the mutants. brown and purpleoid.

PIGMENT CONTENTS OF HETEROZYGOTES BETWEEN PREVOSTI SECOND CHROMOSOME
LINES AND BROWN OR PURPLEOID



of clot, that the same allele is present in both lines. 1/57, the third line to yield "unusual" heterozygotes of clot shows different characteristics. The red pigment content is very close to the standard value, whereas there is a 50% increase in the brown pigment. This is of interest because clot itself, with characteristically dark brown eyes (also observed in the F_1 of $cl \times 1/57$), is an overproducer of brown eye pigment (cl/cl give 55% of Ork red and 117% of Ork brown, table 7).

The second point is that one of the second chromosome lines, namely 2/10, appears twice in the table, showing reductions in brown pigment content with purple (to 43%) and with light (to 65%). There are two possible explanations of this observation which cannot be distinguished on the basis of the evidence given here, namely that either 2/10 carries "minor" alleles of both pr and lt, or that there is some kind of interaction between mutations at these two loci. Neither of these two crosses, pr $\times$ 2/10 nor lt $\times$ 2/10 gave offspring which could be distinguished from wild type by inspection.

Thirdly, P55 puts in two appearances, neither in combination with a "bright-eyed" mutant. This strain, it may be remembered, has a bright red eyed phenotype, which shows segregation patterns consistent with the segregation of a Mendelian recessive. Although insufficient females were obtained from the crosses between this line and light or lightoid to obtain pigment data, the offspring of these crosses appeared wild type on inspection. This was also true of the cross with cinnabar, where the offspring had standard brown eye pigments. However, the offspring of the cross between P55 and purpleoid showed a large reduction in brown pigment content (to 35%). These flies had

somewhat lighter eyes than wild type. This observation is of particular interest because of the difference in phenotype of P55 and pd, bright red eyes contrasting with dull brown. It is possible that pd is a rather less drastic mutation than that carried by P55. The overproduction of brown eye pigment in the F_1 of the safranin x P55 cross is rather surprising in view of the low brown eye pigment contents of the two parents. This also applies in the case of the cross between cinnabar and 1/108, where cinnabar has virtually no detectable brown eye pigment and 1/108 is at the lower end of the range found among the second chromosome lines.

The resolving power of the allelism test as a screen for "minor" alleles of loci on the second chromosome affecting brown eye pigment content may be improved. Paton and Sullivan (1978) have suggested that the third chromosome mutant, red Malpighian tubules may be incorporated in order to reduce the 3-hydroxykynurenine pool in early development. (This mutation causes an abnormal production of ommochrome in the larval Malpighian tubules, which are rusty brown in consequence). They have used this fact in screening for induced mutations at the cinnabar locus and found it to be a useful aid in recovering "leaky" mutants (mutants of low enzyme activity).

As it stands, this simple allelism test would appear to be a useful tool in selecting second chromosome lines which might be amenable to further study. Of course, it would be premature to claim that minor alleles of clot, purple, light, purpleoid, safranin and cinnabar have been proved to be present among the second chromosome lines. Further studies would be necessary to provide rigorous proof, either to rule out or demonstrate the existence of interactions between mutants

at these loci and variation present among the lines at other loci. However, it seems reasonable to suggest on the basis of the evidence here that variation at these loci is likely to be contributing to the quantitative variation observed in the Prevosti population.

Summary, Conclusions, and Suggestions for Further Experiments

This study was undertaken with three aims in mind. One was to confirm that the brown eye pigment content of Drosophila melanogaster behaves as a quantitative character, as Nolte (1955) suggested. If this were indeed the case, then it would be a quantitative character amenable to biochemical dissection. This could be of use, for example, in investigating the effects of selection on the metabolic processes affecting the end character.

A second aim was to enlarge the background of information available on the brown eye pigment system by examining a number of mutants affecting it.

Thirdly, if the brown eye pigment content is a quantitative character, then it should be possible to enquire into the relationships between quantitative and qualitative genes affecting this system. That is, it should be possible to ask what are the differences and similarities between polygenes and major mutants.

Before considering any of the data presented here, it is as well to consider exactly what is meant by "brown eye pigment content". In the strict sense, this is the quantity of xanthommatin and/or dihydro-xanthommatin present in the eye. In the present context, however, "brown eye pigment content" is defined in terms of the extraction procedure of Ephrussi and Herold (1944). Thus this definition includes all those compounds which are eluted from the heads of *Drosophila* by the acid methanol solvent (after pretreatment with acid ethanol) which absorb at 440 n.m. In practice, as "brown pigment" solutions from heads of the mutants white (which has no eye pigment) and scarlet (which has only red eye pigment) give the same low absorbance at 440 n.m., it is unlikely that red pigment related compounds contribute to the brown

eye pigment content. By using the "brown pigment solution" obtained from true white mutants to provide a baseline, some attempt was made to control for any extraneous contribution to recorded brown pigment contents. Although other methods of xanthommatin extraction exist, this method was preferred because it could be applied to single flies.

The first of the three aims of the study was realised. The brown eye pigment content was shown to be a character of high heritability in a number of laboratory cage populations (pages 25-30). Several of the older populations showed very low heritabilities, however. Unfortunately, one of these, Dahomey, was somewhat prematurely chosen as the base population for a selection experiment. The results obtained from this experiment were disappointing. A second attempt at selection, this time using the Prevosti population as the base, proved more hopeful although it was curtailed owing to lack of time. From this work it was concluded that Nolte's claim (1955) that the brown eye pigment content is a quantitative character is justified.

The information available on the effects of eye colour mutants on red and brown eye pigment contents was regarded as less than satisfactory. Nolte, who provided the basic data from which Lindsley and Grell derived percentages of "wild type" values, had failed to control for variation in genetic background. Forty-five mutant strains representing twenty-nine different loci were therefore obtained. Each mutant was backcrossed onto an Oregon K inbred background, and the effects on both red and brown eye pigment contents were studied. With two exceptions, (namely, cinnabar and dark red brown), all the mutants studied affected both eye pigments. Cinnabar had no effect on red pigment and dark red brown had no effect on brown.

Although wild type males have lower eye pigment contents than females of the same strain (about 70-80% of the female value on average), males of first chromosome mutants have the same pigment contents as do mutant females of the same strain. This observation can be explained by assuming that physical constraints on the amount of pigment which can be stored in the eye are important in limiting the amount of pigment found in wild type males. Those constraints assume lesser importance in the case of mutants which greatly reduce the amount of eye pigment synthesis. It is likely that, in spite of the observed sexual dimorphism for pigment colour, dosage compensation at the level of the pathway may be complete in wild type flies.

As the amount of time available for experimental work was limited, a decision had to be made. It was possible either to study a few of the available mutants in great depth, or to examine all of the mutants at a more superficial level. In the event, the latter option was chosen, and this was not a wise choice.

The line of enquiry followed was to examine the three easily measured intermediates of brown pigment synthesis, namely tryptophan, kynurenine and 3-hydroxykynurenine, at three time points fixed relative to time of pupation. Unfortunately, this method of fixing sampling times almost certainly means that the mutants were not all sampled at equivalent stages in development. Again, owing to lack of time, this was not taken into account. The patterns of intermediate accumulation observed in these mutant strains presented a bewildering array. Little or no sense could be made of the results.

A more meaningful approach, and one worth future attention, would have been to concentrate on how enzyme and pool levels throughout development are related to brown eye pigment content. This could be

done by focussing attention on those mutants known to affect brown eye pigment content directly - vermillion, cinnabar, cardinal, scarlet and white.

Indeed, a study of the pigment contents of heterozygotes of these mutants suggests that 50% reductions in enzyme activity have very little affect on brown pigment accumulation.

Detailed investigations of how enzyme and pool levels vary in these mutants, in their heterozygotes, and in double or triple heterozygous combinations, should increase our understanding of the brown eye pigment pathway as an integrated system. Three further mutants, light, lightoid and karmoisin, which also abolish xanthommatin accumulation, might well be worth including in such a study.

Consideration of the eye pigment contents of heterozygotes of a number of pigmented white locus alleles led to the suggestion that the white product is involved in the red pigment pathway as a monomer and in the brown pigment pathway as an oligomer. This suggestion arises from the observation that, while normal white product is necessary for normal functioning of both pathways, complementation is more common in the brown pigment pathway than in the red in heterozygotes of white locus alleles.

After a disappointing attempt at the second aim of the project, the outcome of preliminary experiments designed to tackle the third aim was more hopeful. Making use of a number of lines homozygous for second chromosomes drawn from wild flies, it was possible to show, by allelism tests, that "minor" alleles, either at, or interacting with, major (mutant) loci are present in wild populations. It is possible that the sensitivity of the allelism test (involving extraction and measurement of eye pigments of F_1 s of crosses between

"second chromosome" lines and second chromosome mutants) could be increased. This could be done in this case by incorporating in the background of the strains used in the test a third chromosome mutant, such as red Malpighian tubules, which reduces brown eye pigment content (see Paton & Sullivan, 1979).

This method of sampling wild chromosomes and identifying genes contributing to variation in "wild type" pigment contents is an extremely useful way of obtaining suitable material for studying the metabolic variation underlying the quantitative variation in the character. In this respect, it is worth noting that large variations in brown eye pigment content can be tolerated before the eye appears mutant on visual inspection. In fact, this may be the reason why very few mutants were found to have low brown eye pigment content with high red pigment content. Although such combinations can exist, those found in the course of this work appeared wild type.

In conclusion, therefore, one may state that the brown eye pigment content of Drosophila melanogaster is a quantitative character. It should be relatively easy to examine the effects of selection and inbreeding on the metabolism of the brown eye pigment as the synthetic pathway is short and fairly simple, and as our understanding of this particular aspect of metabolism is increasing.

Some indication was found that alleles at loci where mutation can produce recognisable phenotypic differences may contribute to the quantitative variation in brown eye pigment content.

Little contribution to our understanding of the relationships between the enzymes, pool levels, storage and transport mechanisms and any other aspects of metabolism involved in the synthesis, accumulation and storage of brown eye pigment was made. Some hopeful lines of enquiry were suggested.

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Summary of Known Eye Colour Mutants of *Drosophila melanogaster*

The following pages summarise and give references to the salient facts known about the eye colour mutants in *Drosophila melanogaster*. These are arranged according to chromosome map position, which is given in the top left hand corner of each entry. Abbreviations and rank order (where known) are given in the top right hand corner. The rank order, from 1-5 denotes decreasing ease of visual scoring.

The pteridine patterns of Gregg and Smucker (1965) are as follows:

Class	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
pteridine												
drosopterins	++++	++++	++	(+)	(+)	++	-	-	-	-	++	-
xanthopterin	+++	++	(+)	(+)	(+)	++	++++	-	-	(+)	(+)	(+)
xanthopterin-like	++	++	-	-	-	-	+	-	-	-	(+)	(+)
sepiapterin	++	++	++	(+)	-	+++	++++	-	-	-	(+)	(+)
2-amino-4-hydroxy-pteridine	+++	+++	++	++	(+)	++++	++++	-	-	-	-	++++
biopterin	+++	++	++	++	(+)	++++	++++	-	++	+++	+++	+++

1-0.07 cinnamon cin

The eye colour of cin/cin progeny of cin/cin mothers is similar to that of rosy and maroonlike flies (dull, purplish brown). The pteridine accumulation pattern is also similar to that of these two mutants, and there is no detectable xanthine dehydrogenase activity in cinnamon flies. However, the presence of cin^+ product is almost to be regarded as essential for egg hatch, as $\text{cin}^+/\text{cin}^+$ and cin/cin^+ mothers have normal hatching success, $\text{cin}/\text{cin} \times \text{cin}^+/\text{Y}$ crosses have a 50% reduction in hatch, and $\text{cin}/\text{cin} \times \text{cin}/\text{Y}$ crosses give a hatch rate of about 5% of normal. Further lethality is observed at the pupal stage. cin/cin progeny of cin/cin^+ mothers have normal eyes (Baker, 1973).

1.0 suppressor of sable su(s)

Mutant alleles at this locus act as recessive suppressors of sable, speck and two eye colour mutants, purple and vermillion. Much work has been carried out on the interaction between this and the vermillion locus, where the enzyme involved is known to be tryptophan oxygenase. Jacobson (1971) suggested that suppression of the vermillion phenotype was due to the absence of an isoaccepting tyrosyl tRNA in su(s) flies. Miselke et al. (1975) have questioned this suggestion. At any rate, suppression is specific for only certain vermillion alleles (Tartof, 1969).

1-0.3 deep orange dor PK1

This is a temperature sensitive mutant, the eyes being true orange at 25°C and reddish orange at 18°C. Homozygous females are sterile, and have high accumulations of isoxanthopterin. Male

levels of this compound are lower than normal (Counce, 1957). Development of transplanted eye anlagen is autonomous (Lucchesi, 1964). The time of lethality in the progeny of dor/dor females is post-blastula and before the end of gastrulation (Counce, 1969). The combinations dor; ry and dor; ry² are lethal (Nash, 1971).

1-0.8 prune pn RK1

The eyes are brownish purple. Development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936). The larval Malpighian tubules are normal (Brehme and Demerec, 1942). Nolte reported pigment contents of 26% red and 110% brown (1959), Gregg and Smucker found pteridine accumulations of their type IV pattern (1965). Alleles of prune are lethal in combination with the killer-prune mutant.

1-0.8 murkey mk RK3

The eyes are dull red. Eclosion is delayed, and the flies are smaller than normal. The females are sterile (Fahmy, 1956).

1-1.0 zeste z RK2

The eyes of zeste males are wild type, while those of females are lemon-yellow at 25°C and variegated yellow and red at 19°C. Females heterozygous for mutations in the right hand portion of the white locus have the zeste phenotype suppressed. Males carrying a w⁺ duplication have zeste eyes. Y chromosomes have no effect. Development in mosaics or of transplanted eye anlage is autonomous (Gans, 1948).

1-1.5 white w RK1

The classic white mutants have completely white eyes and colourless ocelli, Malpighian tubules and testes sheaths. More pteridines than normal are excreted in the meconium (Kalmus, 1943). The flies are phototactic, but the optometer response is absent. Response to further stimulus of the eye is inhibited for a period of about three hours by blue light (Cosens & Briscoe, 1972). 3 hydroxykynurenine is accumulated in the pupae, but rapidly lost at eclosion (Howells, Summers and Ryall, 1977). Phenoxazinone synthetase activity is recoverable (Phillips, Forrest and Kulkarni, 1972). The uricase activity of adults is increased, with a reduction in the amount of uric acid present (Auf der Maur, 1960).

A number of pseudoalleles which show varying degrees of pigmentation have been described. Some of these have been mapped to five distinct sites within the locus (Judd, 1964; Green, 1960). The different spatial groups can be discriminated by their behaviour in combination with zeste and enhancer of white^{eosin} (Green, 1959). Differences between different alleles with regard to metal ion absorption have been reported (Kikkawa, 1956). Green (1969) described a "controlling element" associated with white^{crimson} which is capable of spontaneous transposition to an autosome and which may carry a portion of the white locus with it.

1-3.3 ruddle rud RK2

The eyes are a dull, reddish-brown, most easily classified in newly emerged flies. Viability and fertility are both good (Fahmy, 1959).

1-5.4 chocolate cho PK1

The eyes are brown with white highlights. The Malpighian tubules contain a brown pigment which is absent in combination with vermilion, cinnabar and scarlet. cho v eyes are yellowish, cho g are indistinguishable from garnet (Sturtevant, 1955).

1-7.5 ruby rb PK1

The eyes are a clear ruby. Combination with scarlet gives orange, and with brown, brownish red eyes (Mainx, 1938). Development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936). The larval Malpighian tubules are pale yellow (Beadle, 1937). The kynurenine formamidase activity is more or less wild type (Glassman, 1956). Gregg and Smucker (1965) found a pteridine accumulation pattern of type III.

1-12.5 bordeaux bo PK3

The eyes are dark wine in colour, only separable from the wild type with great difficulty. The larval Malpighian tubules are bright yellow (Beadle, 1937). Development of transplanted eye anlagen may not be completely autonomous (Beadle & Ephrussi, 1936). Nolte found pigment contents of 67% red, normal brown (1955). Gregg and Smucker found a pteridine accumulation pattern of type II (1965).

1-18.9 carmine cm PK1

The eyes are a translucent ruby. In combination with scarlet the colour becomes a deep orange, and with brown, slightly lighter than brown alone (Mohr, 1938). The larval Malpighian tubules are very pale yellow (Mohr, 1927). Kynurenine formamidase activity is

more or less normal (Glassman, 1956). The pteridine accumulation pattern is type III (Gregg & Smucker, 1965).

1-20.1 bistre bis RK2A

The eyes and the ocelli are very dark brown. The wings are frequently not extended. The males are sterile, and viability is variable but generally very low (Fahmy, 1958).

1-33.0 vermillion v RK1

The eyes are bright scarlet and the ocelli are colourless. The combination v; bw has white eyes. The eyes of vermillion and wild type gynandromorphs are wild type (Sturtevant & Beadle, 1939). The development of transplanted eye anlagen is non-autonomous (Beadle & Ephrussi, 1936). The v⁺ substance was identified as kynurenine by Tatum and Beadle (1936). The activity of the inducible enzyme, tryptophan oxygenase, is absent (Baglioni, 1959) and tryptophan is accumulated. Some alleles are suppressed by suppressor of sable. Tryptophan oxygenase recovered from suppressed vermillion flies differs kinetically from the wild type enzyme (Kaufman, 1962). Some brown pigment is formed under conditions of partial starvation (Shapard, 1960). Kynurenine formamidase activity is more or less wild type (Glassman, 1956). Phenoxazinone synthetase activity is greatly reduced (Phillips et al., 1973). The pteridine accumulation pattern is of type II (Gregg & Smucker, 1969).

1-43.3 copper cop RK2

The eye colour is brownish-red, most easily classified in newly emerged flies. The wings may show cutaway inner margins. Fertility

and viability are good in both sexes (Fahmy, 1958).

1-44.4 garnet g PK1

Sometimes referred to in early publications as salmon, the eye colour is a deep purplish ruby, lighter in young adults. The g^2 allele has slightly lighter eyes. The development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936). The Malpighian tubules are very pale yellow at the base (Beadle, 1937). With scarlet, the double mutant has dark, yellow-orange eyes, and with brown, brownish pink (Mainx, 1938). Nolte found pigment contents of 38% red and 56% brown (1959). Kynurenine formamidase activity is more or less normal (Glassman, 1956). The pteridine accumulation pattern is of type III (Gregg & Smucker, 1965). In combination with brown, no 2-amino-4-hydroxypteridine is found in adult heads (Schepers, 1962). Garnet hosts can support wild type development of transplanted rosy eye analagen (Schwink & Chovnik, 1960).

1-48.1 riny rm PK2

The eyes are a dull brownish red. Conspicuous white hairs are present between the ommatidia. The wings are longitudinally pleated. Viability and fertility are good (Fahmy, 1958).

1-52.8 raspberry ras PK1

The eyes are dark ruby. Development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936). The larval Malpighian tubules are wild type (Brehme & Demerec, 1942). Nolte (1959) reports pigment contents of 25% red, 114% brown. The pteridine accumulation pattern is of type IV (Gregg & Smucker, 1965).

1-62.5 carnation car RK1

The eyes are dark ruby. In combination with scarlet, the eyes are yellowish pink, with brown they are yellowish brown (Mainx, 1938). The Malpighian tubules become pale yellow in late larvae (Beadle, 1937). Development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936). Kynurenine formamidase activity is more or less wild type (Glassman, 1956). Nolte (1959) reports pigment contents of 33% red and 47% brown. The pteridine accumulation pattern is of type III (Gregg & Smucker, 1965).

1-64.8 maroon-like mal RK3

The eye colour is brownish purple. The larval Malpighian tubules are grossly abnormal, containing yellow-orange pteridine globules (Schwink, 1960). Xanthine dehydrogenase activity is absent, and the pteridines subsequent to this step are missing. There is an increase in the precursors to this step in drosospterin synthesis (Glassman & Mitchell, 1956). Maroon-like and rosy extracts complement to give XDH activity in vitro, but complementation is not found in the double mutant. It has been suggested that the mal⁺ product is a cofactor for XDH (Glassman & Mitchell, 1959). Development is non-autonomous in mosaics or in transplanted eye anlagen (Schwink, 1963).

2-14 pink wing pw RK3

The eyes are dull and reddish in colour, and the wings are short and crumpled (Morgan, Bridges & Sturtevant, 1925).

2-16.5 clot cl RK1

The eye colour is dark maroon, deepening to sepia with age. The development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936). The larval Malpighian tubules are indistinguishable from wild type (Brehme & Demerec, 1942). Seprapterin is present in the pigment granules in addition to ommochrome and drosophterin (Nolte, 1954).

2-24 cream-b cr-b

This mutant acts as a specific dilutor of white^{eosin}.

2-52.5 cream-underscored cru

This acts as a specific dilutor of white^{eosin} and pink. The larval Malpighian tubules of cru individuals are bright yellow (Brehme & Demerec, 1942).

2-54.5 bright bri RK3

The eyes are a slightly brighter red than wild type, and identification is very difficult (Beadle & Ephrussi, 1937). The larval Malpighian tubules are pale yellow (Beadle, 1937). The pteridine accumulation pattern is of type II (Gregg & Smucker, 1965).

2-54.5 purple pr RK1

The eye colour at hatching is ruby, deepening to purplish ruby with age. The eyes are orange in combination with scarlet, and reddish brown with brown (Mainx, 1938). The development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1937). The larval Malpighian tubules are normal (Beadle, 1937). Kynurenine formamidase activity is more or less wild type (Glassman, 1956). The

pteridine accumulation pattern is of type IV (Gregg & Smucker, 1965).

2-55.0 light lt RK1

The eye colour is yellowish pink, lighter at higher temperatures and darker at low temperatures. The ocelli are colourless (Bridges, 1931). At 25°C, Nolte (1954) found 12% of wild type red pigment and 9% brown. In combination with scarlet, the eye colour is only slightly lighter than light alone, and with brown it becomes clear lemon-yellow (Mainx, 1938). Development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936). The pteridine accumulation pattern is of type II (Gregg & Smucker, 1965).

2-55.7 burgundy bur RK1

The eye colour is a dull, darkish brown, like purple. The double homozygote with cinnabar has brilliant orange eyes (Meyer & Edmondson, 1949). Viability is good, as is the fertility of the females. Fertility of the males is variable.

2-56 lightoid ltd RK1

The eye colour is a clear, translucent red, similar to red, and darkening with age. The ocelli and Malpighian tubules are colourless. Nolte (1954) found pigment contents of 38% wild type red and 7% of wild type brown. Baglioni (1958) observed that the combination of brown and lightoid results in white eyes, with no apparent formation of ommochrome in response to feeding with kynurenine or 3-hydroxykynurenine. The pteridine accumulation pattern is of type III (Gregg & Smucker, 1965). The phenoxazinone synthetase activity is reduced (Phillips, Forrest & Kulkarni, 1973).

2-57 specific dilutor dil RK3

This mutant was reported by Bridges as a specific dilutor of brown and a number of white locus alleles.

2-57.1 burnt orange buo RK2

The eyes are bright orange brown in colour. The larval Malpighian tubules are colourless (Brehme and Demerec, 1942).

2-57.5 cinnabar cn RK1

The eyes are bright red, darkening with age. The ocelli are colourless. The larval Malpighian tubules are pale yellow (Beadle, 1937). The development of transplanted eye Anlagen is non-autonomous (Beadle & Ephrussi, 1936). Cinnabar blocks the conversion of kynurenine to 3-hydroxykynurenine (Butenandt, Wiedel & Schlossberger, 1949), identified as the " cn^+ hormone" (Beadle & Tatum, 1936). There is no detectable kynurenine 3-hydroxylase activity, and cn is probably the structural locus for this enzyme. The kynurenine formamidase activity is more or less wild type (Glassman, 1956). The phenoxazinone synthetase activity is reduced (Phillips, Forrest and Kulkarni, 1973). The pteridine accumulation pattern is of type II (Gregg & Smucker, 1965). Kalmus (1943) reports a normal optometer response in cn flies which is absent in cn; bw flies.

2-71.5 safranin sf RK2

The eye colour is a soft, dark brown, most easily classified in males and old flies. The larval Malpighian tubules are pale yellow, but distinct from wild type (Brehme & Demerec, 1942). The allele sf² was found by Nolte (1955) to have 47% of wild type red and 98% of

wild type brown. The development of transplanted eye anlagen is autonomous in transplant (Beadle & Ephrussi, 1936). The Malpighian tubules of this allele appear wild type (Beadle, 1937).

2-73 dark eye dke RK2

The eye colour is similar to that of safranin of which dke is definitely not an allele (Bridges,). Nolte (1955) found 65% of wild type red and 98% of wild type brown.

2-97 punch pu RK2A

The eye colour of heterozygotes is a dilute purple. Punch is a homozygous lethal (Muller, 1930).

2-100 pinkish pkh RK3

This mutant acts as a specific dilutor of white eosin (Bridges, 1919).

2-104.5 brown bw RK1

The eye colour at eclosion is light brown, darkening with age. The red pigments are absent, and Nolte (1954) noted a reduction to 87% of wild type brown. The adult testes and vasa are colourless, while the larval Malpighian tubules are pale yellow (Beadle, 1937). The eyes are white in combination with v, cn, st and ltd (Mainx, 1938). The development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936). The kynurenine formamidase activity is more or less normal (Glassman, 1956). The phenoxazinone synthetase activity is normal (Phillips, Forrest & Kulkarni, 1973). No pteridines are found in adult heads (Gregg & Smucker, 1965).

Certain variegated alleles are known at this locus. Oster (1954) showed that this variegation may be suppressed in females by the presence of fragments of, or whole, Y chromosomes. Brown flies are more phototactic than v; bw flies (Kikkawa, 1948).

2-106.4 purpleoid pd RK2

The eye colour is dark pink or maroon, like purple but less extreme. The development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936). The Malpighian tubules are wild type (Beadle, 1937). The pteridine accumulation pattern is of type III (Gregg & Smucker, 1965).

2-107.0 orange or RK1

The eye colour is bright orange. Orange is not allelic to purpleoid.

3-0.0 sherry she RK3

The eye colour is like that of sherry wine. Sherry flies are sterile inter se but both sexes are cross-fertile (Kaliss, 1937).

3-14.7 syndrome syn RK2

The eye colour in males is translucent brown, in females, darker than wild type. The wings are held at 90° to the body in males, at 45° in females. Viability is low and both sexes are sterile (Wallbrunn, 1964).

3-17 raisin rai RK2

The eye colour is slightly darker than that of sepia. The pupal period is somewhat extended (Hersh, 1955).

3-19.2

Moiré

Me

RK1A

The eye has a shimmering, iridescent pattern owing to a ring of six flecks round the normal one. The eye colour is translucent brown (Muller, 1930). The Malpighian tubules are lighter than normal, but difficult to classify (Brehme & Demerec, 1942). Moiré can be classified when present as a single dose in triploids (Sthultz, 1936). It is lethal when homozygous.

3-23.0

Henna

Hn

RK2A

The eye colour is a homogeneous dark, dull brown. Classification is easiest in older flies. The homozygote is lethal. Henna-recessive (Hn^r). The eye colour of the homozygote is dark brown. Development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936). Sepiapterin reductase activity is reduced and sepiapterin is accumulated (Taira, 1960), giving a type II accumulation pattern (Gregg & Smucker, 1965).

3-26.0

sepia

se

RK1

The eye colour is brown at eclosion, darkening with age through sepia to almost black. The ocelli are normal. Sepia has no red pigment, but accumulates the yellow pterin, sepiapterin. The development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936). The morphology of sepiapterin-containing pigment granules is distinct from those containing the red drosoppterin (Nolte, 1954). The pteridine accumulation pattern is of type VII (Gregg & Smucker, 1965).

3-35.0 rose rs RK2

The eye colour is purplish pink, but approaches wild type. Viability is reduced, and the flies, especially the males, are often sterile.

3-36.5 cream in chromosome 3 cr-3 RK3

The eyes are dilute in colour and mutants of this locus act as dilutors of some white alleles.

3-44.0 scarlet st RK1

The eyes are bright red, darkening with age. The ocelli are colourless. The eyes of bw; st homozygotes are white. Development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936). The Malpighian tubules are pale yellow (Brehme & Demerec, 1942). The kynurenine formamidase activity is more or less wild type (Glassman, 1956). 3-hydroxykynurenine is present in pupae but is excreted at eclosion (Howells & Ryall, 1975). The transport of 3-hydroxykynurenine from the Malpighian tubules to the eye granules appears to be deficient (Sullivan & Sullivan, 1975; Howells & Ryall, 1975). The pteridine accumulation pattern is of type II (Gregg & Smucker, 1965). Feeding scarlet larvae with either kynurenine or 3-hydroxykynurenine does not induce xanthemation synthesis (Baglioni, 1958).

3-46 jelly je RK1

The eyes are dark pink in colour.

3-47.7 dark red brown drb RK2

The eye colour is dark red brown at 18°C and dark red at 25°C. The mutation may be associated with a chromosomal rearrangement.

3-48.0 pink p RK2

The eyes are dull ruby with a purplish tone. The larval Malpighian tubules are colourless (Brehm & Demerec, 1942).

pink-peach (p^P). This allele has lighter eyes than pink, becoming brown with age. Development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936). The pteridine accumulation pattern is of type IV (Gregg & Smucker, 1965).

3-49.7 maroon ma RK2

The eyes are dull ruby, approaching wild type with age. The Malpighian tubules are pale yellow (Beadle, 1937). The development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936).

3-51.7 karmoisin kar RK1

The eyes are similar to those of scarlet, but less bright. The ocelli are white. The Malpighian tubules are lighter than wild type, but difficult to classify in living larvae (Brehme & Demerec, 1942). Kynurenine formamidase activity is normal (Glassman, 1956). The pteridine accumulation pattern is of type II (Gregg & Smucker, 1965).

3-52.0 rosy ry RK1

The eye colour is reddish brown. Xanthine dehydrogenase activity is lacking, as are the pteridines associated with later stages in red pigment synthesis. Putative precursors to this step are accumulated (Chevnik et al., 1962). Extracts of rosy and maroon-like flies complement to give XDH activity, but this complementation does not occur in vivo in double mutants (Glassman & Mitchell, 1959). Electrophoretic variants of XDH map to the rosy locus, supporting the contention

that this is the structural locus for XDH (Gelbart et al., 1974). The development of transplanted eye anlagen is non-autonomous (Beadle & Ephrussi, 1936).

3-53.6 red Malpighian tubules red RK1

The eyes are brown, darkening with age. The Malpighian tubules of larvae and adults are a rusty red. The tubules of double homozygotes with vermilion or cinnabar are colourless, while those of double homozygotes with brown are red. This suggests that the pigment is an ommochrome (Oster, 1954). Development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936).

3-75.7 cardinal cd RK2

The eyes are yellowish vermilion, becoming more like wild type with age. The ocelli are white. Development of transplanted eye anlagen is autonomous (Beadle & Ephrussi, 1936). The larval Malpighian tubules are indistinguishable from wild type (Brehme & Demerec, 1942). Kynurenine formamidase activity is normal (Glassman, 1956). Phenoxazinone synthetase activity is reduced (Phillips et al., 1974). More precisely, a particle bound phe synase activity is absent in the heads, and cd has been suggested as the structural locus for this enzyme (Phillips et al., 1974). Feeding larvae on kynurenine or 3-hydroxy kynurenine does not result in xanthe mmatin synthesis (Baglioni, 1958). The pteridine accumulation pattern is of type II (Gregg & Smucker, 1965).

3-88 mahogany mah RK3

The eye colour is translucent brown in young flies, becoming brown with age. The larval Malpighian tubules are indistinguishable from wild type (Beadle, 1937). Kynurenine formamidase activity is normal (Glassman, 1956). The pteridine pattern is type II (Gregg & Smucker, 1965).

3-95.5 suppressor of purple su(pr) RK3(A)

This mutant completely suppresses the purple phenotype, but the flies are poorly viable and are sterile.

3-100.7 claret ca RK1

The eye colour is a light reddish orange. The Malpighian tubules are colourless (Beadle, 1937). Development of transplanted eye anlagen is more or less autonomous (Beadle & Ephrussi, 1936). Less kynurenine is produced (cn^+ hormone, Ephrussi & Beadle, 1936), although kynurenine formamidase activity is normal (Glassman, 1956). The pteridine accumulation pattern is of type III (Gregg & Smucker, 1965).

2- (left arm) persimmon pers RK2A

The eye colour is dull orange. The larval Malpighian tubules are colourless (Brehme & Demerec, 1942). Viability and fertility are good. The mutation arose in association with an inversion, from which it has not been separated.

Tables of Data

Table A1 - A7 contain in summary form the data obtained on intermediate pool levels of all the stocks examined in the course of this work. Each value represents the mean of at least three independent samplings, and the standard errors are attached. The concentration units are $\mu\text{moles/g.}$ live weight of pupae. The % figures in the column headed "genotype" refer to the % of Oregon K brown pigment found in the respective stocks.

Table A8 gives details of the pigment contents summarised in Table 14.

TABLE A1

Tryptophan, Kynurenine and 3-Hydroxykynurenine Contents of Bright-Eyed Mutants and their Heterozygotes.

GENOTYPE	HOURS AFTER PUPATION	TRYPTOPHAN	KYNURENINE	3-HYDROXY-KYNURENINE
Oregon K 100%	72	0.10 $\pm$ 0.007	0.050 $\pm$ 0.004	0.76 $\pm$ 0.041
	84	0.15 $\pm$ 0.009	0.026 $\pm$ 0.005	0.44 $\pm$ 0.057
	96	0.13 $\pm$ 0.010	0.051 $\pm$ 0.004	0.40 $\pm$ 0.048
vermillion 0%	72	0.19 $\pm$ 0.013	0.002 $\pm$ 0.003	0.02 $\pm$ 0.015
	84	0.22 $\pm$ 0.015	0.003 $\pm$ 0.002	0.03 $\pm$ 0.020
	96	0.20 $\pm$ 0.012	0.004 $\pm$ 0.002	0.02 $\pm$ 0.018
vermillion "heterozygote" 115%	72	0.16 $\pm$ 0.009	0.040 $\pm$ 0.005	0.70 $\pm$ 0.029
	84	0.15 $\pm$ 0.007	0.032 $\pm$ 0.007	0.18 $\pm$ 0.014
	96	0.12 $\pm$ 0.013	0.051 $\pm$ 0.009	0.10 $\pm$ 0.015
cinnabar 0%	72	0.12 $\pm$ 0.015	0.030 $\pm$ 0.004	0.01 $\pm$ 0.010
	84	0.13 $\pm$ 0.007	0.060 $\pm$ 0.002	0.02 $\pm$ 0.017
	96	0.10 $\pm$ 0.009	0.041 $\pm$ 0.005	0.02 $\pm$ 0.021
cinnabar heterozygote 106.7%	72	0.17 $\pm$ 0.010	0.045 $\pm$ 0.003	0.90 $\pm$ 0.023
	84	0.15 $\pm$ 0.011	0.095 $\pm$ 0.008	0.84 $\pm$ 0.029
	96	0.10 $\pm$ 0.015	0.073 $\pm$ 0.006	0.20 $\pm$ 0.015
cardinal 0%	72	0.10 $\pm$ 0.013	0.040 $\pm$ 0.005	0.54 $\pm$ 0.033
	84	0.13 $\pm$ 0.007	0.025 $\pm$ 0.003	0.80 $\pm$ 0.028
	96	0.08 $\pm$ 0.009	0.025 $\pm$ 0.005	0.50 $\pm$ 0.019
cardinal heterozygote 80%	72	0.17 $\pm$ 0.012	0.031 $\pm$ 0.005	1.00 $\pm$ 0.043
	84	0.15 $\pm$ 0.011	0.042 $\pm$ 0.004	0.20 $\pm$ 0.017
	96	0.10 $\pm$ 0.012	0.053 $\pm$ 0.007	0.19 $\pm$ 0.032
scarlet 0%	72	0.10 $\pm$ 0.005	0.020 $\pm$ 0.006	0.58 $\pm$ 0.020
	84	0.17 $\pm$ 0.016	0.030 $\pm$ 0.004	0.60 $\pm$ 0.090
	96	0.18 $\pm$ 0.20	0.041 $\pm$ 0.001	0.21 $\pm$ 0.020
scarlet heterozygote 87.2%	72	0.24 $\pm$ 0.013	0.055 $\pm$ 0.004	1.00 $\pm$ 0.065
	84	0.25 $\pm$ 0.009	0.075 $\pm$ 0.007	0.91 $\pm$ 0.072
	96	0.11 $\pm$ 0.010	0.075 $\pm$ 0.006	0.72 $\pm$ 0.059

TABLE A1 (Cont/d....)

karmoisin 0%	72	0.07 ± 0.008	0.045 ± 0.007	0.61 ± 0.036
	84	0.14 ± 0.015	0.050 ± 0.004	0.50 ± 0.021
	96	0.12 ± 0.003	0.046 ± 0.003	0.23 ± 0.050
karmoisin heterozygote 102%	72	0.71 ± 0.011	0.099 ± 0.006	0.84 ± 0.061
	84	-	-	-
	96	-	-	-
light 0%	72	0.07 ± 0.010	0.047 ± 0.006	-
	84	0.09 ± 0.003	0.045 ± 0.009	0.08 ± 0.009
	96	0.10 ± 0.005	0.045 ± 0.009	0.32 ± 0.035
light heterozygote 121.1%	72	0.19 ± 0.013	0.055 ± 0.004	0.80 ± 0.021
	84	0.13 ± 0.009	0.055 ± 0.009	0.55 ± 0.017
	96	0.10 ± 0.011	0.050 ± 0.007	0.14 ± 0.015
lightoid 0%	72	0.08 ± 0.010	0.025 ± 0.001	0.08 ± 0.009
	84	0.07 ± 0.010	0.54 ± 0.008	0.07 ± 0.004
	96	0.12 ± 0.015	0.025 ± 0.002	0.12 ± 0.045
lightoid heterozygote 92.8%	72	0.17 ± 0.012	0.050 ± 0.006	0.84 ± 0.032
	84	0.12 ± 0.011	0.049 ± 0.003	1.00 ± 0.027
	96	0.08 ± 0.009	0.035 ± 0.007	0.10 ± 0.010

TABLE A2

Tryptophan, Kynurenine and 3-Hydroxykynurenine Contents of white
Locus Mutants.

MUTANT	HOURS AFTER PUPATION	TRYPTOPHAN	KYNURENINE	3-HYDROXY-KYNURENINE
white	72	0.16 $\pm$ 0.013	0.100 $\pm$ 0.005	0.36 $\pm$ 0.027
	84	0.12 $\pm$ 0.015	0.065 $\pm$ 0.003	0.30 $\pm$ 0.032
	96	0.10 $\pm$ 0.007	0.050 $\pm$ 0.004	0.14 $\pm$ 0.010
white ^{buff}	72	0.13 $\pm$ 0.015	0.055 $\pm$ 0.007	0.72 $\pm$ 0.020
	84	0.11 $\pm$ 0.005	0.020 $\pm$ 0.001	0.52 $\pm$ 0.055
	96	0.07 $\pm$ 0.018	0.035 $\pm$ 0.002	0.55 $\pm$ 0.030
white ^{ecru}	72	0.14 $\pm$ 0.012	0.088 $\pm$ 0.006	0.28 $\pm$ 0.015
	84	0.08 $\pm$ 0.005	0.045 $\pm$ 0.004	0.16 $\pm$ 0.020
	96	0.12 $\pm$ 0.010	0.071 $\pm$ 0.003	0.22 $\pm$ 0.019
white ^{honey}	72	0.07 $\pm$ 0.005	0.072 $\pm$ 0.004	0.70 $\pm$ 0.032
	84	0.16 $\pm$ 0.009	0.022 $\pm$ 0.004	0.96 $\pm$ 0.022
	96	0.19 $\pm$ 0.012	0.031 $\pm$ 0.003	0.44 $\pm$ 0.014
white ^{eosin}	72	0.09 $\pm$ 0.008	0.050 $\pm$ 0.007	0.18 $\pm$ 0.015
	84	-	-	-
	96	-	-	-
white ^{blood}	72	0.15 $\pm$ 0.014	0.032 $\pm$ 0.008	0.26 $\pm$ 0.014
	84	0.22 $\pm$ 0.020	0.030 $\pm$ 0.001	0.46 $\pm$ 0.030
	96	0.09 $\pm$ 0.005	0.045 $\pm$ 0.011	0.88 $\pm$ 0.042
white ^{copper}	72	0.23 $\pm$ 0.016	0.085 $\pm$ 0.009	0.42 $\pm$ 0.030
	84	0.11 $\pm$ 0.009	0.080 $\pm$ 0.003	0.20 $\pm$ 0.020
	96	0.15 $\pm$ 0.012	0.242 $\pm$ 0.015	0.29 $\pm$ 0.034
white ^{cherry}	72	0.05 $\pm$ 0.004	0.025 $\pm$ 0.004	0.25 $\pm$ 0.020
	84	0.07 $\pm$ 0.005	0.056 $\pm$ 0.002	-
	96	0.12 $\pm$ 0.018	0.060 $\pm$ 0.001	0.52 $\pm$ 0.017
white ^{coral}	72	0.16 $\pm$ 0.012	0.115 $\pm$ 0.009	0.29 $\pm$ 0.25
	84	0.12 $\pm$ 0.007	0.058 $\pm$ 0.007	0.27 $\pm$ 0.026
	96	0.20 $\pm$ 0.013	0.120 $\pm$ 0.005	0.40 $\pm$ 0.43
white ^{Brownex}	72	0.13 $\pm$ 0.010	0.120 $\pm$ 0.003	0.43 $\pm$ 0.042
	84	0.15 $\pm$ 0.011	0.095 $\pm$ 0.008	0.32 $\pm$ 0.026
	96	0.09 $\pm$ 0.008	0.056 $\pm$ 0.005	0.24 $\pm$ 0.021

TABLE A2 (Cont/d...)

white coffee	72	0.12 ± 0.010	0.122 ± 0.006	0.43 ± 0.049
	84	0.16 ± 0.006	0.095 ± 0.003	0.32 ± 0.020
	96	0.10 ± 0.007	0.055 ± 0.003	0.14 ± 0.010
white satsuma	72	0.09 ± 0.015	0.079 ± 0.008	0.18 ± 0.015
	84	0.24 ± 0.005	0.045 ± 0.002	0.92 ± 0.085
	96	0.14 ± 0.010	0.040 ± 0.004	0.95 ± 0.049

TABLE A3

Tryptophan, Kynurenine and 3-Hydroxykynurenine Contents of First Chromosome Mutants.

MUTANT	HOURS AFTER PUPATION	TRYPTOPHAN	KYNURENINE	3-HYDROXY-KYNURENINE
prune 93.12%	72	0.13 ± 0.009	0.075 ± 0.006	0.45 ± 0.012
	84	0.16 ± 0.007	0.060 ± 0.004	0.55 ± 0.025
	96	0.10 ± 0.002	0.027 ± 0.004	0.30 ± 0.041
chocolate 78.31%	72	0.16 ± 0.010	0.060 ± 0.005	0.35 ± 0.023
	84	0.14 ± 0.009	0.083 ± 0.007	0.19 ± 0.013
	96	0.13 ± 0.011	0.102 ± 0.006	0.30 ± 0.015
ruby 46.65%	72	0.11 ± 0.006	0.050 ± 0.008	0.28 ± 0.017
	84	0.07 ± 0.030	0.029 ± 0.009	0.34 ± 0.014
	96	0.09 ± 0.015	0.058 ± 0.003	0.73 ± 0.041
bordeaux 26.52%	72	0.14 ± 0.016	0.055 ± 0.002	0.42 ± 0.059
	84	0.17 ± 0.013	0.070 ± 0.005	0.58 ± 0.032
	96	0.14 ± 0.015	0.033 ± 0.003	0.94 ± 0.029
carmine 51.52%	72	0.14 ± 0.007	0.050 ± 0.006	0.24 ± 0.015
	84	0.13 ± 0.012	0.059 ± 0.007	0.28 ± 0.021
	96	0.12 ± 0.009	0.045 ± 0.009	0.34 ± 0.035
raspberry ² 77.49%	72	0.18 ± 0.021	0.087 ± 0.009	0.42 ± 0.027
	84	0.16 ± 0.015	0.056 ± 0.005	0.48 ± 0.018
	96	0.10 ± 0.011	0.045 ± 0.009	0.26 ± 0.012
garnet 48.34%	72	0.14 ± 0.009	0.070 ± 0.002	0.49 ± 0.039
	84	0.10 ± 0.007	0.025 ± 0.003	0.47 ± 0.049
	96	0.15 ± 0.012	0.040 ± 0.007	0.26 ± 0.014
carnation 42.84%	72	0.19 ± 0.009	0.045 ± 0.006	0.40 ± 0.015
	84	-	-	-
	96	0.12 ± 0.013	0.025 ± 0.00	0.26 ± 0.020
maroonlike ^{bronzy} 61.87%	72	0.17 ± 0.015	0.094 ± 0.006	
	84	0.16 ± 0.008		
	96	0.16 ± 0.014	0.024 ± 0.004	

TABLE A4

Tryptophan, Kynurenine and 3-Hydroxykynurenine Contents of Second Chromosome Mutants.

MUTANT	HOURS AFTER PUPATION	TRYPTOPHAN	KYNURENINE	3-HYDROXY-KYNURENINE
clot 117.40%	72	0.09 $\pm$ 0.010	0.060 $\pm$ 0.001	0.50 $\pm$ 0.020
	84	0.14 $\pm$ 0.009	0.020 $\pm$ 0.003	0.96 $\pm$ 0.018
	96	0.13 $\pm$ 0.002	0.025 $\pm$ 0.002	0.66 $\pm$ 0.059
safranin 67.67%	72	0.17 $\pm$ 0.013	0.040 $\pm$ 0.002	0.42 $\pm$ 0.080
	84	0.07 $\pm$ 0.003	0.049 $\pm$ 0.002	0.94 $\pm$ 0.020
	96	0.08 $\pm$ 0.005	0.030 $\pm$ 0.001	0.18 $\pm$ 0.009
brown 42.80%	72	0.17 $\pm$ 0.011	0.040 $\pm$ 0.005	0.39 $\pm$ 0.012
	84	0.25 $\pm$ 0.012	0.065 $\pm$ 0.001	0.50 $\pm$ 0.020
	96	0.15 $\pm$ 0.014	0.115 $\pm$ 0.010	0.40 $\pm$ 0.024
orange 77.90%	72	0.24 $\pm$ 0.014	0.105 $\pm$ 0.007	0.40 $\pm$ 0.015
	84	0.08 $\pm$ 0.007	0.040 $\pm$ 0.003	0.26 $\pm$ 0.009
	96	-	-	-
purpleoid 31.22%	72	0.17 $\pm$ 0.020	0.046 $\pm$ 0.001	0.30 $\pm$ 0.017
	84	0.16 $\pm$ 0.010	0.050 $\pm$ 0.003	0.96 $\pm$ 0.033
	96	0.21 $\pm$ 0.002	0.030 $\pm$ 0.005	0.60 $\pm$ 0.011

TABLE A5

Tryptophan, Kynurenine and 3-Hydroxykynurenine Contents of Third Chromosome Mutants.

MUTANT	HOURS AFTER PUPATION	TRYPTOPHAN	KYNURENINE	3-HYDROXY-KYNURENINE
Henna recessive 56.22%	72	0.25 $\pm$ 0.015	0.035 $\pm$ 0.005	0.24 $\pm$ 0.075
	84	0.20 $\pm$ 0.013	0.045 $\pm$ 0.001	0.40 $\pm$ 0.024
	96	-	-	-
sepia 68.78%	72	0.18 $\pm$ 0.002	0.035 $\pm$ 0.006	0.30 $\pm$ 0.048
	84	0.12 $\pm$ 0.015	0.020 $\pm$ 0.004	-
	96	0.18 $\pm$ 0.007	0.028 $\pm$ 0.001	0.64 $\pm$ 0.090
dark red brown 99.00%	72	0.13 $\pm$ 0.015	0.121 $\pm$ 0.003	0.32 $\pm$ 0.020
	84	0.21 $\pm$ 0.013	0.115 $\pm$ 0.005	0.38 $\pm$ 0.023
	96	0.08 $\pm$ 0.006	0.086 $\pm$ 0.002	0.20 $\pm$ 0.010
pink 17.80%	72	0.24 $\pm$ 0.012	0.055 $\pm$ 0.009	1.00 $\pm$ 0.082
	84	0.12 $\pm$ 0.010	0.053 $\pm$ 0.009	0.50 $\pm$ 0.064
	96	0.05 $\pm$ 0.009	0.040 $\pm$ 0.004	0.24 $\pm$ 0.014
red 107.46%	72	0.13 $\pm$ 0.013	0.067 $\pm$ 0.004	0.22 $\pm$ 0.020
	84	0.12 $\pm$ 0.007	0.055 $\pm$ 0.007	0.30 $\pm$ 0.025
	96	0.20 $\pm$ 0.015	0.064 $\pm$ 0.005	0.31 $\pm$ 0.042
mahogany 129.14%	72	0.20 $\pm$ 0.025	0.105 $\pm$ 0.013	0.28 $\pm$ 0.020
	84	0.14 $\pm$ 0.017	0.095 $\pm$ 0.007	0.28 $\pm$ 0.015
	96	0.14 $\pm$ 0.011	0.075 $\pm$ 0.005	0.50 $\pm$ 0.013
claret 33.84%	72	-	-	-
	84	0.15 $\pm$ 0.002	0.045 $\pm$ 0.004	0.50 $\pm$ 0.033
	96	0.18 $\pm$ 0.013	0.025 $\pm$ 0.013	0.58 $\pm$ 0.016

TABLE A6

Tryptophan, Kynurenine and 3-Hydroxykynurenine Contents of Prevost's Second Chromosome Lines.

GENOTYPE	HOURS AFTER PUPATION	TRYPTOPHAN	KYNURENINE	3-HYDROXY-KYNURENINE
Standard	72	0.07 $\pm$ 0.007	0.071 $\pm$ 0.005	0.41 $\pm$ 0.051
	84	0.14 $\pm$ 0.011	0.032 $\pm$ 0.003	0.21 $\pm$ 0.042
	96	0.10 $\pm$ 0.009	0.030 $\pm$ 0.007	0.24 $\pm$ 0.015
P94	72	0.09 $\pm$ 0.010	0.048 $\pm$ 0.004	0.32 $\pm$ 0.040
	84	0.12 $\pm$ 0.007	0.049 $\pm$ 0.005	0.30 $\pm$ 0.023
	96	0.11 $\pm$ 0.006	0.031 $\pm$ 0.003	0.50 $\pm$ 0.065
1/140	72	0.10 $\pm$ 0.001	0.54 $\pm$ 0.001	0.35 $\pm$ 0.025
	84	0.17 $\pm$ 0.008	0.056 $\pm$ 0.006	0.49 $\pm$ 0.020
	96	0.13 $\pm$ 0.004	0.039 $\pm$ 0.001	0.60 $\pm$ 0.010
1/146	72	0.10 $\pm$ 0.011	0.041 $\pm$ 0.002	0.45 $\pm$ 0.010
	84	-	-	-
	96	0.16 $\pm$ 0.015	0.025 $\pm$ 0.001	0.61 $\pm$ 0.013
1/57	72	0.09 $\pm$ 0.005	0.115 $\pm$ 0.003	0.68 $\pm$ 0.015
	84	0.08 $\pm$ 0.009	0.131 $\pm$ 0.001	0.88 $\pm$ 0.025
	96	0.16 $\pm$ 0.010	0.022 $\pm$ 0.005	0.74 $\pm$ 0.005
1/64	72	0.18 $\pm$ 0.010	0.075 $\pm$ 0.001	0.60 $\pm$ 0.010
	84	0.90 $\pm$ 0.01-	0.045 $\pm$ 0.002	0.13 $\pm$ 0.056
	96	0.20 $\pm$ 0.005	0.050 $\pm$ 0.002	0.30 $\pm$ 0.030
P55	72	0.11 $\pm$ 0.013	0.068 $\pm$ 0.009	0.09 $\pm$ 0.030
	84	0.10 $\pm$ 0.010	0.081 $\pm$ 0.002	0.31 $\pm$ 0.015
	96	0.16 $\pm$ 0.003	0.075 $\pm$ 0.002	0.22 $\pm$ 0.002
27	72	0.13 $\pm$ 0.005	0.042 $\pm$ 0.002	0.52 $\pm$ 0.005
	84	0.04 $\pm$ 0.010	0.031 $\pm$ 0.002	0.10 $\pm$ 0.052
	96	0.17 $\pm$ 0.015	0.105 $\pm$ 0.003	0.30 $\pm$ 0.025
2/89	72	0.14 $\pm$ 0.020	0.040 $\pm$ 0.006	0.38 $\pm$ 0.065
	84	0.14 $\pm$ 0.015	0.075 $\pm$ 0.001	0.60 $\pm$ 0.040
	96	0.15 $\pm$ 0.012	0.025 $\pm$ 0.001	0.50 $\pm$ 0.040

TABLE A6 (Cont/d....)

2/103	72	0.13 ± 0.013	0.070 ± 0.005	0.20 ± 0.051
	84	0.14 ± 0.012	0.040 ± 0.007	0.40 ± 0.015
	96	0.06 ± 0.015	0.030 ± 0.005	0.50 ± 0.023
1/108	72	0.10 ± 0.010	0.080 ± 0.001	0.74 ± 0.065
	84	0.17 ± 0.010	0.089 ± 0.006	0.50 ± 0.020
	96	0.19 ± 0.013	0.025 ± 0.003	0.72 ± 0.001
2/10	72	0.18 ± 0.010	0.049 ± 0.003	0.30 ± 0.020
	84	0.16 ± 0.015	0.049 ± 0.005	0.19 ± 0.025
	96	0.15 ± 0.011	0.030 ± 0.002	0.90 ± 0.013

TABLE A7

Tryptophan, Kynurenine and 3-Hydroxykynurenine Contents of
Prevosti Selected Lines.

LINE	HOURS AFTER PUPATION	TRYPTOPHAN	KYNURENINE	3-HYDROXY- KYNURENINE
base	72	0.22 $\pm$ 0.020	0.037 $\pm$ 0.001	0.43 $\pm$ 0.03
	84	0.25 $\pm$ 0.021	0.041 $\pm$ 0.001	1.05 $\pm$ 0.05
	96	0.23 $\pm$ 0.015	0.055 $\pm$ 0.006	1.02 $\pm$ 0.06
c ₁	72	0.27 $\pm$ 0.07	0.025 $\pm$ 0.006	0.77 $\pm$ 0.08
	84	0.22 $\pm$ 0.013	0.053 $\pm$ 0.004	0.56 $\pm$ 0.01
	96	0.18 $\pm$ 0.008	0.044 $\pm$ 0.003	1.06 $\pm$ 0.06
c ₂	72	0.09 $\pm$ 0.081	0.048 $\pm$ 0.002	0.17 $\pm$ 0.01
	84	0.22 $\pm$ 0.011	0.069 $\pm$ 0.005	0.20 $\pm$ 0.01
	96	0.16 $\pm$ 0.093	0.033 $\pm$ 0.002	0.74 $\pm$ 0.03
c ₃	72	0.16 $\pm$ 0.012	0.051 $\pm$ 0.007	0.10 $\pm$ 0.02
	84	0.78 $\pm$ 0.015	0.080 $\pm$ 0.004	0.42 $\pm$ 0.04
	96	0.15 $\pm$ 0.008	0.050 $\pm$ 0.005	0.40 $\pm$ 0.01
H ₁	72	0.24 $\pm$ 0.016	0.041 $\pm$ 0.004	1.08 $\pm$ 0.08
	84	0.20 $\pm$ 0.015	0.032 $\pm$ 0.003	0.33 $\pm$ 0.031
	96	0.19 $\pm$ 0.21	0.033 $\pm$ 0.003	0.78 $\pm$ 0.149
H ₂	72	0.16 $\pm$ 0.013	0.056 $\pm$ 0.006	1.41 $\pm$ 0.089
	84	0.27 $\pm$ 0.019	0.065 $\pm$ 0.010	1.12 $\pm$ 4.05
	96	0.27 $\pm$ 0.018	0.054 $\pm$ 0.011	1.07 $\pm$ 0.162
H ₃	72	0.15 $\pm$ 0.026	0.047 $\pm$ 0.007	0.21 $\pm$ 0.084
	84	0.25 $\pm$ 0.018	0.079 $\pm$ 0.002	0.61 $\pm$ 0.223
	96	0.17 $\pm$ 0.031	0.036 $\pm$ 0.001	0.24 $\pm$ 0.076
L ₁	72	0.14 $\pm$ 0.006	0.036 $\pm$ 0.009	1.21 $\pm$ 0.190
	84	0.20 $\pm$ 0.040	0.041 $\pm$ 0.011	0.75 $\pm$ 0.177
	96	0.21 $\pm$ 0.022	0.048 $\pm$ 0.007	1.01 $\pm$ 0.129
L ₂	72	0.20 $\pm$ 0.039	0.069 $\pm$ 0.011	1.21 $\pm$ 0.147
	84	0.22 $\pm$ 0.030	0.068 $\pm$ 0.006	1.03 $\pm$ 0.125
	96	0.22 $\pm$ 0.021	0.042 $\pm$ 0.003	1.07 $\pm$ 0.109
L ₃	72	0.105 $\pm$ 0.059	0.048 $\pm$ 0.004	0.27 $\pm$ 0.056
	84	0.20 $\pm$ 0.027	0.030 $\pm$ 0.004	0.25 $\pm$ 0.018
	96	0.16 $\pm$ 0.009	0.060 $\pm$ 0.010	1.07 $\pm$ 0.093

TABLE A8

Pigment Contents of F_1 's of Crosses Between Mutant Stocks and Prevosti Lines.

CROSS	RED PIGMENT	BROWN PIGMENT	% RED	% BROWN
Oregon K x Prevosti S	81.50 $\pm$ 0.76	24.70 $\pm$ 1.10	100	100
cl x P55	68.00 $\pm$ 1.53	25.30 $\pm$ 2.60	83.4	102.4
cl x 1/108	55.50 $\pm$ 2.83	29.60 $\pm$ 0.87	68.1	119.8
cl x 1/146	69.00 $\pm$ 4.33	28.50 $\pm$ 1.30	84.6	115.4
cl x 2?	57.50 $\pm$ 1.34	28.80 $\pm$ 1.80	70.6	117.6
cl x 1/57	87.50 $\pm$ 1.54	38.80 $\pm$ 1.63	107.4	157.1
cl x 2/89	64.00 $\pm$ 2.08	25.50 $\pm$ 0.90	78.3	103.2
cl x 2/10	66.50 $\pm$ 1.30	27.70 $\pm$ 1.43	81.6	112.2
cl x 1/64	67.73 $\pm$ 1.04	30.50 $\pm$ 1.17	83.1	123.5
cl x Oregon K	80.00 $\pm$ 1.23	29.85 $\pm$ 1.13	98.2	120.9
cl x Prevosti S	82.50 $\pm$ 2.50	19.00 $\pm$ 1.00	101.2	76.9
pr x 1/146	85.56 $\pm$ 2.42	26.00 $\pm$ 1.10	105.0	105.3
pr x 2?	71.00 $\pm$ 1.63	26.00 $\pm$ 1.45	87.1	105.3
pr x P94	64.50 $\pm$ 2.03	31.10 $\pm$ 0.96	79.1	125.9
pr x 1/57	67.50 $\pm$ 2.14	28.10 $\pm$ 1.13	82.8	113.8
pr x 2/89	70.50 $\pm$ 2.17	33.44 $\pm$ 1.20	86.5	139.2
pr x 2/10	60.50 $\pm$ 1.17	10.50 $\pm$ 1.70	74.2	42.5
pr x 1/64	81.67 $\pm$ 1.67	20.00 $\pm$ 1.34	100.2	81.0
pr x Oregon K	74.49 $\pm$ 1.57	23.34 $\pm$ 1.02	91.4	94.5
pr x Prevosti S	73.07 $\pm$ 2.22	28.93 $\pm$ 0.77	89.6	117.1
lt x 1/108	67.50 $\pm$ 2.14	24.80 $\pm$ 2.31	82.8	100.4
lt x P94	52.50 $\pm$ 1.86	22.50 $\pm$ 1.19	64.4	91.1
lt x 1/57	80.50 $\pm$ 2.37	41.10 $\pm$ 0.60	98.8	166.4
lt x 2/10	68.33 $\pm$ 1.67	16.00 $\pm$ 1.00	83.8	64.8
lt x 2/103	66.67 $\pm$ 1.68	29.39 $\pm$ 1.14	81.8	119.0
lt x 2/102	58.03 $\pm$ 1.32	26.50 $\pm$ 1.36	71.2	107.3
lt x Oregon K	79.00 $\pm$ 0.67	22.70 $\pm$ 0.79	96.9	91.9
lt x Prevosti S	74.17 $\pm$ 0.95	26.23 $\pm$ 1.21	91.0	106.2
ltd x 1/108	73.50 $\pm$ 1.98	17.90 $\pm$ 1.02	90.2	72.5
ltd x 1/146	86.50 $\pm$ 1.98	25.70 $\pm$ 1.08	106.1	104.1
ltd x P94	77.50 $\pm$ 3.75	27.10 $\pm$ 0.74	95.1	109.7
ltd x 1/57	77.22 $\pm$ 3.13	16.78 $\pm$ 1.16	94.5	67.6

ltd x 2/89/

TABLE A8 (Cont/d...)

ltd x 2/89	74.50 $\pm$ 2.29	24.20 $\pm$ 1.03	91.4	98.8
ltd x 2/103	91.00 $\pm$ 1.25	22.50 $\pm$ 0.83	111.7	91.1
ltd x Oregon K	87.50 $\pm$ 1.44	21.75 $\pm$ 1.18	107.4	88.1
cn x P55	78.33 $\pm$ 1.67	20.67 $\pm$ 0.67	96.1	83.7
cn x 1/108	87.20 $\pm$ 2.94	32.90 $\pm$ 0.74	106.9	133.2
cn x 1/146	71.50 $\pm$ 1.98	23.30 $\pm$ 1.05	87.7	94.2
cn x P94	75.00 $\pm$ 2.58	22.50 $\pm$ 1.34	92.0	91.1
cn x 1/57	72.50 $\pm$ 1.86	22.20 $\pm$ 0.79	88.9	89.9
cn x 2/89	57.00 $\pm$ 2.13	30.00 $\pm$ 1.29	69.9	121.5
cn x Oregon K	81.67 $\pm$ 1.67	20.67 $\pm$ 0.79	100.2	83.7
cn x Prevosti S	81.43 $\pm$ 0.92	19.86 $\pm$ 0.94	99.9	80.4
sf x P55	66.50 $\pm$ 2.24	35.30 $\pm$ 1.33	81.6	142.9
sf x 1/108	76.90 $\pm$ 2.25	34.20 $\pm$ 0.55	94.4	138.5
sf x P94	69.50 $\pm$ 2.41	25.50 $\pm$ 0.67	85.3	104.1
sf x 2/89	69.00 $\pm$ 1.00	28.80 $\pm$ 1.28	84.7	116.6
sf x 2/10	64.50 $\pm$ 2.17	25.00 $\pm$ 1.49	79.1	101.2
sf x 2/102	82.78 $\pm$ 1.21	24.10 $\pm$ 0.91	101.6	97.8
sf x 2/103	56.47 $\pm$ 0.95	26.10 $\pm$ 0.79	69.3	105.7
sf x Prevosti S	69.00 $\pm$ 1.56	28.00 $\pm$ 1.11	84.7	113.4
bw x P55	79.17 $\pm$ 1.67	23.33 $\pm$ 0.67	97.1	94.5
bw x 1/146	79.50 $\pm$ 2.17	30.70 $\pm$ 1.90	97.6	124.3
bw x 2?	67.00 $\pm$ 1.70	26.60 $\pm$ 1.01	82.2	107.7
bw x P94	64.00 $\pm$ 1.25	22.50 $\pm$ 1.12	78.5	91.1
bw x 2/89	66.00 $\pm$ 1.63	27.00 $\pm$ 1.11	81.0	109.3
bw x 2/10	58.18 $\pm$ 2.55	29.00 $\pm$ 1.45	62.8	117.4
bw x 1/64	55.50 $\pm$ 2.17	21.30 $\pm$ 1.34	68.1	86.2
bw x Oregon K	77.67 $\pm$ 1.28	28.93 $\pm$ 0.83	95.3	118.1
bw x Prevosti S	72.67 $\pm$ 1.75	27.00 $\pm$ 1.07	89.2	109.3
pd x P55	61.50 $\pm$ 1.30	8.70 $\pm$ 0.52	75.46	35.22
pd x 1/57	72.50 $\pm$ 2.71	30.90 $\pm$ 1.22	88.9	125.1
pd x 2/10	59.17 $\pm$ 2.01	22.50 $\pm$ 1.12	92.60	91.09
pd x 1/140	47.00 $\pm$ 3.43	30.50 $\pm$ 1.17	57.69	123.5
pd x Oregon K	66.67 $\pm$ 1.59	25.33 $\pm$ 1.50	81.80	102.55