

**Some New Eye-Colour Changes in
Gammarus chevreuxi Sexton.
Part II.**

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I. INTRODUCTION.

IN a previous paper (9) attention was drawn to the occurrence of five different "Mutant Stocks" of 'Red-eye' deviations from the normal black eye. Various differences in the characteristics of these stocks were indicated. For instance, Stock I Red-eye is a stable Bright Red behaving as a simple mendelian recessive, whilst in Stock V all kinds of shades of eye-colour occur with various sorts of changes during life.

It has since become clear, from the study of the inheritance of the eye-colours concerned, that we have to deal in each case with the effects of a

single gene* change. Reciprocal crosses have shown that a separate gene is involved at least in Stocks I, II, IV, and V.

In the present paper various hitherto unpublished data are brought together so that all relevant facts may be available for making as full comparison as possible of the five different recessive types to which reference has been made, and one other which has appeared since. Two further additions, the 'Flesh' and the 'Beet' Red-eyes, are being treated in a separate publication (p. 337).

The recessives here considered all show some deviation from the normal Black-eye in the production of pigment in the retinal cells. Though they are characterised as exhibiting redness in the eye to a more or less marked degree, the main characteristic which they share in common is *some kind of inhibition or retardation in the production of the black pigment (melanin)*.† In practice it happens that, since a deposit of melanin below normal density allows the red pigment that is present to show up, the degree of redness is a useful criterion of the state of melanin deposition. Various stages between complete absence and all but full concentration of melanin are represented by a gradation of shades from bright red to dark chocolate. It is useful, however, to focus attention on the state of the melanin in preference to the directly observable resultant effect of the two pigments, because the formation of red pigment is not always maintained at a constant level, and, particularly in some strains, may be subject to wide fluctuation of its own. The red pigment is at least liable to vary in respect to *quantity*, if not also in ways directly affecting the *quality*.

The state of pigmentation of the ommatidia is not only quite readily estimated from the shade of colour observable during life, but it may be known from more direct information given after preservation of the specimen in alcohol. The red pigment having dissolved away, whatever melanin was present is left.

The circumstances of the first occurrence of these recessives are stated here, but discussion of them is deferred until the whole question of the origin of mutant types in *Gammarus chevreuxi* is considered on another occasion.

The genetic details of the Stocks here described are tabulated in the form of pedigree charts, which are too bulky for publication, but which

* The expression 'gene' is used in this paper for no further purpose than implying that the instances under consideration are directly comparable with others which, in the current language of the geneticist, are described in terms of genes. For instance, each Red-eye stock is comparable to familiar examples where a single gene difference is said to be involved.

† The black pigment is denoted throughout as melanin, but it is to be emphasised that this pigment in Amphipods has not been subjected to investigation. While the work on the body pigments of other Arthropods, particularly that of J. Verne on Decapoda (Arch. de Morph. Gen. & Exp. 1923, XVI, p. 1, etc.), would seem to justify the assumption that the pigment is a member of the class of melanins, there is as yet no proof, and very little is known of its specific chemical properties.

are being preserved so as to be available for reference at the Marine Biological Laboratory, Plymouth.

The following abbreviations for eye-colours are used :—

B	.	.	Black
RB	.	.	Reddish Black
PB	.	.	Purplish Black
DR	.	.	Dark Red
DP	.	.	Dark Purple
P	.	.	Purple
RP	.	.	Reddish Purple
DIR	.	.	Dark Intermediate Red
IR	.	.	Intermediate Red.

These eye-colours (except DP and DIR) are figured in 9, Plate VIII.

II. STOCK I RED-EYE.

1. MAIN STOCK.

The designation 'Stock I Red-eye' is given to the original red-eye 'mutant,' which appeared in 1912 and which provided the chief material for the first Mendelian study in *Gammarus* (Sexton and Wing, 1; Allen and Sexton, 2). It has been more thoroughly investigated than any other of the eye-colour recessives, for in addition to the inbred stock which was kept by us for over 15 years, the strain is being maintained in the Zoology Department at Oxford, where it has formed the subject of important investigations by E. B. Ford and J. S. Huxley (3, 6, 7, 8).

At normal laboratory temperatures (i.e. ranging round 14° C., and seldom exceeding 17° C.) the recessives in the main inbred stock are sharply distinguished from the normal dominants, since the eye in them remains bright red throughout life. In appearance this red is typically of a quality (Normal Red, 9, Plate VIII) characteristic of the state when red pigment alone is present in the retinal cells. The production of dark pigment is in fact so completely retarded that either none is ever formed at all or else a small quantity is deposited in the older, central, ommatidia during the later stages of life. In ageing individuals the central darkening may be quite noticeable, especially as it is generally accompanied by a fading of the red.

The evidence derived from the appearance of the eye in living animals has been supplemented by the examination of a number of reds preserved in spirit. The following generalisation on melanic pigment formation is therefore possible. In early stages the eyes are always pure 'Normal' red (9, Plate VIII), that is, all the ommatidia have a full quota of red pigment without a trace of melanin. This condition is typically main-

tained throughout the span of an average adult life, but in long-lived individuals there has been found a certain amount of melanin deposition spreading from the central ommatidia. In more exceptional cases this melanin formation may start earlier, even, in one instance, when the individual was still immature. That some variation, however, of this sort should occur is hardly surprising, for one important environmental condition known to influence melanin deposition, namely, temperature, is not kept at a constant level, and its fluctuations may of themselves be sufficient to account for it, even if no other influences, whether environmental or hereditary, are present. But that the latter are not to be excluded is clear from the work of Ford and Huxley (6) who have discovered three distinct modifying factors which actually do affect the extent of pigment deposition in Stock I reds.

At higher temperatures the production of melanin is much accelerated. We have only recently had the chance to observe this ourselves, but reference may be made to the account given by Ford and Huxley (6) to whom we owe the discovery of the influence of temperature on the phenotypical expression of red-eye recessives and the interpretation of its theoretical significance. There is every reason to suppose that the reds in the strain with which these authors deal behave in essentially the same way as those in our Main Stock, from which they were originally derived. For the present, attention is drawn to the gradual darkening, due to the deposition of melanin, which occurs during life at higher temperatures (well summarised in 7, Fig. 3, p. 71). This darkening is evidently comparable with that which takes place rapidly in the normal black-eyed form during the few days preceding extrusion. A state of equilibrium is eventually reached at early maturity. The darkening starts in the centre of the eye, and, until equilibrium is reached, is here all the time relatively more advanced. The reason for this is that the older ommatidia are situated in the centre, and each ommatidium undergoes a similar process of change from red to dark chocolate.

The effect of the presence of the recessive gene concerned (r_1r_1), as the above authors point out, thus appears in the light of a retardation in the normal process of deposition of melanin and therefore receives expression in physiological or dynamic terms. The fact, however, of the occurrence of a final stable state which is typically lighter than the normal black, shows that other differences are correlated with the mere change in rate, and calls for more detailed interpretation.

2. CROSSES INVOLVING STOCK I RED-EYE.

Various crosses involving Mutant Stock I have been made at one time or another. These include matings with specimens both from other

laboratory Stocks and from the wild. Of more particular interest are the results obtained from crossing Stock I 'reds' and other mutant types. These results are given under the accounts of the mutant type involved and need not be considered here.

In some cases the progeny of the crosses have not been carried beyond the F_2 or F_3 , but in others they were in-bred for several generations, so giving rise to independent strains.

As far as the effects of the Stock I Red-eye gene are concerned, in none of the strains so derived is any deviation from the expected simple mendelian behaviour shown. There remains the question of the phenotypic character of the Stock I recessives.

It is noteworthy that, whereas in the Main Stock the recessives are of a remarkably uniform type and subject to little variation, when however all the r_1r_1 types from other strains are taken into consideration, a considerable range of variation is to be found. Illustrations may be given of those strains in which occur some of the more striking deviations from the "normal" Red-eye.

(1) A conspicuous example is provided by the descendants of a cross between a Stock I "red" and a heterozygous "black" from Mutant Stock III (p. 313). The r_1r_1 types, of which a number have been reared, are characteristically distinct from the typical "normal" reds Main Stock I. At laboratory temperatures they develop considerable darkening in the centre of the eye. The process starts not long after extrusion, but progresses slowly. Even in long-lived individuals the eye never passes beyond the state of dark red centre with light red periphery. (The effect of higher temperatures has not so far been tested.) Practically all individuals behave in the same way, and so bear an absolute, and not merely average, distinction from those of the Main Stock. The distinction is particularly definite in immature stages.

The genetic relations of this strain of reds, the numbers of which have become much reduced through cannibalism, are now being examined. Evidently some modifying factor, or set of factors, has been introduced in the cross. But whatever the actual genetic interpretation, the empirical result affords an example of how the effect of a particular gene may be modified when introduced into a new strain.

In certain other strains there seems to be a greater tendency for darkening to develop than in the Main Stock, but not to the extent seen in the Stock III Cross.

It may be added that in this strain a tendency of the red pigment to diminish somewhat in intensity has been noticed.

(2) Another strain which has been carried on for several generations was derived from a cross between the recessives of Stock V and Stock I ("HC," see p. 321). The Stock I "reds" which have segregated out have

been of the "normal red" type with no unusual tendency to darken, but most show deficiency in red pigment. Some adults indeed have so little red pigment as to be almost colourless. This is noteworthy, because the strain is composed entirely of Stock V recessives (see p. 321), among which loss of red pigment during immature stages is characteristic (p. 331).

(3) A certain range of variation within a single strain ("CHC" 788) is shown in the F_2 and F_3 of a mating between one of the above-mentioned HC Stock (this specimen did not carry the Stock I gene) and a Main Stock I "red." Among a number of surviving Stock I recessives hardly any two are alike. There is not only variation in intensity of red pigment, but in the extent to which darkening, of which almost all specimens have some, occurs in the centre of the eye. At extrusion several were noted as being pale,—orange instead of bright red; but this condition does not seem to have had any influence on the pigmentation in later life.

By comparison with other stocks which have been investigated more extensively, it is very probable that the variation in the intensity of the red pigment, as seen here, in (2), and in other strains, is partly non-hereditary. Yet, as a difference is shown between some strains, there is evidently a hereditary distinction somewhere. What, indeed, can be said to be inherited is a greater or less susceptibility to "environmental" influence. (For a fuller consideration of the inheritance of red pigment deficiency in another Stock, see p. 331).

(4) The issue from a cross between a Stock I "red" (from the Oxford Main Stock) and a 'Flesh' red (see p. 347) provides a further example. These animals were reared in the incubator at an average temperature of from 21° C. to 23° C. In the F_2 , there was variation in the degree of redness among specimens proved recessive for r_1 . Some came almost to lack pigment altogether, having a somewhat lilac colour, with the centre a darker purple. Since the 'Flesh' strain at these temperatures is characterised by tendency to great reduction of red pigment, no doubt here again some modifying factor introduced in the cross was at work.

From these illustrations it is seen that in some strains the character of the recessives is more or less uniform, in others liable to fluctuation, particularly in respect to the red pigment. Instances where there is comparative uniformity are provided (1) by the Main Stock (with which may be classed strains derived from certain crosses), in which full red pigmentation is maintained and only little, if any, darkening occurs; and (2) by the strain derived from the cross with Stock III, in which occurs conspicuous central darkening starting early in life, and a certain tendency for the intensity of the red to diminish. The latter case is no doubt susceptible of interpretation in terms of the action of modifying factors of the sort, described by Ford and Huxley (6, 7), which influence the rate of darkening

of r_1r_1 types at higher temperatures. But in this case the influence is greater than that of any of the modifying factors hitherto described.

III. STOCK II RED-EYE.

An account of Stock II and strains derived from it is under preparation (a preliminary note is contained in 4 and a short reference to the Stock in 9, p. 191). An in-bred "Main Stock" and strains derived from cross matings with Stock I have been investigated.

The recessives are characterised by a red-eye resembling that of Stock I. Their simple Mendelian behaviour has been fully verified, as has the fact that the hereditary factor involved (which is designated as gene r_2) is distinct from, and its action quite independent of, that of Stock I (r_1).

Matings between reds of the two Stocks give *all blacks*, with a 9 : 7 F_2 ratio of black to red.

The actual figures are :— 585 : 451

expected : 583 : 453

The phenotypical expression of r_2r_2 types of the Main Stock II, at laboratory temperatures, is identical with the r_1r_1 types of Main Stock I, giving typical 'Normal Red' eyes. The formation of the black melanin is completely inhibited in all but a few exceptional examples of adults, in which a faint deposit of melanin could be seen in the centre of the eye after preservation. It is generally characteristic of Main Stock II r_2r_2 types that at laboratory temperatures no melanin is deposited. And indeed just as complete an inhibition of melanin was seen in the earlier families which were kept at an average temperature of between 20° and 21° C. The effect of a temperature of higher than 21° C. is not known.

IV. ACCOUNT OF STOCK III.

A number of wild pairs were brought into the laboratory in Sept., 1922. Some were placed in an incubator at 21° C., and some kept under laboratory conditions. Of the former, four pairs gave an F_2 , two containing red-eyed specimens and giving rise to Stocks II and IV respectively; while of the latter, 16 pairs gave offspring, and in five cases there was some sort of deviation from the normal black eye-colour.

In one case Bright Reds appeared in the F_1 generation. Out of 5 survivors from a family of 89, 2 were found to have bright red eyes. All of these died without issue. The same ♀ gave a second family of 357 with another ♂, but all the survivors were black, and so were all the F_2 obtained from at least 4 pairs.

In three cases a slight reddening was noticed in blacks of the F_1 generation, but in neither case was the family carried beyond a scanty F_2 .

In the fifth case, which came to be known as Stock III, the F_1 were all black but some F_2 changed to Reddish Black. In the F_3 and F_4 three different gradations of red were found at birth, which were classified as Reddish Black, Dark Red, and Light Red. The main stock soon died out through cannibalism, but the strain has persisted in a cross (with Stock I, which is still under investigation).

The red colour that appeared here is peculiar to this Stock. It is not the vermilion "Normal" Red of Stocks I and II, but a tone between that colour and the "New" Red of Stock V with a concentration of slightly darker colour in the centres of the eyes.

Details of the appearance of redness in the eye are as follows. Out of a large F_1 family, 39 became mature and all were black. There was only one F_2 family of any size, in which 8 out of 26 survivors to maturity were found to be Reddish Black.* (It does not look as if these reddish specimens were distributed uniformly among the broods.)

From this F_2 family, 7 matings gave offspring, which are tabulated as follows:—

Parents.	Young at birth.	Survivors.
Black \times Black	4 B + ? 2	—
Black \times Black	1 B	—
Black \times Black	15 B	—
Black \times Black	9 B	—
Reddish Black \times Reddish Black	7 B	—
Black \times Reddish Black	58 B (of which a number were slightly reddish)	$\left\{ \begin{array}{l} 3 \text{ B} \\ 3 \text{ RB} \end{array} \right.$
Reddish Black \times Reddish Black	$\left\{ \begin{array}{l} 32 \text{ B (of which} \\ \text{a number} \\ \text{were slightly} \\ \text{reddish)} \\ 1 \text{ DR} \\ 1 \text{ Red} \end{array} \right.$	$\left\{ \begin{array}{l} 1 \text{ Mosaic eye} \\ 3 \text{ irregular RB} \end{array} \right.$

It is this last family that is of main interest. The first brood contained 5 B and 1 with the left eye "Mosaic," i.e. an eye with some of the ommatidia jet-black and some bright red (in this instance the 3 anterior were red and the 3 others black); the second brood were 3 Reddish Blacks, all with irregular-shaped eyes and 1 Dark Red; in the third brood 1 Light Red appeared with 6 Blacks; the fourth consisted of 5 B and 6 RB; the fifth of 4 B and 2 RB. There was no F_4 from any of these families.

Of the remainder of the small F_2 families (which altogether gave only 11 Black survivors), only one, the eye-colours of which are not known, produced young, giving an F_3 family of 21 B and 1 RB with mosaic left eye. Seven of the Blacks survived, one changing to RB, its left eye

* Three of these Reddish Blacks had irregularly shaped eyes, and one had a Mosaic Right Eye with jet-black ommatidia in the centre and some bright red round the margin.

becoming 'mosaic' (Fig. 2 on Plate III of this Journal, Vol. XVIII). The matings obtained in this F_2 family gave the following results:—

Parents.	Young at birth.	Survivors.
Black \times Black	93 B (one or two slightly reddish)	{ 6 B, 1 RB
Black \times Black	{ 235 B (a few slightly reddish)	{ 31 B 6 RB
	8 DR	{ 5 DR un. 2 DR \rightarrow IR
	2 Light Red	2 unchanged
Above Black φ mated in bowl	{ 6 B (one slightly reddish)	1 B
	3 DR	1 DR \rightarrow RB
	1 Light Red	

Very few F_5 and F_6 broods were obtained.

One Light Red was crossed with a Stock I Red, and another with a Stock II Red, the young in each case being black. Other crosses were also made.

Altogether 4 'mosaic' eyes appeared, and one or two others in crosses.

On analogy with Stock V we may ascribe the appearance of reddish eyes to the influence of a gene r_3 , the independence of which from r_1 and r_2 has been shown.

V. ACCOUNT OF STOCK IV.

Stock IV, a small stock which did not survive very long, was descended from one of the pairs brought in from the wild in September, 1922, and kept at a constant temperature of 21° C. (Stock II arose at the same time). The only F_1 pair produced a large family of 19 broods, and among this F_2 a certain number of red-eyed specimens were found. The young were not examined at birth for eye-colour, but, judging from the survivors, it is probable that the reds were occurring in a 1 to 3 ratio. The number of matings among the F_2 blacks producing families containing reds is such as would be expected if a proportion of one homozygous to two heterozygous occurred among the blacks. The families from these matings in which reds occurred gave a total of 157 Black and 41 Red, thus approximating to the expected 3 to 1 ratio.

Though a number of F_3 families were obtained, the stock dwindled rapidly in the 4th and 5th generations, and then died out. Altogether no more than 1463 specimens were concerned in the Main Stock, but a considerably greater number resulted from various crosses in which specimens of the Main Stock were used.

The results of various matings fall in line with the hypothesis that, as in the case of previous red-eye types (Stocks I and II), the red eye-colour was behaving as a simple mendelian recessive character, involving

a single gene, which may be named r_4 . The recessive, r_4r_4 individuals have red eyes.

(a) "*reds*." A feature concerned with the "*reds*"* of this stock was a certain variability in the shade of red. About half were born with the eye a very dark red, and the rest either bright red or slightly darker than bright red. There was always a clear distinction between the 'Dark Reds' (DR) and the rest, which are classed as 'Light Reds' (LR). The survivors of the former were found to lighten to 'Light Red' as they grew up. The darker shade of red colour was due to the presence of some dark pigment mixed with the red. Such a shade is represented at a stage in the darkening of the normal black eye in the embryo, and the gradual darkening in the later stages of Stock I "*reds*" when kept at a high temperature (6).

The form taken by the "*reds*," both in the main stock and those that appear in crosses—a total of 834—is summarised below.

1. MAIN STOCK

Total extruded.	Survivors.	
	Died before maturity.	Reached maturity.
Dark Red 244	11 DR→LR	13 DR→LR
Light Red 296	7 unchanged LR	31 LR

2. STRAINS FROM CROSSES

Dark Red 139	16 { 7 unchanged 9 DR→LR	18 { 4 DR unchanged 14 DR→LR
Light Red 160	7 unchanged LR	22 unchanged LR

There is thus a strong tendency to redden; it is the rule in Dark Reds, and among Light Reds also there is a higher proportion of true bright reds at maturity than in the early stages. There is no instance of darkening.

Whatever genetic relation, if any, there exists between the Dark and Light Reds, is at present obscure.

In the *main stock*, among the few matings that were made, Light × Light gave all Light, and Dark × Dark gave Dark and Light. The figures of matings between reds are as follows:—

Light × Light. Main Stock.

10 Lights (258)
5 Lights (258)
6 Lights (514a)
2 Lights (473)
5 Lights (106 × 111)
65 Lights (106b)

* The term "*red*" is applied to recessive individuals (rr). In the same way "*black*" refers to individuals with the dominant gene R . 'Black' and 'Red' refer to special types of eye-colour in the same way as 'Reddish Black,' 'Dark Red,' etc. In other words, 'Black' refers to a phenotype, while "*black*" refers to a genotype.

Dark × *Dark*. Main Stock.

17 Darks	..	42 Lights (216)
36 Darks	..	19 Lights (153b)
11 Darks	..	6 Lights (1658)

Dark × ? *Dark*.*

142 Darks	..	80 Lights (115d)
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But 4 Light × Light matings that were made in the *crosses* each gave both Darks and Lights.

Light × *Light*. Cross with Stock I Albino.

4 Darks	..	5 Lights (3786b3)
10 Darks	..	16 Lights (3786b2)
7 Darks	..	1 Light (3786b)
20 Darks	..	22 Lights (3954b)

Dark × *Dark*. Crosses.

3 Darks (2053)

A further point is that the distribution of Darks and Lights, when they occur together among different broods of a family, certainly does not appear to be as regular as would be expected on the assumption that a genetic relation existed between them.

(b) "*blacks*." In the great majority of cases pure and impure alike have unchanged black eyes. Some, however, show a tendency to redden, gradually changing to a Reddish Black shade (9, Plate VIII). The figures, for the main stock, are as follows:—

Total extruded.	Died before maturity.	Survivors.	Reached maturity.
Black 916	74 { 68 Black unchanged 6 B→RB	147 { 143 B. unchanged 4 { 3B→RB 1→DR→RB	

Out of 221 survivors, 10 showed signs of reddening. This is known to occur occasionally among heterozygotes of Stock I and II Red-eye, but here it seems that the individual need not necessarily be heterozygous.

The evidence for this:—Out of the very few matings involving this type, in two cases a B → RB mated with a specimen carrying r_4 has given all black young.

(i) Brood 365 B → RB ♀ × Red ♂ 375, gave family of 16 B (2 to maturity, unchanged). (ii) Cross 307, B → RB ♀ × B ♂ 584 (impure for IV Red) gave a family of 90 Black.

* In this mating the ♀, TH 115d, was hatched Dark Red and lightened to Red. The ♂, from an earlier brood of the same family, was not examined until mature, and was then recorded as Light Red. There seems little doubt but that it was of the same constitution as the female.

(c) *Contrast between "blacks" and "reds."* While the majority of the "blacks" are of the typical normal appearance, a few, as noted above, developed slight reddening. Coming to the "reds," we have seen that all are not uniformly similar. Two distinct classes exist—those born Dark Red and those Light Red throughout life. Though in the Main Stock all the Dark Reds lightened as they grew up, a few in strains derived from crosses remained Dark Red. Those born Light Red varied among themselves to a certain extent, some resembling bright Normal Red, and others almost as dark as Intermediate Red (9, Plate VIII). A slight, but definite, variability is thus shown by each class, especially the second. Yet if ample allowance is made for all variation among the "reds," in no case does the eye colouration fall outside the extreme limits, Dark Red unchanged to Normal Red unchanged. There is therefore always a clear distinction between the red-eye recessives and even the most reddish of the dominant "blacks."

(d) *Further consideration of Stock IV recessives.* We have hitherto followed the methodological practice of referring to the recessives as being 'red-eyed' in contrast to 'black-eyed.' The contrast, however, as in the case of other 'red-eye' recessives, is more justifiably stated in some such terms as 'eye greatly lacking in black pigment' as distinct from 'eye with full quantity of black pigment.' For the sake of comparison with other Stocks we may attempt to summarise what is known of the presence or absence of the melanic pigment in the reds.

It is safe to assume that all eyes classed as 'Dark Red' owed their dark appearance to the presence of a certain amount of the melanic pigment. Unfortunately there is only a single preserved specimen to which reference can be made—a ♀ which was born Dark Red and changed to Light Red (TH 115d). This specimen, as expected, showed a faint dark deposit. It cannot be said whether Light Red eyes all contain a dilute deposit of melanin, but, while a number of them probably contain a little, the brighter of them do not differ in appearance from Stock I reds and evidently contain none. At any rate it can be said that those which were Dark Red at extrusion and subsequently lightened did not maintain the melanin production exhibited at first. It may be noted that in these examples the relative concentration of melanin, rising to maximum round about the period of extrusion and then declining, follows a strikingly different course from that to be seen under any conditions in darkening Stock I recessives.

The earlier families of the Stock were kept at an average temperature of between 20° C. and 21° C., but no difference was observed between any of the "reds" of these families and those reared at laboratory temperatures. The effect of higher temperatures is not known.

The concentration of the red pigment showed no fluctuation in any

r_4r_4 types from the Main Stock (and in only one instance in strains derived from crosses—broods TH3786 and TH4244—in which the red decreased, giving a purplish appearance to the eye). A full colouring of red was characteristically maintained.

VI. STOCK V RED-EYE.

Since the account of this stock given previously (9) a great deal of further information has been accumulated. It is now seen that a much simplified description is possible. The origin of the Stock and character of the early broods which were kept in the heat at temperatures between 20·8° C. and 28° C. can be summed up shortly. The F_2 from a certain wild pair, brought into the laboratory in February, 1928, and kept at a temperature gradually increasing from 20·8° C., gave individuals with various shades of red eyes, some of which darkened and some of which lightened during life. In subsequent families there appeared all shades intermediate between Black and Red, involving changes in either direction and of various extents, as well as temporary or permanent change to some shade of purple. (The purple colour we consider to be the result of a decrease in the red pigment without a corresponding increase in black.) On the whole, black was dominant to any kind of red, and reds mated together nearly always gave reds of some sort, but even here there were exceptions (H558, H748, Family H577). The discovery of genetic ratios among the families presented many difficulties.

After a time the numbers fell off rapidly and it was thought advisable to remove the Stock to cooler laboratory conditions. Not long after this was done the previous account was given.

1. LATER FAMILIES OF THE MAIN STOCK KEPT AT LABORATORY TEMPERATURE.

Since removal from the incubator one very noticeable character of all the families generally has been a distinction between individuals that may be called "black" and "red" respectively, the ratios conforming with either all "blacks," or all "reds," or "black" to "red" in a proportion of 3 to 1, or "black" to "red" in equal numbers. In fact, "reds" behave as simple recessives to "blacks." Matings give, in all cases, expected results. That is, "reds" mated together give all "reds"—quite a large all "red" branch of the Stock is being maintained (descendants of F_6 broods in 9, Chart I); "black" mated with "red" gives either all "black," or "blacks" and "reds" in approximately equal numbers; and "blacks" mated together give either all "blacks," or a 3 to 1 ratio. In all cases where a black is known from its parentage to be impure, it has given results according to expectation.

The eye-colour of "reds" at birth is frequently Reddish Black, sometimes Dark Red, frequently Intermediate Red, and quite frequently Bright Red. There is in most cases at least a temporary change to some shade of purple. The Reddish Blacks generally lighten during life, but some remain a Purple Black (=an RB in which red pigment has disappeared); while Bright Reds, though a few remain unchanged, generally darken to some extent. There is often a considerable fluctuation in colour shade.

"Blacks" usually have black eyes unchanged throughout life, but sometimes they gradually redden to a Reddish Black, and occasionally are decidedly reddish when young or when just extruded. The behaviour of the 112 "black" individuals which survived to maturity is shown below.

*B unchanged	91
B→RB	16
B→RB→PB	1 (984)
RB→B→B	3 (1053 and 882)
RB→B→RB	1 (1053)

In addition, among 101 specimens which survived but died before maturity, there was 1 case of a B→RB and 4 of RB→B.

It may be mentioned here that there is good evidence that the blacks which show a certain redness at some stage or another are not necessarily heterozygous. In the above list one of the B→RB specimens behaved genetically as a pure black. Other instances will be noted later, and if, as is highly probable, a certain section of Stock V (9, Chart 2) is a pure 'dominant' strain, 4 other examples would be provided, including the above B→RB→Purple Black. Of 30 "blacks," from various families, known to be impure, 27 were Black unchanged, and 3 changed to RB. There is no indication here that heterozygotes have any greater tendency to redden than the homozygous blacks.

Generally speaking, individuals fall readily into one or other of the two classes—"blacks" or "reds." But doubtful cases occur now and then. It is easy to see how ambiguity may arise. It is always possible, for instance, that a "red" born black may sometimes occur (but, for that main section of Stock V with which we are dealing, it would clearly be at most only a very occasional occurrence and does not invalidate the reckoning of all specimens born black, which do not survive, as "blacks"). Reddish Blacks which do not survive are, however, often doubtful. Usually those that survive turn out to be "reds," but certain instances have been given above in which they darkened and proved themselves "blacks." When such borderline cases occur, the general character of the brood is often a sure enough guide of the category to which the

* State at maturity in thick type.

individual belongs. There is one other exceptional case to be mentioned, namely, an Int. Red which darkened to almost black. This proved to be a "red."

Summarising, it may be said that the families of Stock V kept at laboratory temperatures show clearly enough the presence of a recessive gene with which is associated instability in pigment deposition in the eye. Later it has to be considered how this gene was behaving when the Stock was kept in the incubator, whether any modifying genes are influencing its expression, or whether the effect of any other gene at all can be discovered within the stock.

2. RESULTS FROM A CROSS BETWEEN STOCK V AND STOCK I (HC STRAIN).

Some useful information has been obtained from a strain derived from a cross between reds of Stock V and reds of Stock I. Several hundred specimens are involved.

The bulk of this strain is descended from a single pair of Stock V "reds" (one of which was impure for Stock I red), and consequently consists entirely of specimens recessive for the Stock V factor.* Here then is a chance of seeing how far Stock V "reds" may vary in eye-colour. It should be mentioned that the original Stock V individuals involved in the cross came from the F_3 , that is, the next generation after that in which Stock V "reds" first appeared, and the early families, including the first generation after the above-mentioned pair, were reared in the heat.

The specimens of this recessive stock show a tendency towards the darker shades of red and purple. One striking character is the frequent occurrence of black, or almost black, eyes, particularly at birth. In these cases there is always a falling off in black pigmentation, the colour generally becoming Purple or Reddish Purple. The darkest individuals were some born Black and not lightening further than Purple Black. There are also one or two cases of $RB \rightarrow B$.

It can summarily be said that at birth individuals could be nearly Black, Dilute Black, Reddish Black, Dark Red, Dark Intermediate Red, Intermediate Red, Bright Red, or Mixed Red.† RB and IR are most frequent. Survivors show a great variety of changes, e.g. $Black \rightarrow$ various

* Among these will, of course, be specimens which carry Stock I Red-eye and some recessive for that factor. The latter are double recessives for both Stock I and Stock V Red-eye, and in appearance are indistinguishable from Normal Stock I "reds." The double recessives are not included in this account, but reference has been made to them above (p. 311).

† Certain individuals at extrusion appeared intermediate between 'New Red' and 'Normal Red,' and to these the term 'Mixed Red' was applied. It is probable that they were extreme examples of Stock V recessives with an unusually large supply of red pigment.

forms of purple or RB; RB→B,→PB, unchanged, → lighter reds or purples; DR→PB, →other purples, →B, unchanged, or →lighter red; IR→RB, or purples. On the whole there is considerable uniformity in the eye-colour of members of the same brood, both at birth and subsequently, but the broods of one family may differ from each other considerably, as do also successive generations.

In this cross, therefore, the "reds" are on the average darker than in the red strain of the main stock described above and show a wider range of variation. It shows particularly the sort of difficulty to be expected in distinguishing "reds" from "blacks" when they occur in the same family.

3. RESULTS OF MATINGS OF VARIOUS STOCK V SPECIMENS WITH OUTSIDE BLACKS.

Six "blacks" and six "reds"—superfluous or cannibal ♂♂—were mated with outside wild black ♀♀. From these matings quite large F₂ generations were obtained.

Members of each F₁ generation, as is to be expected, are all Blacks, but in some cases there is reddening later in life. Those reaching maturity that are known to be impure included

37 Black unchanged.

7 Black reddening to RB.

Another case of Black reddening to RB proved to be pure.

The F₂ generations fall into three categories. (1) All families (i.e. offspring from each F₁ pair) are entirely "black"; (2) one family out of every few contains some "reds"; (3) all families contain some "reds." These types of F₂ occurred according to expectations. That is to say, all families contained "reds" when the original Stock V parent was a "red," and the other two types of F₂ occur when it was a "black." In other words, the results come out as expected if it is supposed that some "blacks" are pure and others impure, while the "reds" are all recessive. In one case all F₂ families would be black, in the second one out of every four F₁ matings should give "reds" in a 3 to 1 ratio, and in the third case all F₂ families should be 3 to 1 ratios of "black" to "red."

One of the Stock V specimens involved was a Black which became Reddish Purple after maturity (OH745). The results of the cross showed that this specimen was a pure recessive. The other five recessives were: RB→RP (723); RB→P→IR→R (786); RB→IR→R (713); RB→DR→IR→R→RP (725); RB→P→RP (748). Among the six "blacks" one B unchanged (715), and a B→RB (774) proved pure; and two B unchanged (717 & 746) impure, the other two (739 & 761) being still doubtful.

The F_2 families in which reds appear clearly approximate to 3 to 1 ratios, but the actual proportions are confused by the frequent occurrence of Reddish Blacks. Though some,* and probably most, of these are to be reckoned as recessives, yet in some cases they are almost certainly to be included with the blacks. The families are useful in showing the type of 3 to 1 ratio family to be expected in the Main Stock.

These crosses, then, confirm the supposition that the deficiency of melanin which occurs in Stock V, can be referred primarily to the effects of a single recessive gene.

A special point in connection with one of these crosses remains to be mentioned. The original Stock V parent concerned was the $B \rightarrow RB \rightarrow RP$ (OH 745) ♂ referred to above, which proved to be a pure recessive, reds appearing in all of five F_2 families. Now in one of these families there appeared individuals with approximately 'Normal Red' eyes, resembling those of Stock I in lacking dark pigment entirely. They were regularly distributed among broods of the family, which was constituted as follows :—

85 Black ; 5 RB ; 25 DR and IR ; 33 Normal Reds.

The Normal Reds occurred in a proportion of 1 to 3 of all other colours, among which there was a typical 3 to 1 ratio of Stock V "reds" ; and it looks very much as if a new genotype had been introduced.

In the descendants of this family, Normal Reds have segregated out in mendelian proportions, independently of Stock V "reds," and back crosses with "reds" from the Main Stock V have proved that a separate gene must indeed be involved. This gene is designated r_6 . A branch of the strain which is free from the Stock V recessive gene has been set apart as Stock VI.

The appearance of the r_6r_6 types in one out of 5 F_2 families indicates that one of the original parents (Stock V ♂ and Outside ♀) was heterozygous for r_6 . Suspicion is at once thrown on the ♀, which would therefore be regarded as either having mutated, or as having been heterozygous in the wild.

4. THE EARLY FAMILIES OF STOCK V KEPT IN THE HEAT.

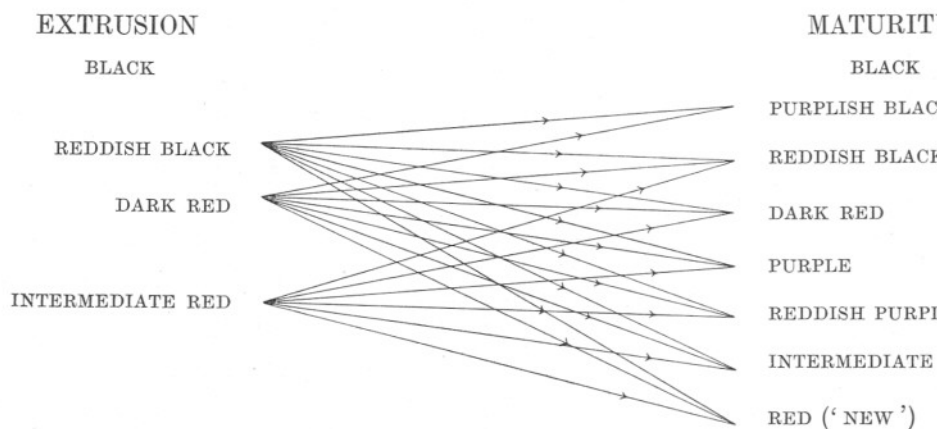
(a) *The recessive 'red-eye' gene.* Among the later families of the stock the effects of a certain red-eye-colour gene have been discovered (see Section 1). It has now to be considered whether this gene can be traced back within the early families of the Stock kept in the incubator, and how far this assumption of its presence helps in elucidating the inheritance—or absence of it—of Stock V eye-colours. Different crosses with outside blacks (Section 3) have shown that various red-eyed individuals

* Tested by mating back to Stock V recessives.

actually are recessives, all apparently for a single gene. On the face of it, then, it is likely that one principal recessive gene has been handed down through the stock and lies at the bottom of the eye-colour instability. Considerable variation has so far been found among the "reds" (i.e. recessives) even when it is possible to distinguish fairly definitely between them and the "blacks." But in these earlier families no clear distinction can be drawn, which means that still greater variation occurs here. The results of the cross with Stock I (Section 2) have also given a warning. So we must be prepared for recessives born black, and perhaps also for a variation towards redness in the heterozygotes; in short, for an 'overlapping' between the redder "blacks" and the darker "reds."

To cut the story short, it is in fact found possible to trace the career of the recessive gene through most of the families of the Stock. There is some uncertainty in several branches owing to the small number of survivors or smallness of the families, but reasonable certainty has been obtained sufficiently often to have shown up any discrepancy in this method of interpretation. If it be assumed that the same genotypes occur in the earlier families, the following are the conclusions reached on the phenotypic character of recessives and dominants.

(b) "*reds.*" The colour shown by specimens known to be recessive for the greater part varies within the limits of the range of colour change represented by the following scheme.



Among 142 individuals every one of these changes has occurred, nearly all of them several times. The course of change is not always uniform and direct, but subject to fluctuation. For example, a specimen starts, say, at RB and at maturity is RP. Before this it may have passed through an IR stage, or a PB stage, or both. Redness may fall off and later increase again. Also a gradual change after maturity may occur. But the above

scheme covers all changes that have been found to occur over *any* chosen period.

There remain certainly 3, probably 12, cases where "reds" have assumed a darker colour than any included in the above scheme. The three include a B→RB→RP (OH745) (see Section 3), which is known to be recessive from the result it gave when bred from, a Black→Red, and a Black→PB, both the latter belonging to a brood (748) in a pure recessive branch.

Now, in the later families of the Stock there has been no case of "reds" ever having a colour even approaching Black; on the other hand, in the cross with Stock I (Section 2) some broods were black at birth. It is therefore valuable to have corroborative evidence on whether there is a right to expect black "reds" in these early families of the Main Stock. There is some evidence that this is so—and that the occurrence might even be a common one.

The evidence is as follows:—(1) Family 628, in the 5th generation of a pure "red" branch, was composed of 4 Blacks and 15 Reddish Blacks (9, Chart 4). Of the two surviving Blacks one changed to Purple Black, and the other went all the way to Bright Red. (2) Family 658a, which contains the Black→RP specimen mentioned above and in Section 3, might be considered a 3 to 1 ratio, yet the eye-colours at birth were 113 Black and 8 Reddish Black, in which case a number of "reds" besides the particular specimen were born black. (3) There is a certain branch which almost certainly should rank as pure recessive. It arose from an F₃ pair, one DR unchanged, the other B→RB. Their family contained, at birth, 16 B, 43 RB, and 31 IR, most irregularly distributed among the broods (9, p. 216). Of the blacks, the 2 survivors reddened to IR. The next generation contained mostly RB's (at birth) and 1 Black; but the only third generation family, derived from 2 RB→Purplish, was composed of 21 B and 5 RB. (4) It is probable that family 563b, containing 18 B (2 unchanged) and 16 RB (2→PB), is all recessive. If this is so (and considerable difficulties in the interpretation of related families arise, if it is not), then an instance would be provided in which "reds" may even be Black unchanged!

It is noteworthy that in these small apparently recessive families which have given blacks (at birth), the family as a whole is on the dark side, viz:—

1 B	13 RB (448-3)
21 B	6 RB (577)
18 B	16 RB (563b)
4 B	15 RB (628)

(c) "*blacks*." The form taken by the "blacks" is generally unchanged Black. The 10 specimens known to be pure are all Black unchanged, as are also 29 out of 41 impure. Among the impure are also the following:—

4 B→RB
 1 B→RP
 6 RB unchanged
 1 IR→RB.

So it appears that a certain range must be allowed for variation in the heterozygous "Blacks." This range overlaps that of the darker forms of the "reds." An unchanging Reddish Black, for instance, may equally well be a "black" or a "red," and does in fact occur for both in the same brood of one of the F_2 families. Fortunately abnormal specimens like the $IR \rightarrow RB$ do not seem to occur often.

(d) *Character of the original F_2 .* The original F_2 family (9, Chart I) in which red-eyed individuals first appeared is found to contain the following recessives:—

$IR \rightarrow DR$	IR unchanged
$DR \rightarrow RB$	$DR \rightarrow RB$
$RB \rightarrow Red$	$RB \rightarrow IR$
RB unchanged	

and the following heterozygous "blacks":—

RB unchanged	RB unchanged
RB unchanged	$B \rightarrow RB$
B unchanged	B unchanged
B unchanged	

while one B unchanged is known to be pure.

It is not possible to discover the character of this F_2 family. The 5th, 6th, and 7th broods seem to be producing "blacks" and "reds" in a 3 to 1 ratio, but it is not easy to see where many recessives might be found among the first 4 broods, of which unfortunately few survived.

(e) *Conclusion.* The effects of a recessive gene can be traced back into the early families of Stock V. The higher temperature seems to have had the effect of widening the range of phenotypic expression, thus very largely obscuring the difference between recessives and dominants.

5. VARIATIONS IN STOCK V RECESSIVES.

It has been seen that r_5r_5 types of the Main Stock V show considerable variation in eye-colour shade and in the course of change during life. The explanation of the wide range of observed colour shades in terms of pigmentation is that both the red and the black pigment vary, and do so independently of each other (9, pp. 204–206).

The question next arises as to whether there are hereditary relations underlying any of these variations. In considering this point it is convenient, and indeed necessary, to treat the two pigments separately. With each pigment the general behaviour has first to be examined.

Black Pigment. At extrusion there is always at least some melanin present, even when the eye is bright red. Confining attention to the Main Stock at laboratory temperatures, we find that the amount present may

vary from very slight (New Red) to considerable (Reddish Black). This implies that there has occurred in the few days preceding extrusion a process of melanin deposition similar to that which occurs at this time in the normal black eye, but retarded to a greater or lesser extent. During life, the intensity of melanin is usually either maintained at a constant level, not changing from its condition at extrusion, or else shows a gradual decline. The course followed is generally uniform, involving, so far as can be seen, no larger fluctuations than would be expected to result from variations in growth rate, etc. It is to be noted that the intensity does not *increase* to any great extent beyond the condition reached at extrusion. If, indeed, the earliest growth stages are excepted, it can be said that no general increase in intensity occurs. All the more conspicuous examples of darkening after extrusion are confined to cases of darkening in early life. It thus appears that at a certain time, which may coincide with extrusion or come not very long after, the concentration of melanin reaches a point after which it increases no further, but remains at equilibrium or gradually falls away.

To this extent, then, is it possible to generalise on the behaviour of the melanic pigment in Stock V recessives. A slow accumulation of melanin continues until a certain critical stage, after which deposition suffers a check. From this point deposition may be sufficient to maintain the same relative concentration, but more often is so retarded that the intensity declines steadily. The amount of melanin accumulated before the critical stage may vary considerably, and the time of occurrence of the critical stage varies over a period between extrusion and some time during early life. The extent of subsequent fall of intensity may vary from nothing to that seen in eyes which are Reddish Black at birth and New Red at maturity.

A striking characteristic is that in all eyes,* whatever the shade and whatever the course of change in pigmentation, the melanin is uniformly distributed through all the ommatidia. This state of affairs contrasts strongly with that seen in a darkening Stock I "red," in which the melanin concentration decreases from the centre to the periphery (see p. 310). The latter case is susceptible of the simple explanation that each ommatidium undergoes the same cycle of change, and the older ommatidia in the centre will therefore be at a more advanced stage of darkening. It follows that the slower or the more limited the course of darkening, the less contrast will be shown between the centre of the eye and the periphery and the more uniform the distribution of pigment. Accordingly among Stock V recessives, in which darkening is either negligible or restricted to

* With possible exceptions among early growth stages. This statement is based mainly on the examination of preserved material, which consists almost entirely of later immature stages and adults. It may be expected that specimens which do not reach the maximum darkening till, say, the 2nd moult should show a darker centre.

a period of short duration in early growth stages, there is no opportunity for the centre to become appreciably darker than the more peripheral parts. Here expectations are fulfilled. If, however, all ommatidia follow the same course of change in eyes in which the concentration steadily falls, it is to be expected that the central ommatidia would be at a more advanced stage of lightening than those situated nearer the periphery, so that the eye would show a pale centre and a darker outer border. Such a condition is never found. The newer ommatidia on the periphery acquire the concentration of melanin that exists in more central ommatidia but do not surpass it, even though the older have previously passed through a considerably darker stage. It is therefore clear that the process which leads to decrease in melanin concentration prevents the ommatidia from following their individual course of change. It is a process which affects the whole eye, and not the ommatidia as discrete units.

We are therefore brought to the recognition of two distinct phases in the deposition of melanin. Phase 1, evident in earlier stages, results in accumulation of melanin, and is comparable to the darkening process occurring in the normal black eye and in Stock I "reds" at higher temperatures. Phase 2, coming into effect at, or not long after, extrusion, is marked by a check in the further accumulation of melanin, affecting the eye as a whole, and results in uniformity of pigment distribution over the whole eye, which may remain in a state of equilibrium or gradually grow paler. There are reasons for supposing that at the onset of phase 2 a physiological change in the process of melanin formation is involved. This is considered at a later stage.

The above consideration is based on the character of the recessives in the Main Stock kept at laboratory temperatures. It should however be mentioned that the behaviour of r_5r_5 types in other strains, and in the earlier families of the Main Stock kept in the incubator, falls into the same general category. In the case of recessives reared at incubator temperatures, it is noticeable that at extrusion the average level of melanin concentration is decidedly higher than in those hatched in the laboratory. This accords well with expectations; for if the early phase of melanin deposition is comparable with that occurring in the normal eye and in reds of Stock I, then it should be accelerated with increase in temperature. During the second phase, however, the higher temperature does not appear to have brought about darkening; and further tests substantiate the supposition that during this phase heat has no effect on the rate of melanin deposition. In the strain derived from a cross with Stock I (see p. 321), the eye-colour sometimes reaches Black before phase 2 sets in.

To summarise the effect of the recessive gene r_5 on the formation of melanic pigment would involve repetition of the second paragraph of this section. More briefly stated, the influence of the gene is seen in (1),

the retardation of melanin accumulation in phase 1, and (2), the inhibition of phase 1 at an early stage by the introduction of whatever influences underly the occurrence of phase 2.

The main variations that are found in r_5r_5 types are in respect to the following:—(a) degree of retardation of melanin deposition in phase 1, as evidenced by eye-colour at extrusion; (b) time of onset of phase 2, indicated by the extent of darkening during the first week or two of life; (c) in the extent of decrease in concentration of melanin during phase 2. The presence of modifying hereditary factors may be sought in reference to these sources of variation. In respect to (a), eye-colours recorded at extrusion constitute the available data. These can be investigated for indications of hereditary relations. As regards (b), the information available is not adequate; but what is known of (c) affords a possible basis for further study.

(a) That eye-colour at extrusion is at least partly dependent on non-inherited influences is evident from the striking dissimilarity that often exists between broods of the same family. Some examples are given in Table I.

(In these examples eye-colours are grouped as follows:—Red, IR (Intermediate Red), DIR (Dark Intermediate Red), DR (Dark Red), RB (Reddish Black), and B (Black). Since all eyes at extrusion contain red pigment, the darkness of the red is a fair indication of the concentration of melanin present.)

In all instances there is a statistically significant irregular distribution among the broods of the eye-colour types contained in the family. This means that the occurrence of different eye-colour types cannot be explained wholly in terms of genetic segregation.

These instances illustrate a general tendency among recessives both of the Main Stock and of other strains. It is clear that differences in eye-colour at birth cannot be simply related to genotypic differences until at least some correction can be introduced which eliminates distinctions between different broods of the same pair.

There is also a noticeable tendency for members of the same brood to exhibit identical characters, while a similar uniformity does not exist among the whole family.

As regards the relation between parents and offspring of successive generations, the results are again negative. No correlation is apparent.

When these facts are taken together, it must be concluded that underlying hereditary factors could only be detected by statistical treatment requiring prohibitively extensive numerical data.

(c) During the second phase, the concentration of melanin may remain uniform or gradually decrease. The information on this matter is however of a much more limited kind than that treated in (a). In the first place,

TABLE I.

FAMILIES REARED AT LABORATORY
TEMPERATURES.

No. 838 b—i.			
Brood			
No. 1.		4 DR	5 IR
2.			9 IR
3.	9 RB		9 IR
4.		7 DR	13 IR
5.	15 RB	1 DR	
6.			3 IR
7.	7 RB	6 DR	

No. 830 a—ii.			
Brood			
No. 1.		9 IR	
2.		18 IR	
3.	8 RB	18 IR	
4.	2 RB	19 IR	
5.	5 RB	7 IR	
6.	11 RB	16 IR	
7.	16 RB	4 IR	

No. 1004—ii.			
Brood			
No. 1.			4 IR
2.	8 RB		
3.		9 DIR	
4.		14 DIR	
5.			10 IR
6.	8 RB		
7.			12 IR
8.	5 RB		3 IR
9.	4 RB		

No. 831 c 2.			
No. 1.		12 IR	
2.		22 IR	
3.		12 IR	8 Red
4.	20 RB	3 IR	

FAMILIES REARED IN INCUBATOR.

No. 515 a.			
Brood			
No. 1.		3 IR	
2.		2 IR	
3.	5 DR	2 IR	
4.		15 IR	
5.	10 DR	2 IR	
6.	9 DR	2 IR	
7.	5 DR	2 IR	
8.	11 DR	1 IR	
9.	12 DR	8 IR	
10.	25 DR		
11.	8 DR	5 IR	

No. 314 b.			
Brood			
No. 1.			15 IR
2.		15 RB	2 IR
3.	9 Black		
4.		12 RB	10 IR
5.	7 Black	16 RB	4 IR

No. 330 a & b.			
(a 1: 1 ratio of $R_S r_S$ to $r_S r_S$.)			
Brood			
No. 1.	6 Black		4 IR
2.	7 B		5 IR
3.	8 B		14 IR
4.	13 B	5 RB	
5.	11 B	6 RB	1 IR
6.	6 B	6 RB	5 IR
7.	9 B	4 RB	10 IR
8.	10 B	11 RB	1 IR
9.	8 B	5 RB	
10.	7 B	10 RB	
11.	18 B	3 RB	

attention is necessarily confined to individuals which survive for some length of time after extrusion. The numbers in each family are thus greatly reduced. Secondly, the amount of the red pigment varies considerably. This means that the resultant eye-colour gives only an indication of the concentration of melanin. Owing to the fluctuation of the red pigment, it is only possible to derive an approximation of the course of change in the melanin, which at the most is not subject to very wide variation.

All that can be said is that while on the one hand there is a noticeable tendency for members of the same family to behave in the same way; on the other hand no relation can be discovered between successive generations, and there are indications of other non-hereditary fluctuations.

The conclusion on the matter of variations in melanin pigmentation of Stock V recessives may be stated thus: while no hereditary relations among them have been discovered, the variations are at least partly dependent on non-hereditary influences.

Red Pigment. While fluctuation in the concentration of red pigment occurs very frequently among Stock V recessives—and indeed is responsible for the more striking changes in eye-colour—there is no reason to suppose that the gene r_5 has necessarily any direct effect upon it. It is only because the r_5r_5 types are deficient in melanin that in them alone can changes in the red pigmentation be observed. It is not therefore possible to go beyond the statement that, in Main Stock V and in strains derived from it, fluctuation in the red pigment is prevalent. This characteristic in itself contrasts Main Stock V with, for example, Main Stock I, in which the r_1r_1 types exhibit an ample and unvarying concentration of red pigment.

An opportunity for observing the variation that occurs with respect to the red pigment is afforded particularly by branches of Stock V composed entirely of r_5r_5 individuals. The strain HC derived from a cross with Stock I is also useful in this respect (see p. 321). Confining attention, however, to the Main Stock, we may briefly refer to such definite statements as can be made at the present moment.

It may be recalled that eyes frequently acquire a purple shade of greater or lesser degree. (The shades are classed as Purplish Black, Purple, and Reddish Purple.) The cause of this is a loss of red pigment. In Purplish Black and Purple eyes it may be almost or entirely absent. The dull purple colour, given by a dilute deposit of melanin, becomes brighter and more reddish as the concentration of red pigment increases. The appearance, then, of a purple shade may be used as an indication of decrease in red pigment.

At extrusion, no instances are known in which red pigment is sufficiently lacking to give a purple shade. For the course of change after extrusion, generalisation is difficult. Some examples retain a more or less ample concentration throughout life; in others, however, a decrease becomes evident some time during the earlier immature stages. The course of change which is more usual than any other is a loss of red pigment during immature stages followed by a revival in its production coinciding approximately with the onset of maturity. Examples exhibiting this type of change are sufficiently numerous to deserve special comment, and invite further investigation. It sometimes happens that the revival in the production of pigment is delayed, or is only slight, or does not occur at all. Also the amount present after the revival may fluctuate.

It is exceptional for those examples which appear to have retained their

red pigment all the time up to maturity to lose it afterwards (at any rate, before signs of ageing set in). Therefore two main classes of individuals may be distinguished. One typically retains pigment through life; the other shows loss during immature stages, and usually a revival about the time of onset of maturity. The genetic relationship between these two classes may be tested.

The information available suffers from certain limitations, such as its concern with survivors only, and the comparatively infrequent examination of specimens. It cannot on this account be expected to give more than indications.

There is an unmistakable tendency to resemblance between members of the same family, even though many broods be involved. Thus many families are constituted predominantly of one or the other of the two types of individuals. This is a point in favour of the presence of underlying hereditary factors. On the other hand there is no obvious relation between parents and offspring of successive generations. The question of hereditary relations therefore remains open. In any case at least minor non-hereditary fluctuations have to be admitted.

Examination of Stock V recessives in other strains does not give any additional help. In some families both 'purpling' and 'non-purpling' individuals occur, apparently unequally distributed among the broods, but the numbers are not sufficient to warrant any definite statement on this point.

The above consideration of the variations among Stock V recessives justifies the following conclusions. Differences in eye-pigmentation in part at least cannot be explained in terms of hereditary differences. The clearest piece of evidence for this is the frequent significant disparity between broods of the same family. That is, the variations are at least partly of an 'environmental' nature. At the same time no modifying hereditary factors have revealed their presence. If any are at all directly concerned, then they exert their influence—on the average character of the family—in such a way as to be detected only by very extensive numerical data.

Elucidation of the inheritance of Stock V eye-colours cannot proceed further until more is known of the environmental factors which influence the particular form of unstable eye-colour associated with individuals recessive for the gene r_5 . Consideration has to be given not only to obvious external factors such as temperature, conditions of the water in the culture bowl, state of food, and so on; but also to 'internal' factors of the kind that have been demonstrated by Ford and Huxley (7, pp. 71–76) who, in emphasising the dynamic aspect of eye-pigmentation, show how variations in rate of pigment production, growth of ommatidia, and general body-

growth, in relation to one another, may be sufficient to give marked variation in eye-colour. Also something may depend on the cytoplasmic legacy with which the individual started off, thus introducing as a possible effective factor the physiological condition of the mother at the time the eggs are formed.

VII. STOCK VI RED-EYE.

The origin of a new type of red-eye among the F_2 from a mating between a Stock V recessive ♂ and Outside ♀ has been described on p. 323.

The eye-colour of Stock VI "reds" is of a Normal Red, very like that of Stock I. At laboratory temperatures no melanin develops in the eye at least before maturity (older specimens have not yet been investigated). As in the "reds" of all strains that are connected with Mutant Stock V, there is a tendency for the amount of red pigment to diminish.

Stock VI Red-eye behaves as a simple mendelian recessive character, and its appearance is ascribed to the presence of a gene r_6 . So far r_6 has proved distinct from r_1 as well as from r_5 . Investigation is being continued.

VIII. SUMMARY OF "RED-EYE" RECESSIVE TYPES.

A description has been given of 6 Stocks (Mutant Stocks I to VI), each of which contains red-eyed individuals of some form or another.

It is found that in each case the occurrence of redness is associated with a single recessive gene which influences the process of melanic pigment production. A separate gene is involved in at least Stocks I, II, IV, and V. The genes are denoted respectively r_1 , r_2 , r_4 , and r_5 , replacing the 'normal' genes R_1 , R_2 , R_4 , and R_5 . Stock III evidently contains a recessive gene (r_3), which is definitely distinct from r_1 and r_2 , and probably also, judging from its phenotypic expression, from r_4 , r_5 , and r_6 . The gene r_6 is distinct from at least r_1 and r_5 .

Each Main Stock consists of the inbred strain derived from the mating from which the recessive form originally arose. Investigation of the recessive types has been focussed on the respective Main Stocks, but at the same time it has been extended to strains derived from cross-matings or matings with animals from the wild. Each recessive type, in fact, has been studied in one or more inbred strains. Since differences are sometimes noticed in the phenotypic expression of one recessive type in different strains, a distinction between strains is maintained in discussion as well as in practice.

The effect of these genes is seen in some form of retardation or inhibition of the melanic pigment in the retinal cells. That they may also have some direct effect on the production of red pigment is not impossible,

but evidence on this point with respect to observed variations is so far to the contrary. It has been found that the quantity of red pigment may greatly decrease or otherwise fluctuate; but these fluctuations are characteristic of certain strains and it seems that they can be observed in any of the rr types contained in those strains. So far no special investigation has been carried out with the view to determining what hereditary differences may underly the variation of the red.

r_1

The recessives (r_1r_1) of Main Stock I, at laboratory temperatures, have bright red eyes—with few exceptions melanin production is completely inhibited until old age. At 23° C., however, melanin appears a few days after extrusion, and gradually accumulates, until well before maturity the colour of the central ommatidia has reached a reddish brown or even black (see 6, Fig. 1a). Strains darkening at different rates have been separated and the influence of genetic modifying factors has been discovered (6, p. 115). It is characteristic of the Main Stock that the red pigment retains a full concentration through life.

In some strains derived from cross-matings melanin deposition takes place more readily. A conspicuous instance of this is afforded by a strain derived from a cross with Stock III, in which the r_1r_1 types characteristically showed darkening already in immature stages. In certain strains a marked deficiency in red pigment occurs.

The effect of the gene r_1 is greatly to retard the normal process of accumulation of melanin in the eye. The extent of its action is influenced by temperature, as well as by the presence of modifying genes.

r_2

The r_2r_2 types of Main Stock II resemble, at laboratory temperatures, the r_1r_1 types of Main Stock I, failing to develop melanin to the same degree. The effect of higher temperature is, however, not known. A full quantity of red pigment is maintained in recessives of the Main Stock. So far as is known, r_2 exerts the same effect as r_1 .

$? r_3$

On analogy with Stock V, the occurrence of red-eyed forms in Stock III is ascribed to the action of a gene r_3 . Different grades of eye redness occur. The action of the gene r_3 is probably to be compared with that of r_5 .

r_4

The r_4r_4 types were either Dark Red at extrusion and subsequently lightened to a Light Red, or they were Light Red through life. Among certain strains derived from crosses, there occurred a few specimens which remained Dark Red. The brightest of the Light Reds evidently contained no melanin, but probably some of those born Light Red and certainly all the Dark Reds carried a deposit.

The effect of the gene r_4 is rather different from that of r_1 . There may

be a slow accumulation of melanin before extrusion, but afterwards production is inhibited, if not altogether, at least to such an extent that it does not keep pace with growth-rate. There is a suggestion of two distinct phases, as in the case of r_5r_5 types.

The recessives of Stock V (r_5r_5 types) show partial inhibition of melanin formation, the extent varying considerably among individuals. The colour shades and the changes which occur during life owe their variety to the fact that both the black and the red pigment are subject to independent variation. The red pigment fluctuates in all strains investigated.

Concerning the course of melanin production in recessives, the following generalisation seems justifiable. The pigment slowly accumulates (phase 1) until a point is reached beyond which no further relative increase takes place, but either a state of equilibrium is maintained, or the intensity gradually declines (phase 2). The intensity attained at the critical point, which occurs at some time in early immature stages, varies considerably. During phase 2 it is characteristic that the melanin is distributed uniformly over the eye, and there is evidence that temperature has no accelerating influence.

The effect of the gene r_5 may summarily be stated as follows. The normal process of melanin accumulation is retarded to a varying degree, and at a certain point is checked by the introduction of whatever conditions underlie the equilibrium state characteristic of phase 2.

No other genes, either modifying the effect of r_5 , or underlying the fluctuations in the red pigment, have been detected. Significant differences in the composition of broods of the same family, and other indications, show that the variations among r_5r_5 types are at least in part of a non-hereditary nature.

The r_6r_6 types have a bright red eye. The gene r_6 appears to inhibit melanin production as effectively as r_1 . So far it has been proved distinct from r_1 and r_5 .

Reference may be made to two further recessive types which are dealt with in a separate paper (p. 337 of this Journal, Vol. XVIII). These are 'Flesh' Red-eye and 'Beet' Red-eye, associated with the genes f and t respectively (Stock VII). The effect of f is very similar to that of r_1 , but has been proved distinct; while t has a different effect from any previously encountered, and may be reasonably regarded as definitely distinct from any recessive genes hitherto described.

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