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

By

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With Plate VIII.

1. INTRODUCTION.

Summary of Mutant Stocks giving Red Eyes.

The eye-colour of the wild *Gammarus chevreuxi* is always black and no variation from the normal Black has ever been observed in the many thousands which have been taken in the native habitat of the species. This, however, does not preclude the possibility that mutations may have arisen in the wild stock and have gone under in the struggle for survival.

The difficulty we have had throughout our work in establishing mutant stocks that have arisen in the Laboratory indicates that the mutations of the type used for genetical study would have but little chance under normal conditions in nature, of survival through the early critical period. Each new mutation has shown greatly lowered vitality during its earlier generations, accompanied by marked abnormalities in breeding.

But once established, the mutants tend to become healthier with each generation, some, such as the Red-eye and the Albino-eye of Mutant Stock I, for example, being able, in time, to hold their own with the normal wild Black-eyed animals in vigour, length of life and number of offspring.

As has been shown before (**5**, p. 46), the black pigment in normal cases starts as bright scarlet in the embryo and passes through various darkening stages from "intermediate red" to "dark red" and then to jet black on hatching.

In all the different mutating stocks there have arisen Red-eyed specimens in which the red colour that normally appears only for a short time in the embryonic stage persists after hatching. It is noteworthy that the Reds from the five stocks with which we work are all genetically distinct from one another, for in whatever way we cross-mated the Reds from different stocks the young have always been born Black-eyed. In other stocks started at the same time as Stock V, in which Red-eyes appeared, none survived to be cross-mated ; the details of their occurrence are given below (p. 00).

The term "Mutant Stock" we apply to the offspring of any one particular Black-eyed pair in which mutations from the normal have developed. They may be summarised briefly.

Mutant Stock I.

One of the pairs brought in from the wild on June 12th, 1912, and kept in laboratory conditions, gave the first Red-eyed young in the F_2 generation from one Black-eyed female only—the Red a bright scarlet (Plate VIII—Normal Red). Other mutations in this stock were, the Albino degenerate eye from a mating of Black with Red, the Irregular Coloured eye, the No-white, the Blind, and the One-eyed. This is the stock with which Mr. E. B. Ford is working at Oxford.

Mutant Stocks II, III, and IV.

These three stocks came from a dredging brought in on September 12th, 1922. Nineteen pairs were set out in an incubator kept as nearly as possible at 21° C. Only two of the pairs gave redness in their offspring (Stocks II and IV), both in the F_2 generation.

Sixteen other pairs were started in laboratory conditions, with the temperature varying through a wide range, from two to six degrees centigrade in the twenty-four hours ; the highest recorded was 27° C. in July, the lowest 5·7° C. one day in January, when the heating apparatus failed. Five of these pairs, one the Mutant Stock III referred to below, gave some redness in the offspring. Of the others, one showed slight reddening in F_1 and F_2, two in F_1 only, and the fourth gave two bright Reds out of a total of 574 offspring.

Mutant Stock II. The Red-eye* in this, as in Stock I, is bright scarlet associated, as in all the stocks, with normal pale-green body-colour. The most striking feature appeared with the Red-eye, in the F_2 generation, viz., the White-body mutation, in which the body and eye are completely devoid of coloured pigment. The eyes connected with it are, the homozygous White-eye, the Changeling White which later develops red-eye and body colour, the Flushed White, and the Purple White eye.

Mutant Stock III differs from the others in that the Red came gradually, some Black-eyed parents becoming Reddish Black and giving different shades of Red-eye in their offspring. This Red is not the bright scarlet of Stock I but a deeper tint, like the New Red figured on Plate VIII. Another point of resemblance with our new mutation, V, is that the eye-colours sometimes change with growth, but with these it is usually only in the one direction, a *lightening* of the colour, e.g., Reddish Black to Dark Red, Dark Red to brighter Red.

The "Mosaic" eye appeared in this stock, i.e. an eye with some of the ommatidia jet-black and some bright red.

In *Mutant Stock IV* also there are different kinds of Red eyes, bright, intermediate, and dark. Like the preceding, the Dark Red lightens in varying degree. The colours are heritable, and an animal hatched Dark Red functions always as Dark Red, however much the colour may alter during its life.

In our new *Mutant Stock V* with which this paper deals there is much greater variation in eye-colour and, particularly, in colour-changes. All intermediate stages between Black and Red have occurred. Changes undergone after birth include both darkening and lightening, as well as either permanent or temporary changes to some shade of purple. Intermediate Reds have been crossed with Reds of Stock I, Stock II, and Stock III and have given a Black F_1 with all.

In comparing these five mutant stocks, it is seen that there are two distinct categories of mutants producing red eye-colour. In one, represented by Stocks I and II, the Red-eye appears as a typical mutation, arising suddenly and persisting as a simple recessive. Its mode of inheritance is comparable with that of other familiar recessives, occurring, for example, in Drosophila or Primula, and interpretable in terms of a change

* This Red is referred to as "New Red" in "Inheritance in *Gammarus chevreuxi* Sexton" (**3**, p. 119). Since then, we have found that the different Reds of the five stocks fall into two distinct classes of colour—the bright scarlet of Stocks I and II, which we call "Normal Red," and a deeper, more intense shade of red which appears in Stocks III, IV, and V, to which we now confine the term "New Red" (see Plate VIII).

in a single gene. Attention may be called, firstly, to the manner in which the Red-eye of Stock I originated : there were altogether 9 reds in the progeny of a certain black-eyed F_1 female,—2 out of 13 survivors from two broods with a brother, 4 out of 44 from a mating with a freshly captured male, and 3 out of 39 from another mating with the same brother. Since neither of these males threw any red offspring when mated with other females, it suggests that the female parent was in some way responsible for the occurrence of the red mutation in the offspring (**1**, p. 22). Secondly, to the physiological difference between "Black-eye" and "Red-eye" as demonstrated by Ford and Huxley (**4**, p. 115), who showed that when kept at a temperature of 23° C. the red-eyed individuals gradually darken to black, undergoing a process similar to, but much slower than that of normal blacks before hatching. The Red-eye is thus a case in which the rate of deposition of black pigment is very much reduced, in fact indefinitely reduced at normal temperatures. A more precise idea of the action of the gene concerned in the Red mutation is thus obtained. The mutation "Red-eye" is now expressed as a certain degree of retardation of pigment deposition.

In the other category, represented by Stocks III and V, redness emerges somewhat gradually, as described below. Intermediate stages between black and red occur and it seems as though segregation is necessary for the production of a bright red eye. Colour change during the course of life, involving either darkening or lightening, is of usual occurrence and characteristic of these stocks.

The manner in which red eye-colour appears is as follows. A certain number among the offspring from a wild pair are found to develop a reddish tinge and may become "Reddish Black" or even redder. Amongst the next generation, whether derived from these specimens or from unchanged Blacks, there occur individuals born in various stages of redness, i.e. "Reddish Black," "Dark Red," "Intermediate Red" (for explanation of these colours see below, p. 193 and Plate VIII), and bright "Reds" may also appear. Those that survive of the various reds may keep the same colour throughout life, but they are more liable to change, either in the direction of red (lighten) or black (darken). In Stock III lightening was the rule, but in Stock V both darkening and lightening occur. One other point common to the Stocks III and V should be mentioned—namely, that the shade of the red colour in the "New Red" eye is different from that in the original "Normal Red" (see Plate VIII). Examination of specimens in Stock V indicates that this is because the New Red is rendered slightly impure by a dilute deposit of a dark pigment, apparently some form of melanin.

Stock IV holds an interesting intermediate position. The red is evidently a simple recessive to black, but the red may exist in different shades, i.e. bright Red, Intermediate Red, Dark Red, or even Reddish Black. In so far as this stock has been investigated no tendency for the reds to darken has been found,—on the other hand nearly all the darker shades have lightened to red. Among the blacks, there are some which develop a certain degree of redness. It may be supposed that these are heterozygotes showing not quite complete dominance, but, if so, not all heterozygous specimens behave thus. The quality of the red colour is more akin to that of the " New Red " than to that of the " Normal Red."

It is hoped to enter more fully into the comparison of the various red mutants on a future occasion. The present investigation aims at elucidating the mode of inheritance of this second category of eye redness. Owing to its manner of origin there are obvious difficulties in thinking of the "New Red " eye as a simple mutation involving only one gene, however much the action of the latter may be modified by accessory genes. If, on the other hand, a group of genes is involved, then the " New Red " is not a simple, but a multiple recessive. The comparison, therefore, of the " New Red " with the " Normal Red " becomes a matter of considerable interest, and may well throw light on the general problem of the nature of mutations.

The Types of Eye-colour.

We may now summarise the various shades of eye-colour that have been distinguished and used in description.

The symbols relating to each are inserted below in brackets, and the colours are shown on Plate VIII.

Pure Black (B), the normal wild type ;

Reddish Black (RB) ;

Dark Red (DR) ;

Intermediate Red (Int. in Charts 1–4, I.R. in Table II) ;

Bright Red (R), the " New Red " of this new Mutant Stock V ; the bright Red of Stock I is shown on the Plate as " Normal Red " ;

Purplish Black (PB) ;

Dark Purple (P) ;

and Reddish Purple (Rp).

Red, Intermediate Red, Dark Red, Reddish Black and Black form a series of progressive darkening, such as, for instance, occurs in the embryo of normal Blacks. Purplish Black has roughly the same depth of colour as Reddish Black, and Reddish Purple that of Intermediate Red.

Mr. E. B. Ford, in his work on *Gammarus chevreuxi* at Oxford, has distinguished a series of fourteen stages of darkening in the red eye-colour (**4**, p. 114). A proof of our Plate VIII was sent to him, and he has very kindly compared the eye-colours on the Plate with those in his series.

He allows us to state that his " stage 3, Light Red " is the same as our Intermediate Red, his " stage 6, Brown " is the same as our Dark Red, and his " Chocolate, stage 9 " as our Reddish Black.

The selection of these particular colours is purely a matter of convenience. Every gradation, both among the reds and between the reds and purples, has been observed. The standards chosen are sufficiently far apart to be determinable even in a live, wriggling specimen, but they are sufficiently close to indicate any marked change in the colour of the eye.

If a " No-white " eye (i.e. lacking the reticulation of white pigment) were being used no doubt a greater accuracy in differentiation of colour shades (if indeed desirable) could be obtained. In these eyes with the normal white pigmentation, not only is the colour of the pigment cells largely hidden or shaded, but the optical effects of the white pigment when the animal is moving quite clearly give a brighter, redder shade than is seen when the eye is perfectly still. Care has to be taken especially with regard to this latter point. Previous work on eye-colour changes (Ford and Huxley, **4** and **6**) has been carried out upon No-white eyes.

2. HISTORY OF THE EXPERIMENT.

The opportunity for this investigation occurred when a number of wild pairs brought into the Laboratory in January and February, 1928, for temperature experiments, gave offspring which showed a tendency to redden, and an F_2 containing individuals of various shades of red at birth. Altogether thirty-nine pairs were set out in an incubator, thirty-two of these gave offspring and it is noteworthy that in 20 of these stocks this tendency to redness appeared. Whatever mutations are concerned therefore occurred independently in at least 20 cases. It is difficult not to conclude that some common environmental factor was instrumental in inducing them. The change over from the wild conditions in winter to those of the incubator room (starting at a temperature of 20·8° C. and rising in four months to 28° C.) is the obvious possible environmental factor.

Mutant Stock V with which this paper is mainly concerned is descended from one of these pairs.

Before giving the details of the history of the experiment, mention must be made of the many difficulties encountered in the handling of the stock, quite apart from the problems connected with the characters investigated. The broods were usually very small, numbers such as 1 to 10 being often recorded, whereas in the normal stocks the average number hatched is from 30 to 40. Then there was frequent ineffective mating and other irregularities in breeding ; the proportion of young surviving to maturity was small; but the greatest difficulty was the cannibalism of the males, which was carried on to such an extent that few of the females died a natural death.* Adults available at the right time for mating were always scarce, and therefore continual cross-matings between members of different families of the stock, often different phenotypically, were necessary in order to keep the stock going, with the consequence that progress in the direction of establishing pure lines has been slow. The mortality after eighteen months in the incubator was so great that a reduction in the temperature was necessitated. The stock has therefore been kept under the more normal laboratory conditions, which has meant that, though its viability has increased, it is no longer subject to a regulated temperature.

The incubator referred to was a small room 10 ft. × 8ft. × 7 ft. high, in which the temperature could be kept approximately constant. It was heated by hot-air pipes, the gas furnishing the hot air being regulated by a Hearson's thermostat placed in the room. Air and water readings were taken daily by means of a thermograph in the air, and Negretti and Zambra's maximum and minimum thermometers immersed in a covered glass dish of water, the difference between the readings for air and water averaging about half a degree centigrade, those in the water being the lower. The broods were always examined in the incubator, so that

* An instance from one of the F_4 families may be given in illustration of these points. Seventy-six young were hatched, of which only 18 survived to maturity, six males and twelve females. Only one of the females died a natural death, the other eleven were eaten by their mates.

Normally a male pairs with a female towards the end of a reproductive period, some days before her moult is due, mating as soon as this is accomplished. The eggs are laid almost at once; if not laid before the cuticle hardens, that is to say, within about 24 hours of casting the old skin, oviposition becomes impossible till the time for moulting comes again. The term "mating" therefore implies a complete reproductive period. The danger to the females comes during or just after the moult while the cuticle is still soft.

The matings of this family were as follows:—

One female mated, ate the male; mated with another male, same stock, laid eggs which were thrown off, mated again four times with no results; then ate the male; mated twice with a brother, and was eaten.

One male mated with sister female, no results, mated four times with eggs laid and broods hatched, numbering 1, 11, 14, and 21 respectively, then ate the female; mated once again with another female and died.

The second male mated with a sister, female died; mated with another sister, ate her; with a third female, had a brood of 1; mated again, brood of 8, ate the female, and died.

The third male had two matings with a sister, broods of 1 and 3, ate the female; two matings with another female, brood of 1, mated again, ate this female, and another, and then died.

The fourth male mated twice, ate the female, and died.

The fifth had three matings with a female, ate her; mated with another, ate her; mated twice with a third female, ate her; then as it had grown to a large size, it was given a female from the wild. With this one, it has had four broods, numbering 17, 46, 38, and 26 respectively, followed by three matings with no results, and now, after a fourth mating, eggs laid.

The sixth male ate two sisters in the brood bowl; mated three times with a third female, broods of 2, 9, and 8, mated again and ate the female; mated with another, ate her; mated with a female from the wild, ate her; mated with a second one, a brood of 15; mated again, brood of 22, and male was eaten.

they were not subjected to any change of temperature during the examination.

The temperature of the room when the experiment started on February 24th, 1928, was 20·2° C. The heat was gradually and steadily increased until in April it reached a mean of 25·8°* From May 1st to June 11th the mean was 26·8° C. From June 12th to June 26th it stood at 28°. This was found to be the limit of endurance for the species. Only 5 broods were hatched from all the pairs in the incubator, and these were very small numbers, 8, 3, 2, 3, 1,—all died within a few days. An alteration was therefore made, and the heat was lowered to 23·8° C. and kept at this from June 26th to July 6th. It rose again through July, as will be seen by the mean temperature given below for the incubating periods, until on August 16th it was decided to keep it constant at about 23° C. From August 16th, 1928, to June, 1929, it was kept almost constant between 22° and 23° C. In June it rose one degree and was constant through July and August till September 6th when the gas was turned out. The stock had grown so weak that it had to be re-established in healthier conditions.

The heat averaged 18° C. through the rest of September, and 15° through the winter months.

On April 9th, 1930, the bowls were brought out into the Laboratory, temperature averaging 15·7° C. The heating of the Laboratory was stopped on April 30th, and since then the mean was 14·5° for May, rising in June to about 17° C.

The first four generations, with the exception of one F_4 mating were hatched and reared in the heat. The broods hatched since are marked with the symbol † on the Charts.

Mutant Stock V is derived from one of the pairs brought in on February 13th, 1928, and kept at first in laboratory conditions for the females to extrude their young. In this pair, the female extruded her young, and laid eggs on February 16th. They were placed in the incubator on February 24th, the temperature then standing at 20·2° C. On the 28th this brood was extruded and the first batch of eggs to be laid in the heat was deposited.

Twelve Black-eyed F_1 were extruded on March 8th, the mean temperature for the incubation period, 8 days, being 20·8° C. Ten of these reached maturity about six weeks later, 6 males and 4 females, and mated in the brood bowl, but only one pair gave any results. From this pair all the stock is descended. The others mated again and again,

* At 10.30 every morning the temperature at the time was read, and also the maximum and minimum temperature for the previous 24 hours. It was found that the mean of the temperature readings at 10.30 gave a result which differed only slightly from the mean of the maximum and minimum readings. When considering the temperature over a period we have therefore used the mean of the readings taken at 10.30 a.m.

seven batches of eggs were laid and thrown off, only 1 young, Black-eyed, was hatched and it died. The pair referred to mated and were taken out in a separate bowl on April 27th, and the *first* batch of eggs was laid on April 28th.

The heat was being increased, and the mean temperature for the incubating period was 25·8° C. It was evidently too much for the female, for though she carried the eggs until they showed orange in the pouch, i.e. to within a few hours of hatching, they were thrown off.

The *second* brood, laid on May 5th, was also thrown off.

The *third* brood, laid May 12th, hatched, and 5 young were extruded on May 20th, two Black-eyed and three with a slightly reddish tinge, the first sign of redness in the stock. Four survived, eye-colour unchanged, and two, a male and female, were seen to be mature on July 6th, a period of 47 days. By July 16th both females had eggs.

The matings gave different results, viz. the Black male mated with the Reddish Black female gave 47 Black, 21 Reddish Black, 1 Dark Red, and 10 Intermediate Red; while the other pair, Reddish Black male with Black female, gave 112 Black, 37 Reddish Black, and 16 Intermediate Red.

The *fourth* brood, laid May 20th, extruded May 27th, consisted of 7 Black and 1 Reddish Black. Four survived, three males and one female, maturing in about 40 days, the first eggs, only 2 or 3 in number, being laid on July 6th. The offspring given by this female were 97 Black and 1 Reddish Black.

The *fifth* brood, laid May 27th, and the *sixth* on June 4th were both thrown off—most likely because of the rise in temperature to 26·3° C. The *seventh* brood, laid June 11th, and extruded June 18th, was also a failure, for only 2 young, Black-eyed, were produced and these died almost at once.

The temperature was still rising, the mean from June 12th to June 26th being 28° C. Two batches of eggs were laid during this period, the *eighth* on June 18th, *ninth* on June 26th, but were thrown off, and as it was evident from the mortality in all the stocks that the conditions were too severe, the gas was lowered. The next brood, *tenth*, laid July 5th and extruded on the 12th, with a mean temperature during the incubating of 23·6° C., died before the young could be examined for eye-colour.

New eggs, brood *eleven*, were laid on July 13th, extruded July 20th, the mean for the seven days being 25·3° C.; 15 Black and 1 Reddish Black were hatched, but none reached maturity.

Brood *twelve*, July 21st to July 28th, mean temperature 24·4° C., contained 13 Black, 5 Reddish Black, and 1 Intermediate Red, the first Red-eyed young of this mutation.

Five Black survived, one of which lightened to Reddish Black; five

Reddish Black also, three of them unchanged, while the other two had lightened, one to Intermediate Red, and the other first to Intermediate Red, and still further to bright Red. The one Intermediate Red of the brood, almost normal red at birth, had darkened to Dark Red by August 14th. This is the first instance of lightening and darkening both occurring in the same brood, and noteworthy too for the fact that the changes took place within a comparatively short time after extrusion, 17 days. The young were an exceptional length of time in reaching maturity, the first to be noted, a female, took 65 days to October 1st. Some of the others took 93 days, and others even longer, a period of time comparable with that taken by the young maturing in the open Laboratory.

The *thirteenth* brood, July 29th to August 6th, took eight days hatching in a mean temperature of 23·4° C. Fifteen Black and 3 Reddish Black were extruded, the only survivor, a Black female, mated, eggs thrown off, mated again and was eaten by her mate.

The *fourteenth* brood, laid on August 7th, was extruded on August 14th and 15th, mean temperature for the seven days, 23·4° C. There were 11 Black, 2 Dark Red which darkened, and 1 Intermediate Red which grew up unchanged. The first to mature took 52 days, to October 5th, the first eggs were laid on October 8th.

The male parent of these broods died on August 15th, and the female on August 16th.

3. EYE-COLOURS AND COLOUR CHANGES IN MUTANT STOCK V.

The Appearance of Red Eyes in the Stock.

From the wild pairs brought in during January and February, 1928 (see p. 194), close on 4000 offspring were hatched in the F_1 generation. All were born with black eyes, but of the 432 survivors (representing 32 families), 33 (from 14 families) showed a tendency to redden later in life. Fifteen developed a reddish tinge ; fourteen became definitely Reddish Black ; and four lightened still further, two to Dark Red, one to Dark Red and then to Intermediate Red, and one still further to Red.

Among the 1890 specimens in the F_2 generation, twenty-eight were born Reddish Black, two Dark Red, ten Intermediate Red, and seven bright Red. Some of these reds were born from parents that had developed a reddish tinge and some from parents that had remained black. Of the four stocks that produced these reds, namely, H. I, VIII, XXV, and XXXI (our Mutant Stock V), one, H. VIII, had shown reddening in the F_1, the other three had not.

In three stocks, H. I, XIII, and XXX in which the F_2 were all born black, subsequent reddening appeared for the first time in the F_2. Only one of these stocks, H. I, was carried on, and in it red-at-birth specimens arrived in the F_3.

Apart from Mutant Stock V, the only survivors of all the specimens which showed any redness at birth, behaved as follows :—

One Reddish Black unchanged,
One Reddish Black became Purplish Black,
Four Reddish Blacks darkened to Black,
One Intermediate Red darkened to Black.

The figures for these stocks are given in Table I.

TABLE I.

Table Showing Simultaneous Appearance of Eye-Redness in the Progeny of 20 Wild Pairs Brought in January and February, 1928.

N.B.—All the pairs which gave broods are included, except 4 which gave an F_1 of under 50 and went no further, and 3 which gave still smaller F_1 with no survivors.

For explanation of the symbols see p. 193.

Stock No.	Generation.	Colour at Birth.	Survivors.
H. I	F_1	20 B	4 B un.
	F_2	102 B	10 B→**RB**.
	F_3	284B: 5**RB**: 10**Int.**	25B un.: 9 B→slightly **RB**.
	F_4	132 B: 3 slightly **RB**: 2 **Int.**	26 B un.: 4 B→**RB**: 1 B→**PB**. 1 **RB**→B: 1 **RB**→**PB**.
	F_5	26 B: 1 **RB**	4 B un.
H. VIII	F_1	127 B	26 B un.: 2 B→slightly **RB**.
	F_2	282 B: 13 **RB**: 8 **Int.**: 6 **Red**	16 B un. 1 **RB** un.: 3 **RB**→B. 1 **Int.**→B.
	F_3	221 B: 3 **RB**	22 B un.
H. XIV	F_1	55 B	26 B un.: 1 B→**RB**: 1 B→**DR** (?)
	F_2	244 B	9 B un.: 5 B→**RB**: 1 B→**RB**→B.
	F_3	62 B	11 B un.
	F_4	55 B	6 B un.
H. XII	F_1	348 B	37 B un.; 3 B→slightly **RB**.
	F_2	175 B: 2 **RB**	9 B un.
H. II	F_1	264 B	26 B un.: 4 B→**RB**.
	F_2	169 B	6 B un.: 4 B→**RB**.
H. V	F_1	369 B	32 B un.: 1 B→**RB**.
	F_2	167 B	11 B un.: 1 B→slightly **RB**.
H. XXV	F_1	234 B	19 B un.
	F_2	64 B: 1 Red	1 B un.
H. III	F_1	126 B	16 B un.: 3 B→**RB**: 1 B→**RB**→**DR**; 1 B→**DR**→**R**.
H. XLI	F_1	38 B	1 B un.: 1 B→**Int.**
H. VI	F_1	108 B	8 B un.
	F_2	9 B	4 B un.

Stock No.	Generation.	Colour at Birth.	Survivors.
H. VII	F_1	268 B	9 B un.: 2 B→slightly **RB**.
H. X	F_1	297 B	23 B un.: 3 B→**RB**.
	F_2	5 B	4 B un.
H. XI	F_1	232 B	33 B un.
	F_2	55 B	3 B un.
H. XIII	F_1	2 B	2 B un.
	F_2	73 B	9 B un.: 1 B→slightly **RB**.
H. XV	F_1	242 B	12 B un.: 1 B→**RB**.
	F_2	17 B	4 B un.
H. XX	F_1	306 B	15 B un.: 1 B→**RB**.
	F_2	69 B	3 B un.
H. XXI	F_1	141 B	8 B un.
H. XXIII	F_1	178 B	23 B un.
	F_2	65 B	9 B un.
H. XXVI	F_1	230 B	17 B un.
	F_2	41 B	8 B un.
H. XXIX	F_1	16 B	3 B→slightly **RB**.
H. XXX	F_1	23 B	3 B un.
	F_2	33 B	5 B un.: 2 B→**RB**.
H. XXXIII	F_1	33 B	8 B un.
	F_2	160 B	11 B un.: 1 B→**RB**.
H. XXXIV	F_1	43 B	4 B→slightly **RB**.
H. XL	F_1	43 B	9 B un.: 1 B→slightly **RB**.
H. XXXI	F_1	12 B	10 B un.
	F_2	65 B: 13 **RB**: 2 **DR**: 2 **Int.**	12 B un: 1 B→RB.
			7 RB un.
			1 RB→B.
			1 RB→DR.
			1 RB→Int.
			1 RB→Int.→Red.
			2 DR→RB.
			1 Int. un.
			1 Int.→DR.
	F_3	425 B: 125 **RB**: 60 **DR**: 105 **Int.**: 1 **Red**	43 B un.
			3 B→PB.
			4 B→RB.
			1 B→Rp
			1 B→Int.
			6 RB un.
			1 RB→B→PB.
			2 RB→PB
			1 RB→PB→P.
			1 RB→P.
			1 RB→DR:
			3 RB→Int.

Stock No.	Generation.	Colour at Birth.	Survivors.
			1 DR un.
			7 DR→Rp.
			1 DR→Rp.→R.
			2 DR→Int.
			1 DR→Int.→Red.
			11 Int. un.
			1 Int.→RB.
			5 Int.→P.
			5 Int.→Rp.
			1 Int.→DR
			6 Int→DR→Rp.
	F_4	397 B : 252 **RB** : 233 **DR** : 131 **Int.** : 2 **Red**	113 B un.
			6 B→PB.
			9 B→RB.
			2 B→Rp.
			1 B→DR.
			2 B→Int.
			1 B→Red.
			26 RB un.
			2 RB→B→PB.
			10 RB→PB.
			1 RB→PB→P.
			1 RB→PB→Int.
			1 RB→P.
			2 RB→Rp.
			3 RB→Rp→Int.
			6 RB→DR.
			2 RB→DR→Int.
			1 RB→DR→Int.→Red.
			9 RB→Int.
			2 RB→Int.→DR.
			2 RB→Int.→Red.
			22 DR un.
			3 DR→PB.
			15 DR→RB.
			4 DR→RB→DR.
			3 DR→P.
			21 DR→Rp .
			1 DR→Int.→Rp.
			10 DR→Int.
			3 DR→Int.→Red.
			1 DR→Red.
			13 Int. un.
			1 Int.→PB.
			11 Int.→RB.
			2 Int.→P.
			1 Int.→P→DR→Rp.
			12 Int.→Rp.
			1 Int.→Rp→Int.
			2 Int.→DR→P.
			1 Int.→DR.
			1 Red→Int.
	F_5	389 B : 225 **RB** : 21 **DR** : 106 **Int.** : 18 **Red**	91 B un.
			2 B→PB.
			3 B→RB→Rp.
			1 B→DR→Int.→Red.

Stock No.	Genera-tion.	Colour at Birth.	Survivors.
			6 RB un.
			18 RB→PB→P.
			11 RB→PB→P→Rp.
			1 RB→PB→P→Rp→Int.
			1 RB→PB.
			1 RB→PB→P→Int.
			5 RB→PB→Rp.
			1 RB→PB→Red.
			1 RB→PB→DR→Int.
			2 RB→PB→Int.→Red.
			12 RB→P.
			1 RB→P→PB.
			5 RB→P→Rp.
			1 RB→P→Rp→Red.
			1 RB→P→Int.
			2 RB→P→Int.→Rp.
			1 RB→P→Red.
			2 RB→Rp→Int.→Red.
			1 RB→Int.
			1 RB→Int.→Rp.
			1 RB→DR→P→DR.
			2 RB→DR→Int.→Red.
			1 DR un.
			1 DR→RB.
			6 DR→Rp.
			2 Int. un.
			4 Int.→PB.
			1 Int.→RB.
			12 Int.→Rp.
			2 Int.→P.
			2 Int.→Rp→Int.
			2 Int.→Rp→Red.
			1 Int.→P→Rp→Red.
			3 Int.→Red.
			4 Int.→Rp→Int.→Rp.
	F_6	97 B : 12 **RB** : 266 **Int.** : 124 **Red**	10 B un.
			6 B→RB.
	Still going.		

In Mutant Stock V the 12 F_1 were all Black and remained so. One of the pairs, from which all the rest of the stock are descended, produced an F_2 containing, at birth, 65 Blacks, 13 Reddish Blacks, 2 Dark Reds, and 2 Intermediate Reds (see Chart 1). Of the 2 Intermediate Reds, one remained unchanged and one darkened to Dark Red. Of the 2 Dark Reds both darkened to Reddish Black. Of the 13 Reddish Blacks, two died young, one darkened to Black, one lightened to Dark Red, one to Intermediate Red, and one to Red, seven remained unchanged, but of these two died immature. Of the 13 Black survivors one turned Reddish Black. In this family, therefore, both lightening and darkening occurred.

The Appearance of Purple Eyes in the Stock.

The results of the matings of various members of this family with one another produced, in the F_3, an even greater variety of eye-colour changes, which included not only darkening and lightening but also, for the first time, changes to *purple*. Purple eyes arose from various types of red and from black as well. Three shades of purple were distinguished—" Purplish Black," " Dark Purple " (or simply, " Purple ") and " Reddish Purple " (see Plate VIII).

In no case were young born with purple eyes, the colour in question always coming as a change later in life. Most frequently there was no further colour change after the purple condition had been reached, but this has not been the rule in subsequent generations, in which purple appears as an intermediate stage at one time or another in almost every type of colour change. It is noteworthy, however, that there is no instance at all of an eye purple at birth, the earliest onset of purpling observed up to now being at 14 days, e.g. a number of young hatched Red on April 25th, 1930, darkened to Intermediate Red by April 29th, and by May 9th were all Reddish Purple.

In the companion stocks, nearly all of which, it should be mentioned, were dying out in the F_3, purpling occurred in one family, namely, the F_4 of H. I (see Table I). One Black and one Reddish Black changed to Purplish Black.

Changes of Eye-Colour in Individual Animals.

In Stock V eye-colour changes in the individual animals have occurred in great variety. With very few exceptions they are all in the one direction—either of darkening or of lightening—that is, they do not change over from one direction to the other. Lightening is much commoner and proceeds to greater lengths than darkening, which when it occurs seldom goes beyond one stage. Purple may intervene at any point in the course of a change, as has been particularly evident since the stock was brought out of the incubator room. A glance through the accompanying charts will show the variety of occasions on which Purple eyes have appeared.

In the case of Blacks which have changed, the majority have gone no further than Reddish or Purplish Black, but there have been cases of Blacks giving Dark Red, Purple, Intermediate Red, Reddish Purple, and even Red.

Reddish Blacks have lightened to various extents, in several cases going as far as bright Red. Purple is commonly involved in the changes, especially in certain families. There are only two instances of darkening to Black.

Dark Reds have darkened to Reddish Black and in one case to Black. Of those which have lightened some have gone as far as Red.

Intermediate Reds have lightened to Red, but many more have darkened either to Dark Red or Reddish Black. As in the other types of Reds, a number have remained unchanged, 81 changed to 27 unchanged,—curiously enough, the total proportion of changed to unchanged, taking all the Reds together, gives an actual 3 to 1 ratio, i.e. 288 changed to 96 unchanged.

Only three isolated cases of individuals born bright Red occurred in the earlier generations (F_3 and F_4). Of these two died young and the survivor darkened to Intermediate Red as it grew older. In F_5 and F_6 there have been four families which have produced a number of Reds (see Chart 1), 122 individuals altogether, but the survivors are still immature. The majority have turned Reddish Purple or Intermediate Red. Up to now, therefore, no permanent Reds, such as, e.g. those in Mutant Stocks III and IV, are known from this stock.

The Red eye of Mutant Stock V has thus occurred in two different circumstances. (1) Individuals are born red and subsequently darken or turn purple ; (2) Red occurs as the final result of a process of lightening from any of the darker reds or even black. In no case yet has the red produced by lightening undergone any further change.

4. INTERPRETATION OF COLOURS IN TERMS OF PIGMENTATION.

Darkening and Lightening.

With a view to bringing this work in line with previous work on eye-colour changes (see p. 193), it is now necessary to examine more closely the question of eye-colours and the changes described : to express them, in fact, as far as possible in terms of the pigmentation in the retinal cells. By doing this there is every chance that the problem of the colour changes will be simplified and the manner of action of whatever genes are concerned made more evident. At the least the characters will have been expressed in a less empirical form. Further, some explanation is required on the relation of the purples to other types of colour.

In the normal eye the first pigment to appear in the embryo is red. As the eye grows dark pigment is deposited in increasing quantity, the rate of deposition of red pigment rapidly falls off, and in one to two days the eye is quite black. After extrusion the rate of black pigment deposition keeps pace with the growth of the eye, so that it remains a jet-black. All the time quite probably a certain amount of red pigment is also being formed—in fact it may be a necessary precursor of the black.

The gradation of intermediate stages between the red and the black is more easily observed in cases of Normal Reds which, kept at 23° C., gradually darken during life (Ford and Huxley, **4**).

As the melanin accumulates so the shade of the eye becomes darker and the darkening is an approximate measure of the amount of melanin accumulated. Such fluctuation as may have occurred in the concentration of the red pigment has been evidently unimportant. It should be mentioned that the extent of melanin deposition can, if necessary, be observed directly by placing the specimen in alcohol—the red pigment dissolves out, leaving behind a deposit of the insoluble dark.

In the changes occurring in Mutant Stock V and falling within the series of Black→Red, evidently a reverse process is taking place—the rate of deposition of dark pigment falls off and that of the red increases, or at least fails to decrease. Since there is reason to suppose that dark pigment, once laid down, remains always, dilution can only be caused by the growth of the ommatidia. At any given stage, therefore, there must be sufficient dark pigment to account for any earlier darker stage. Thus a specimen born Black and changing later to Reddish Black should in the latter stage contain sufficient dark pigment to account for the earlier blackness. So unless a Black starts to change early it has little chance of lightening very far. It is in fact found that few lightened Blacks pass beyond the Reddish Black stage. Again, Red individuals which have lightened from darker stages should contain a deposit of melanin. This is precisely what has been found. Several Reds have been preserved in alcohol at death, and have all shown a dilute deposit of dark pigment after the red pigment has dissolved out.

The Interpretation of Purple.

The explanation of the purple eye-colours has proved a simple matter. It was found, on examining a series of the various stages of red-eyes preserved in spirit, that the colour of the diluted dark pigment deposit which remained after the red had gone was in all cases a chocolate. The intensity of the chocolate colour, of course, varied with the quantity of pigment present, being dark for the darker red eyes and pale for the lighter red. A complete gradation, too, between dark chocolate and jet-black was found, but the quality of the colour was approximately the same all through.

Purple eyes left just the same kind of chocolate colour.

Comparison of Purple eyes in the living animals with Purple eyes preserved in spirit showed that the colour of the two was essentially the same, namely, a shade of chocolate. In fact "Purplish Black" and "Dark Purple" eyes after treatment with alcohol were found to remain unchanged; while "Reddish Purple" eyes became a duller chocolate, losing the redness. These facts go to show that the Purple colour is given by eyes in which diluted dark pigment is not covered over by red pigment. The red element in these eyes is much reduced or entirely negligible; when there is an appreciable quantity present the purple is brightened up.

Purplish Black corresponds to Reddish Black in which the red pigment has disappeared; Dark Purple similarly to Dark Red; while a small admixture of red with a dilute black pigmentation gives Reddish Purple. Reddish Purple merges into Intermediate Red as the concentration of red increases.

Further examination of the effects of alcohol on different eyes has borne out this conclusion. We are indebted to Mr. E. B. Ford for the loan of spirit specimens of the Normal Red of Stock I in various stages of darkening. These specimens also showed that the dark pigment when diluted is of a chocolate colour.

To sum up, purple colour is produced by eyes in which the red pigment has fallen off without a corresponding increase in dark pigment.

The Relation between the Various Eye-Colours.

It is thus possible to express all colour variations occurring in this stock in terms of variations in the concentration of the two pigments present in the normal eye—the red and the black;* and all changes in terms of variations in the rates of deposition of each of these two pigments relative to the rate of growth of the eye (or, rather more precisely, of the ommatidia).

The relations between the various colours may be expressed by the following scheme:—

↑		BLACK	
	REDDISH BLACK		PURPLISH BLACK
INCREASE IN DARK PIGMENT	DARK RED		DARK PURPLE
	INT. RED	REDDISH PURPLE	LIGHTER PURPLE
	BRIGHT RED		

→ DECREASE IN RED PIGMENT

5. RESULTS OF MATINGS WITHIN MUTANT STOCK V.

General.

The matings within Stock V have at present reached the eighth filial generation. Difficulties encountered in the behaviour of the stock have already been mentioned; and, when to these are added the complications

* As a matter of fact, in the original water-colour drawings for the colour series on Plate VIII, all the different shades were obtained by using two tones of red with black.

exhibited by the characters under investigation, it can be realised that progress towards elucidating the mode of inheritance has necessarily been slow.

For the present, therefore, we go no further than setting out the results of the various matings within the stock as they stand, in order to show the sort of results that have been obtained and the data available up to now.

Charts 1 to 4 show the cases in which in-mating within the families has been possible through to the F_8. Certain cross-matings are omitted as well as unproductive matings or matings which produced very small numbers of young. Table II, however, gives a record of every mating that has produced a brood. It is arranged so as to bring together as far as possible, irrespective of the generation, matings of a similar kind.

The known colour changes of the survivors are in all cases given. It should be pointed out that often a long period elapsed, especially in the case of the earlier generations, in between examinations of a specimen for eye-colour. Consequently in cases of colour change intervening stages have been easily overlooked. So, when a certain specimen is designated, for example, B→IR, it is not implied that there is any difference from a case denoted B→RB→DR→IR. It only means that the intervening stages that must have occurred happen to have been missed. In later generations eyes have been examined more frequently.

In each of the charts a special note is made of those families which have been reared since the stock was removed from the incubator room. It is impossible as yet to say what difference this change to a lower temperature has made. Though no doubt it may have affected the *rate* of eye-colour change, yet perhaps after all it has not made much difference to the *kind* of change. It is perhaps worth mentioning that the occurrence of purple seems much more frequent since the stock has been kept in the cool.

Chart 1.

Chart 1 shows the ancestry of the most promising of the F_6 families. Starting with the ancestral F_2 in which reddening and darkening both occur, we pass to an F_3 derived from a mating between a Black and a Reddish Black, both of which showed reddening. At birth this F_3 contained a mixture of Black, Reddish Black, and Intermediate Red of which the survivors either remained unchanged or else turned Purple. One Black changed to Intermediate Red, and there is also an indication of lightening (of the dark pigment) in the Reddish Black which turned from Purplish Black to Purple; darkening is indicated by the five Intermediate Reds which became Dark Red before purpling, and an unusual mixture of darkening and lightening in the RB→B→PB. But it is seen that, with the exception of the one B→Int.R., there is no *reddening* such as occurred in both the parents, and as is conspicuous in subsequent generations.

In contrast to the F_3, in the two families of the F_4 (in production of which purple parents were involved) a strong tendency to redden is shown and no change to purple appeared at all.

The three families of the F_5 are all in many ways similar. Judging from the behaviour of the high proportion of survivors, it looks as though almost every individual was doing its best to redden and turn purple at the same time. Thinking more precisely in terms of concentrations of red and black pigment, one sees that the F_5 specimens almost without exception show a steady dilution of dark pigment, while the red pigment fluctuates independently. The red pigment on the whole diminishes after birth and tends to reappear later in life, by which time the black pigment is dilute enough to produce the Intermediate Red or the bright Red condition. Those born Reddish Black approximate in their behaviour closely to a mean represented by the expression (RB→Purple stages→Int. or Bright Red), and the Intermediate Red to (IR→redder purple stages→Red). What is of main interest is that all the individuals are behaving in very much the same sort of way. This homogeneity is most refreshing.

There are eleven families in the F_6, of which nine are still going and so far have given up to four broods. It is seen that the young at birth are either all Red, or all Intermediate Red ; or a mixture of the two. It is hoped that the homogeneous nature of some of these families is a sign that pure lines are in the process of emerging.

The F_2, F_3, and F_4 families were reared under the incubator conditions, and F_5 and F_6 in the cool. It is impossible to say what difference this has made. At any rate the contrast between the F_3 and F_4 cannot be put down to this account.

This chart therefore shows (1) the appearance of Purples in the F_3, and how purple parents may give offspring that show no signs of purple ; (2) how more or less homogeneous families are gradually making their appearance in the stock.

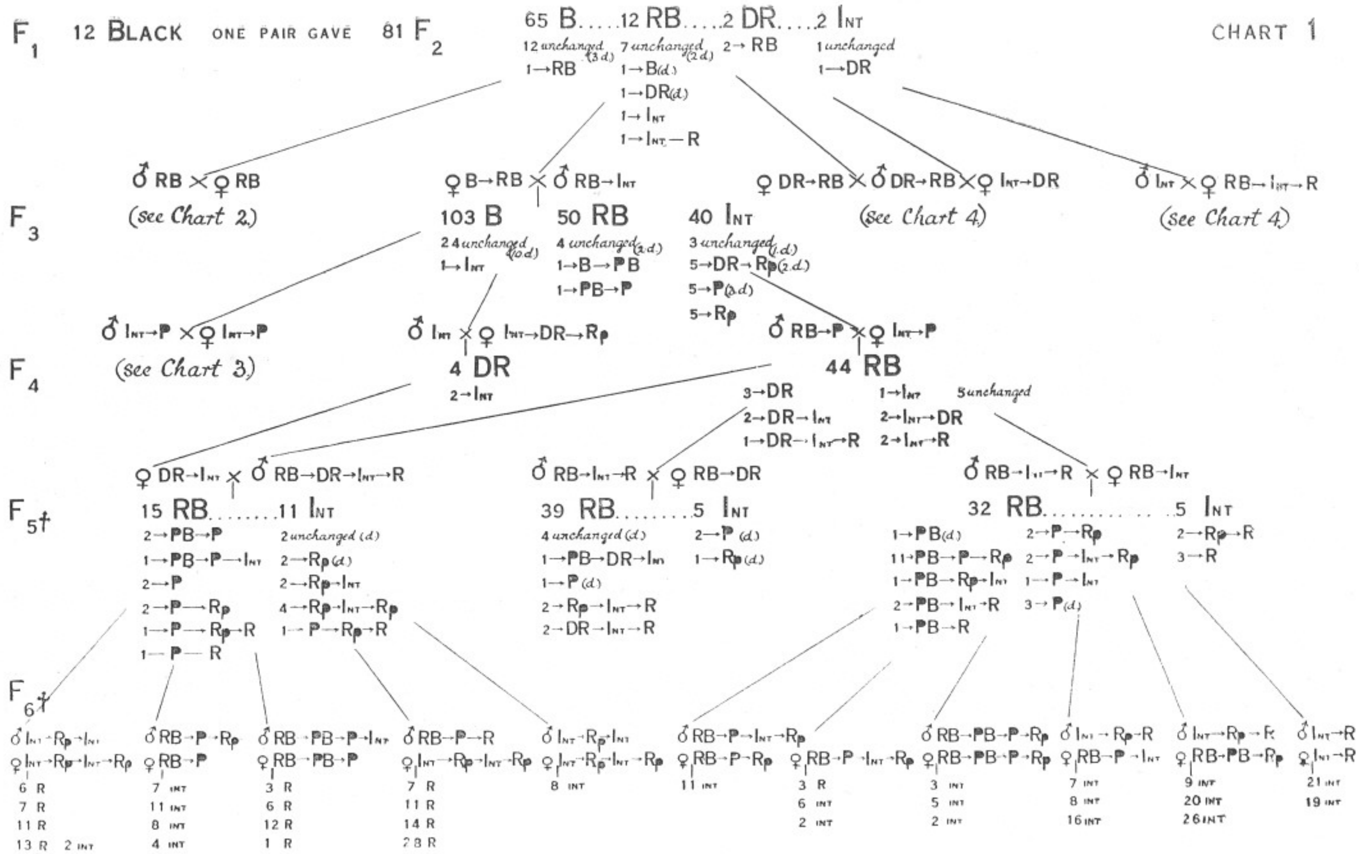
Chart 2.

This shows the stage so far reached in an attempt to establish a pure Black stock from an original pair of F_2 Reddish Blacks. Such other matings as gave any result are also included.

Chart 3.

An early attempt at establishing a Red strain is shown here. It gradually died out. Attention is called to the appearance of Blacks in one of the F_5 families. However, the two survivors did not remain Black ; one changed right over to bright Red, the other to Purplish Black.

CHART 1



† Broods hatched after the heat was turned off.

CHART 2

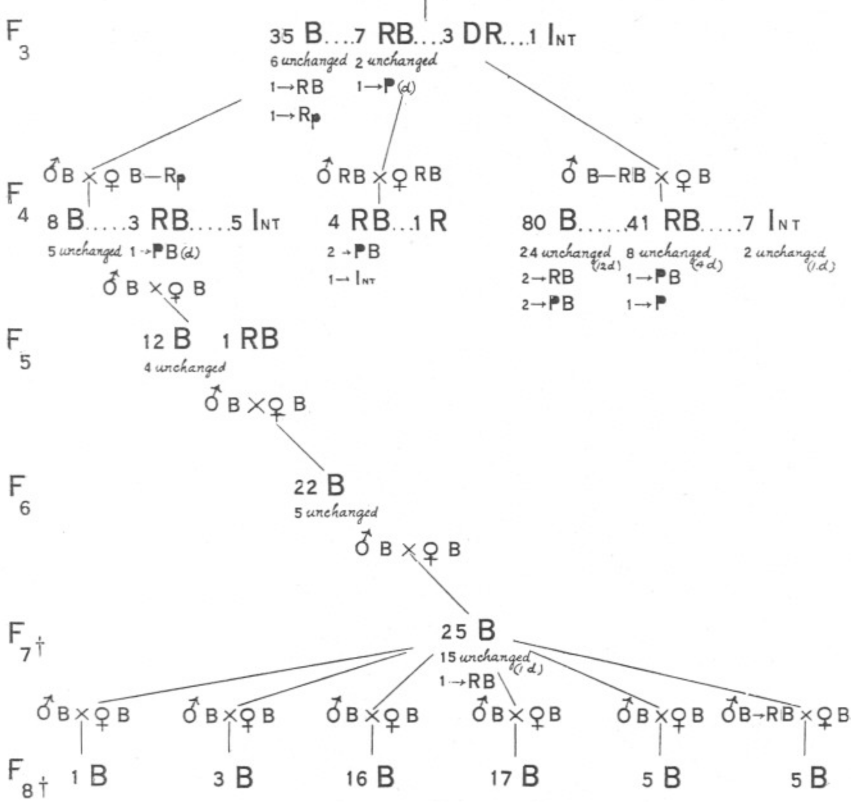
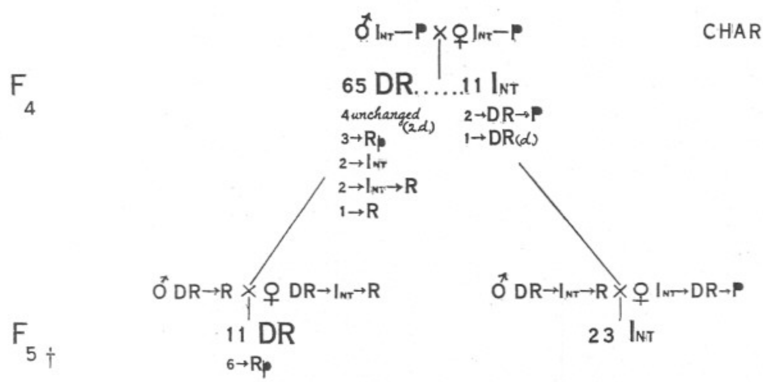
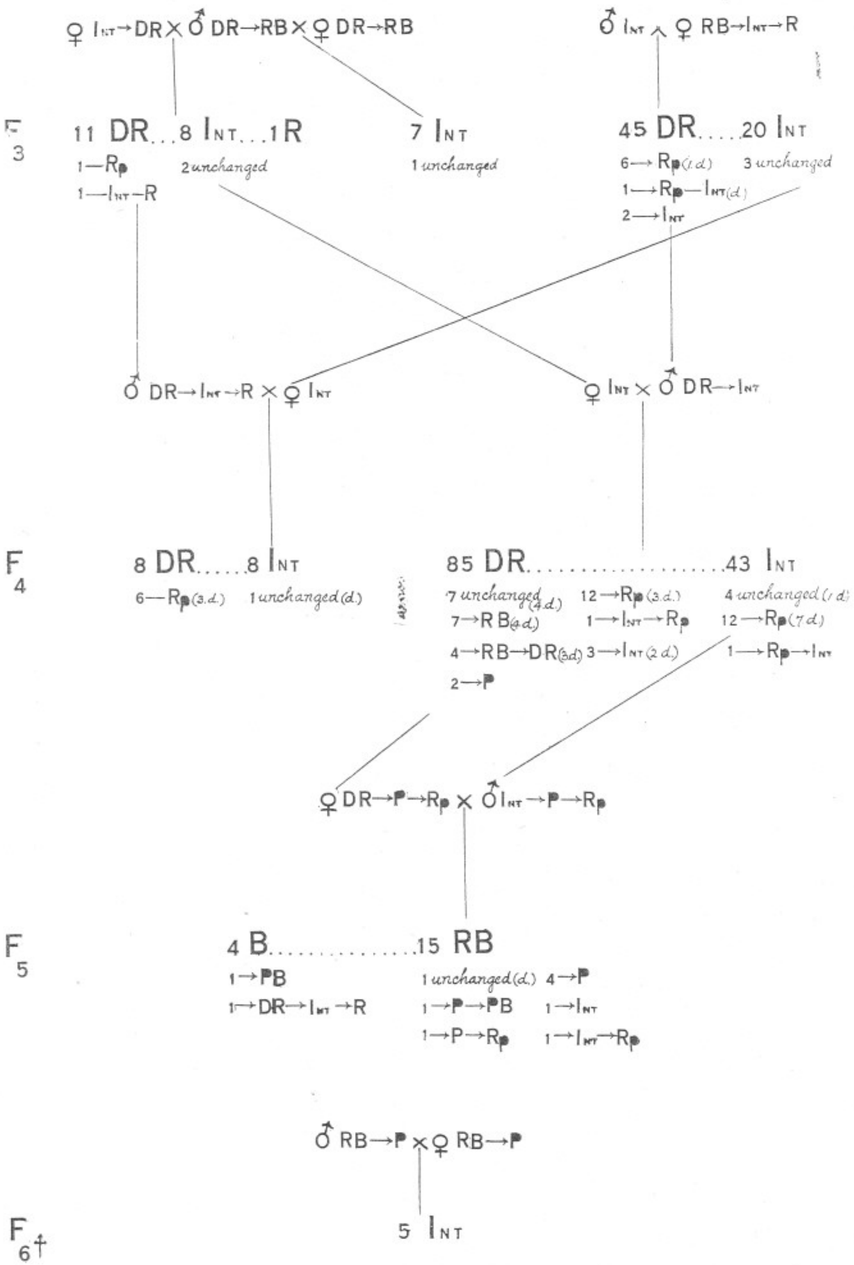


CHART 3



† Broods hatched after the heat was turned off

CHART 4



† Brood hatched after the heat was turned off.

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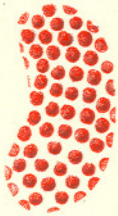
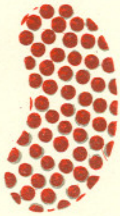
Normal Red

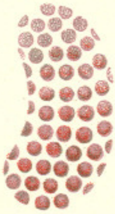
New Red



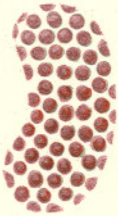
Inter. Red



Dark Red



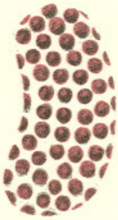
Reddish Purple



Dark Purple

Reddish Black

Black



Purplish Black

E. W. Sexton del.

Chart 4.

Here a small branch derived from the F_3 family set out in Chart 1 is shown. It starts from a mating between two IR→Purple specimens of the F_2 which might reasonably be expected to have been genetically alike. Yet the family produced by them is quite mixed—some born DR, some IR ; some turn purple, some redden. The F_5 shows one of the very frequent cases in which purple colour appeared in the progeny of non-purple parents. The other F_5 family is still going and in it so far only Intermediate Reds have appeared.

Table II.

All the matings up to June 1st, 1930, are contained in this table, including several not figured in the previous charts, and many derived from other matings within the F_2. It is set out in table form so as to bring together matings of a similar kind.

It will be noticed that on the whole the mean of the redness of the progeny corresponds to or varies with the mean of the redness of the parents. Thus if the matings of Blacks with various kinds of Red eyes are considered, it is seen that the proportion of Reds in the offspring on the whole increases with the redness of the Red parent. But this does not apply rigidly and scarcely holds at all as far as matings between Reds are concerned.

Again, on the whole, Black is dominant to all the forms of Red and therefore matings between Reds give only Reds. But there are a number of exceptions. For instance, Reddish Blacks not uncommonly give Black among their offspring (e.g. matings between two RB unchanged ; two RB→P's ; RB unchanged × IR→P). Other cases are : a mating between two IR→RB's which gave one Black to 13 Reddish Blacks, and a mating between an IR→RP and DR→RP, to which allusion has been made in Chart 3.

If Black were a simple dominant to all forms of Red, then there would be even chances that a mating between a Black and a Red should produce all Blacks or a mixture of Blacks and Reds. Actually only one out of 19 of such matings has given all Blacks. Even making allowance for a preponderance of heterozygotes, owing to special attention to the redder forms, a higher proportion than 1 out of 19 would be expected.

Value of Data for Genetical Interpretation.

Attention has been called to the small numbers in the earlier broods. Only a few families are large enough to give adequate indications of ratios even of eye characters at birth. (The small number in the broods apparently does not always mean that numbers of eggs die off. It has been observed that many of the females giving small broods have small ovaries. There is probably not much risk of ratios being upset by a differential death-rate among the eggs.)

But more than this, since the changes undergone after birth are clearly important, it is not sufficient to know the eye-colour of the broods at birth alone. It is only from the survivors that adequate information is to be obtained. The proportion of survivors in the stock has been small, working out at 27%.* That no more than one specimen in three or four survives to maturity is a distinct handicap when so many phenotypes may occur in one family. Recently, however, the proportion has shown a marked increase.

Then there is the question, to which reference has already been made, as to how far the change of temperature has affected the phenotypes.

Finally, attention is called to a rather disturbing feature all too common in this stock, namely, disparity between the composition of and proportions within different broods of the same family. Here is a case in point :

Successive broods from an F_3 DR × B→RB mating had the following composition :

1. 15 IR (11→RB)
2. 15 RB (2→DR, 1 unchanged, 1→IR) ; 2 IR (1→PB).
3. 9 B
4. 12 RB. 10 IR
5. 7 B (2→IR) ; 16 RB (3→IR) ; 4 IR (2 unchanged).

Normally in stocks of Gammarus various phenotypes in a family are fairly evenly distributed among the broods. In this stock there is therefore clearly some irregularity which requires explanation. This at least arouses suspicion of the ratios given by families of only one or two broods.

So far, in spite of the number of families reared, the facts are still inadequate for genetical purposes. No pure lines of any sort of Red eye have yet been produced, and until this is possible there is of course little chance of testing to what extent external factors (in a wide sense) may affect a constitutional type of eye-coloration. For example, throughout the stock there is much variation in the rate of growth, and this variation may well affect the balance between the rate of growth of the ommatidia and the rate of deposition of eye-pigment. Any alteration in this balance means a change in eye-coloration.

Further discussion on the behaviour of this stock and the possibilities

* It is incidentally of interest to note that there has been no marked variation from the 27 per cent survival when the different eye-colours (at birth) are taken separately. Black gives 25%, Reddish Black 27%, Dark Red 32%, and Intermediate Red 29½%. The lower figure for Black, whether it actually means anything or not, might be expected since Blacks occur in larger numbers and hence are kept in more crowded conditions and more at the mercy of cannibals. At any rate, the point is that there is no evidence that the bright Red individuals are less viable than the Blacks.

of genetical interpretation of the results is deferred until additional facts are available. It has been the aim of this paper to give an indication of the problems which have arisen and on which investigation is proceeding.

6. SUMMARY.

For the present, attention is called to the following points :

1. In a large number of experiments with *Gammarus chevreuxi* when the animals were kept under similar conditions at temperatures ranging from 20° to 28° C., twenty instances occurred in which red-eyed mutations appeared independently and simultaneously. The most important of these (Mutant Stock V) is described in detail in this paper.

2. The Red eyes which have arisen in this Stock V and in the four previous Stocks, I to IV, have proved to be all genetically distinct from one another.

3. There are at least two different classes of "red-eye," the one simple, the other with intermediate stages. Mutant Stock V, here described, represents the second type. Differences in the method of origin of the two classes have been pointed out.

4. The inheritance of the red-eyedness of the second class—namely, that in which intermediate stages and various colour changes occur—is of a complicated kind. For an interpretation in terms of Mendelian genes, even if allowance is made for an extent of variation in members of one particular genotype, clearly several of them are involved. Further, there must be considerable interplay of one kind or another among the different genes.

5. Although so many different colour shades have appeared in Mutant Stock V, all can be interpreted in terms of varying concentrations of two pigments—the black and the red. Dilute black pigment with little or no red gives a purple eye.

We are greatly indebted to Dr. E. J. Allen for his constant assistance and advice throughout the whole course of the work; and our best thanks are due to Mr. E. B. Ford of Oxford for the help he has been so ready to give us.

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EXPLANATION OF PLATE VIII.

All the eyes have been figured the same size, so that the colours could be more easily compared.

The colour of each was taken from a living specimen, all the tints in the original water-colour drawings being obtained by using two shades of red with black.

Normal Red from a female of Stock I, sent by Mr. E. B. Ford. This is a pure Red, leaving no deposit of melanin when treated with alcohol, or any other preservative.

New Red from Stock V. A male, H. 814 (2), which was hatched Reddish Black and lightened through Reddish Purple to Red. All these Reds, whether born Red or lightened to Red, contain a slight deposit of melanin, as can be seen on treating them with alcohol. The red pigment, which in life obscures the melanin, dissolves out and leaves a layer of the insoluble dark.

Intermediate Red from a male, H. 813, Pair 1, hatched Reddish Black, and lightened through Reddish Purple to Intermediate Red, and afterwards to Reddish Purple again. This is an example of the fluctuation of colour shown by some specimens.

Dark Red from a young specimen, H. 859b, hatched Dark Red.

Reddish Black from a male, H. 811, hatched Black and lightened to Reddish Black.

Reddish Purple from a female, H. 844, hatched Intermediate Red, changed to Reddish Purple, then back to Int. Red, and later to Reddish Purple again, another example of the fluctuation of colour through life.

Dark Purple from an immature animal, H. 813, hatched Reddish Black.

Purplish Black, H. 835 a.a., hatched Black, lightened to Purplish Black.

Black from a female, H. 811, unchanged through life.