A Summary of the work on the Amphipod *Gammarus chevreuxi* Sexton carried out at the Plymouth Laboratory (1912–1936).

By

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CONTENTS.

INTRODUCTION.

The work began in June, 1912, simply as a study of the life-history of some of our common amphipods. The genus Gammarus was chosen, because of the number and the wide distribution of its species in the neighbourhood. Seven of these species, all black-eyed, from marine, estuarine, brackish and fresh waters, were kept in the laboratory for investigation until the first red-eyed specimens were discovered in one of the brackish species, later described as *Gammarus chevreuxi* (see p. 360). Since the manner in which these red-eyed individuals occurred raised points of considerable interest, it was decided to confine investigation to *G. chevreuxi*, and a series of experiments was started. The different variations involving both the structure and the pigmentation of the body and of the eyes appeared from time to time, some behaving as simple mendelian characters and attributable to the presence of a single recessive gene, others with a more problematical hereditary basis. These variations have been recorded as they occurred (see bibliography). For ten years, no second mutation appeared, although frequent dredgings were made and the animals thus obtained were kept for several generations in laboratory conditions. Because of this apparent stability of character the wild Gammarus was regarded as a homogeneous population until, in 1922, it became increasingly evident that the results showing in the laboratory cultures could only be explained on the supposition that many recessive factors must be present in the natural conditions.

The evidence steadily accumulated, but direct proof was very difficult to obtain partly because (as we found in our experience with the cultures) the recessive types are often less viable than the normal, and therefore probably less able to withstand the competition in the wild, and partly perhaps because we had no criteria by which to assess the influence of the laboratory conditions (changes of temperature, food, salinity, pressure, etc.) on the constitution of the Gammarus, and so could not decide whether a variation was caused by the inherent action of the recessive genes, or by the untoward environment producing a change in the action of the normal genes.

It was not until March, 1933, that direct proof of the existence of heterozygosity in the wild stock was obtained. A Black-eyed female, which had mated and laid eggs before being brought into the laboratory, hatched her brood a few days later, 11 young, of which 9 were Black-eyed and 2 were Red-eyed recessives (**39**, p. 27). Up to the present, however, only one Red-eye recessive has ever been captured in the wild (**47**, p. 836). This was on October 22, 1935, when one Red-eye was found in a dredging amongst 2,040 Black-eyed. None have appeared since that date although thousands of Gammarus were brought in afterwards for investigation.

The present paper gives a history of this Gammarus work, and of the many recessive characters and other variations affecting the structure and colour of the body and the eyes which have been detected from time to time in the laboratory cultures.

The signs and abbreviations used throughout the paper as descriptions of types according to their appearance are :—for the colours, **B.**, Black ; **RB.,** Reddish Black ; **DR.,** Dark Red ; **Int. R.,** Intermediate Red ; **R.**, Red ; **W.**, White ; **A.**, Albino.

For the "Nowhites," **N.** added to the colours, e.g. **BN.**, Black Nowhite ; **AN.**, Albino Nowhite : in practice this symbol is only used for animals which are recessive for the Nowhite factor, i.e. Mendelian Nowhites.

Spd., spotted, i.e. with spots of white pigment on the head apart from the eye. The numbers of spotted are recorded throughout the paper where figures for broods are quoted.

The sign → = became, e.g. RB.→B.

Letters and figures italicised and bracketed refer to the designation of the experiments and the brood numbers in the records kept at the laboratory.

Note.—The term "filial generation" is used to cover all the members of the same generation within a given stock, the numbers F_1, F_2, etc., indicating which generation in descent from the parent pair is being discussed.

CONDITIONS IN THE WILD.

Gammarus chevreuxi was first found* (June, 1912) inhabiting the ditches traversing a low-lying salt-marsh formerly part of the tidal estuary of the River Plym (**1**, p. 542). This land, reclaimed about a hundred years ago and known as Chelson Meadow, is enclosed on the river side by a long embankment, and drained by sluice-gates into the Plym. It was used as a race-course and was well-drained and kept in good order. The conditions at that time were very favourable to the increase of the Gammarus, with

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* *G. chevreuxi* has recently been found by Crawford (Journ. Mar. Biol. Assoc., XXI, No. 1, p. 102) in similar habitats on the coasts of Devonshire. It was recorded in 1931 by Pentelow from inland brackish waters, Wyken Slough, near Coventry (**20**, p. 797), an observation which has just been confirmed by Dr. R. W. Butcher. Other localities, on the French coast, are given by Chevreux and Fage (**13**, pp. 255–6). All the material used in our work has from the first been derived from the salt-marsh, Chelson Meadow, and any notes on the conditions in the wild refer to this place.

the water never above a certain level, food in abundance and few enemies. The worst to be endured was a very occasional drought, such as in the hot summer of 1911 (and again in 1921) when most of the ditches dried up, but even then shelter and moisture, sufficient to prevent the species being wiped out, could be found in places. The Gammarus population was at that time apparently a very stable one, consisting, generation after generation, of normal Black-eyed animals. Also, with the exception of the Red-eyed mutation referred to above (known later as Stock I Red-eye), not one of the many hundreds of pairs brought in from the wild gave anything but normal Black-eyed offspring.

In later years, however, great changes have taken place in the ditches, in depth, temperature and salinity (**39**, p. 27). Little attention has been given to the drainage, the Meadow itself is under water at times before the sluice-gates are operated, and, on such occasions it becomes impossible to reach the ditches for dredging.

The Gammarus, too, have changed their habitat to the shallower ditches farther inland, and dredgings taken at different times show extraordinary fluctuations in the numbers of the population. They have changed also in that many different recessive factors have appeared in the stock. The eye-pigments do not appear to be as stable as heretofore, partial or complete absence of the white pigment occurs and reddening or decrease in the amount of the black pigment (p. 376) is also found. It seems to us that these changes may perhaps be related in some degree to the change in the conditions.

If, when the ditches are overflowing, the sluice-gates are opened, and a great volume of water pours out, it must carry with it most of the inhabitants of the ditches. Only a small nucleus would be left and therefore any recessive factors that were present would be multiplied by interbreeding, and rapidly conveyed through the whole of the Stock to reappear again and again. That this is what happens appears to us probable from the evidence obtained, e.g. the r_2 gene was found for the first time in the laboratory cultures in 1922 (p. 372), reappeared in 1930 (p. 380), again in 1931 (p. 387) and 1932 (p. 399), in numbers in 1933 (pp. 401, 402), and now in 1935 one Red-eyed specimen carrying the r_2 gene has been found in the natural conditions in the ditches (p. 402).

DESCRIPTION OF *GAMMARUS CHEVREUXI*.

Gammarus chevreuxi is a small species of amphipod, the largest male recorded measuring 14·5 mm., and the largest female 9 mm. from the tip of the rostrum along the dorsum to the tip of the telson. The body is laterally compressed and semi-transparent with the internal organs showing through the thin chitinous cuticle. In the normal wild type the pigmentation of the body is pale green, yellow-green in the males, bluer green in the females with bands of brown on the antennæ. The gonads and eggs in the female are dark green, almost black. The sessile eyes are compound* and well developed, reniform in shape with the margin entire and clearly defined, lightly convex, the corneal cuticle smooth and not faceted. Each eye or ommateum is composed of a number of ommatidia arranged in regular rows, the numbers increasing on the periphery at the growth-stages, and rising from 10–12 in the newly hatched to 70 or 80 in the adult. Each ommatidium has an oval lens formed of two crystalline cones, with the rhabdome, immediately below the cones, surrounded by 5 retinular cells, 4 large and 1 small, containing the coloured pigment. The interstices between the ommatidia are filled with the large so-called "accessory-pigment cells" carrying the opaque chalk-white pigment; seen from above, this has the aspect of a superficial raised white reticulation spread over the eye-surface, with the ommatidia showing as round coloured spots deep in the meshes of the network.

The granular retinal pigment in the normal wild eye when first laid down in the embryo is a pure bright Red, which darkens rather rapidly through Intermediate Red and Dark Red to jet-Black just before extrusion from the egg-capsule.

Two pigments are involved, of different origin and constitution. The black is permanent, and insoluble in any of the preservatives tried. The red is soluble in alcohol, and fades completely out after a time, whilst the white interommatidial pigment is evanescent, and instantly soluble in water or any of the other liquids used. It disintegrates as soon as death takes place, and rises in irregular masses to the surface of the eye, thence to disappear within a few hours. In any disturbance of the eye involving the different pigments, such as the "Clotted-eye" (**48**, p. 695), it has been noted that the granular retinal pigment sinks, while the white pigment rises to the surface.

MOULTING AND DEVELOPMENT (12 AND 21).

The development of Gammarus after leaving the egg may be described as a series of growth-stages each terminated by a moult, or casting of the hard chitinous cuticle. The period between moults is short in the immature, and the increase in size and change in proportions considerable at each stage up to the time of sexual maturity. "The difference in the *proportion* the various parts of the body bear to each other is very marked, e.g. the head in the newly hatched young is much larger in proportion, about a seventh of the total length of the body measured dorsally, and the

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* For a description of the structure see G. H. Parker, The Compound Eye in Crustaceans. Bull. Mus. Comp. Zool. Harvard, XXI, No. 2, 1891.

peræon and pleon are practically equal in length, whilst in the mature animal the head is an eleventh of the total length, and the peræon is a third as long again as the pleon " (**12**, p. 365).

The *rate* of development is affected to a great degree by temperature. In summer the time taken to reach sexual maturity may be from 34 to 40 days, in winter from 2 to 4 months, but the characters are not affected in any way by the difference in rate.

The development of the young *Gammarus chevreuxi* takes place in a series of seven growth-stages before sexual maturity is reached. The sexes are indistinguishable by any external character up to the fifth stage ; they are identical in size, proportions and even to the exact number of setæ and spines. Even at the fifth stage they are still almost indistinguishable, for though minute broodplates are present in the female, it needs a high power to see them. At the sixth stage the differentiation of the secondary sexual characters becomes more evident, the broodplates are larger, and one or two curved hairs of the peculiar male type appear on the male's lower antennæ, but it is not until the next stage, the seventh, that the sexual difference can be seen with the naked eye. Pairing generally takes place during this period, but no mating is possible until after the moult. In the next stage, the eighth, the eggs are laid and fertilised. The female's development is now practically finished, but the male undergoes two further stages and great modification of the secondary characters before it reaches its definitive adult form (**12**, p. 385).

At this period (Stage 8) the animals although sexually mature have only reached about half their growth. From then onwards until they attain the maximum length of the species they moult at longer intervals, and the increase in size, though constant, is much more gradual and therefore less noticeable at each stage. After full growth is established the difference between the stages is hardly perceptible ; a few more setæ may appear, the colour darken, or the chitin become thicker.

For a further discussion of this section, see Appendix I, page 407.

THE QUESTION OF TELEGONY.

As soon as the first mutant, the Red-eye, appeared in the 1912 Stock, and before any mendelian experiments were started, the question as to possible telegony in the species had to be settled. The structure and breeding-habits of *G. chevreuxi*, the species chosen for the work, were therefore studied, and compared with six other species of the genus Gammarus. The conclusion reached was " that it is absolutely impossible for a male to fertilise two broods of eggs with one deposition of sperm " (**3**, p. 19).

Briefly stated, the facts for *G. chevreuxi* are as follows :—

(*a*) the female never lays eggs in the absence of a male ; (even in those species, e.g. *G. locusta* and *G. duebeni*, where batches of eggs are sometimes laid without a male present, the eggs are always infertile and are thrown off within a very short time);

(*b*) the animals never pair until the female is in the right physiological condition;

(*c*) once paired, they remain together, the male carrying the female till mating is accomplished;

(*d*) mating is always preceded by the female's moult;

(*e*) in moulting, the old cuticle cracks across behind the cephalon, on the dorsal surface, and the female draws herself out backwards through this dorsal opening leaving the ventral surface unbroken, so that the old brood pouch (which is attached ventrally to the 2nd, 3rd, 4th and 5th pairs of legs) is sloughed off as a whole;

(*f*) fertilisation takes place externally,* the male ejecting the sperm into the open new pouch before the eggs are extruded;

(*g*) the eggs are laid into the pouch, where they develop;

(*h*) during the whole period of incubation of the eggs the pleopods drive a steady current of water through the pouch from behind forwards;

(*i*) and, finally, the young when hatched do not emerge for at least 24 hours, but keep in constant movement in the pouch feeding on anything that may be washed in through the openings at either end (**45**, p. 477).

This seemed sufficient proof that the sperm from one mating could not be carried over to the next, but other tests were devised to make assurance sure.

If it were possible that sperm could remain in the pouch and active through one reproductive period to the next (and it must be remembered that these periods last sometimes for three months in the winter), then an ovigerous female kept by herself should be capable of laying a second and fertile batch of eggs in the absence of the male. Numbers of females were therefore taken at different seasons of the year, and mated, and then, after the eggs were laid, the males were removed from the brood-bowls, and the females kept separate for one, two, or more breeding periods, sometimes up to six months. Never once were eggs laid in the absence of a male: but all these females, when mated again later on, gave the expected results with their new mates.

A number of cross matings were also made (Red female mated first with a Black male and then with a Red one), but in these, as well as in

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* Le Roux makes the same observation with reference to another Gammarus species, *G. duebeni* (**37**, p. 31). "On se rend compte sur coupes, que la plus grande partie des spermatozöides se trouve dans la poche incubatrice, autour des orifices genitaux et *qu'ils ne pénètrent pas dans les oviductes*."

all the cross matings that have since been made from 1912 till now, we have never had a single instance of a male influencing any offspring subsequent to his own.

MUTANT CHARACTERS AS THEY APPEARED IN THE LABORATORY CULTURES.

Gammarus chevreuxi has proved an exceptionally good material for a study of the nature of the variations in structure or in physiological processes. So far, the experimental data on this question can be sorted into three distinct groups, though others may appear later.

I. First, there are the variations which are inherited in a strictly mendelian fashion and which are evidently the result of the presence of recessive genes. They may be classed as follows as affecting

a. the pigmentation of the body, e.g. *White-body* (**16**, pp. 194–195) ;

b. the structure of the eye, e.g. the *Albino* (**41**, p. 274) ;

c. the coloured retinal pigments, e.g. *Red-eye : White-eye* (**16**, p. 194), *Lilac* (**38**, p. 201), etc. ;

d. the white interommatidial pigment, e.g. *Nowhite* (**3**, p. 43) ;

e. both the coloured retinal and the white interommatidial pigments, such as the *Nowhite-Red* (**38**, p. 201).

II. Secondly, there are the variations which appear more or less to affect all the members of certain stocks, where the line of demarcation is not so much between normal and aberrant as between the degrees of intensity of the abnormality. The precise genetical interpretation of the transmission of the variations has not yet been elucidated. Such are

a. Gradual Nowhite, with reduction of the white pigment ;

b. Gradual Normal, with increase of the White pigment ;

c. Irregular Coloured-eye, with its furthest manifestations, the *One-eyed,* and *No-eyes,*

d. and the *Clotted-eye* (**48**, pp. 692–695).

III. The third group consists of those variations which have not been proved to have a direct connection with " genes," but which, because they occur in some stocks, often in succeeding generations, and not at all in others, must have an hereditary basis of some sort. Such, for example, are :—

a. Spottedness, i.e. presence of spots of white pigment, apart from the eye, on the head and first peræon segment ;

b. Half-Nowhite, in which one eye has the normal white reticulation and the other eye none ;

c. Non-mendelian Nowhites, which arise sporadically in certain stocks ;

d. Variations in the intensity or amount of the coloured pigments.

THE 1912 EXPERIMENT.

STOCK I.

The first mutation arose in 1912 in the F_2 from a black eyed pair brought in from Chelson Meadow. Nine of the first brood of the pair came to maturity, three males and six females, and were mated *inter se* and with outside mates. All gave black-eyed offspring with one exception, a female. She was mated first in her own brood with male A and hatched two broods, in each of which one red-eyed appeared among the black. She was then tested with a male from a freshly brought in dredging and gave 40 Black- and 4 Red-eyed young; put back again with male A, she had another brood, 39 Black and 3 Red. Both the males, and the other animals in the cultures and in the fresh dredging were tested for Red, and matings were carried out for several generations but no Reds appeared, only normal Black-eyed young were hatched.

The Red was tested and proved recessive in the simple mendelian ratio 3 : 1. In one experiment, where the F_2 broods were examined for eye colour as they hatched, out of 1004 young, 251 were Red-eyed. It is now known as **Stock 1 Red** (r_1 gene).

All our stock in the laboratory, together with what we have distributed to colleges and teaching institutions, has been derived from the third brood of this one female. The mutation has never again arisen from the wild.

The colour, a full bright clear red, is very stable through life and has not varied in the pure Main Stock I in all the years since its first appearance, though it was noted (**6**, p. 348) that in very old animals the red colour tended to darken with age. It has not been affected by the temperatures at which we keep our cultures, but that darkening can be induced by constant high temperature (23° C.) has been shown by Ford and Huxley (**20**, p. 115, and **26**, p. 68).

Other mutant characters which arose later in this Stock I were connected with the structure of the eye, and it is noteworthy that these, like the Red r_1 gene, only occurred once and have never appeared again.

The second departure from the normal was the **Albino*** (**4**, p. 274), a sharply defined and heritable mutation, which behaves as a simple mendelian recessive. In this form, the whole structure of the eye is degenerate, the ommateum is broken up, the retinular cells are lacking and only a few scattered cones and interommatidial cells remain. The white pigment forms irregular curd-like masses in the eye, and spots, streaks or splashes of white, apart from the eye, on the cephalon and first

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* For a discussion of the structure of the Albino eye see Wolsky and Huxley (**42**); and particularly Wolsky (**49**).

peræon-segment. The "spotted" condition appears to be closely connected with instability of eye-structure, the spots appearing in certain definite positions (**6**, p. 353).

The third variation, again a case of defective eye-structure, the **Irregular Coloured** eye (**33**, pp. 355–393), arose in the offspring of a mating between one of the dorsally spotted Albinos and a Black Nowhite male heterozygous for Red; i.e. on the one side brought in by the male: the normal regularity of shape and structure in the ommateum and individual ommatidia, the presence of the retinular cells containing the coloured pigment, the presence of the interommatidial cells, but absence of white pigment; and, on the other side by the female: the ommateum degenerate and broken, no retinular cells present, the interommatidial cells present and scattered and greatly reduced in number, but containing white pigment and the white pigment also in spots on the head.

The range of variation in the succeeding generations was extraordinarily wide, extending at birth from the typical normal-eye—perfect in shape and pigmentation—through all stages of degeneracy to the complete loss of one or of both eyes. The extreme stages of degeneracy, the **One-eyed**, and the **No-eyes** or Blind forms, were usually associated with malformation of the head and brain. **Intersexuality** in varying degree (**7**, pp. 549–550: and **12**, pp. 388–396), and other abnormalities in the reproductive organs were frequent throughout this stock.

The line of inheritance could not be clearly defined, owing to the gradual onset of the irregularity, even animals born "normal-eyed" becoming affected later, and to its tendency to increase in intensity throughout life, as well as to the high mortality in the young stages especially amongst those born "abnormal-eyed." It is interesting to trace the series of degenerative stages in a perfectly normal-eyed race which ended in the loss of one or of both eyes, and the rate at which it was accomplished—only eight years. The first Black-eyed pair was brought in from the wild in June, 1912; the first Red-eyed arose in the F_2 generation in August, 1912; the Albino appeared from heterozygous parents in June, 1915; the Nowhite later also from heterozygous parents; the mating referred to above, heterozygous Black Nowhite × Albino was made in 1918; and the first completely Blind form appeared in the F_3 in May, 1920.

All these genetic variations in our stock were produced in laboratory cultures, and had never been encountered in the wild. But, in a very striking example of eye-degeneration, recently recorded in the case of another amphipod, the same amount of variation can be parallelled in a wild population and in a much greater frequency.

The authors, Gallien and David (1936, **50**, pp. 184–196) have described collections of the common sandhopper, *Talitrus saltator* Montagu, brought in from the wild, in which the range of mutant characters extended from Red-eye to the loss of one eye, and included practically every departure from the normal known in *G. chevreuxi*. To gain an idea of the frequency of the occurrence and the proportion of normal to un-normal, a collection of 700 Talitrus was examined ; 3 were " spotted " " taches cephaliques blanches " and 30 were found with " malformations oculaires," but if the lesser cases, such as occasional depigmented ommatidia, had been included, it would have worked out at 8% not strictly normal.

Another genetic variation in Stock I was the **Nowhite** which affected the interommatidial white " accessory pigment," completely inhibiting its appearance. This also proved to be a mendelian recessive, with the 3 : 1 ratio. It is now known as the *Genotypic Nowhite* to distinguish it from the other Nowhite types which developed later, such as, e.g. *Phenotypic* (**48**, p. 692). The genotypic Nowhites hatch nowhite, and remain so throughout life ; they give nowhite young when mated *inter se* or with other genotypic nowhite stocks ; and an F_2 ratio of 3 : 1 when mated with normals.

The phenotypic nowhites are indistinguishable in appearance from the genotypic. They also hatch nowhite and remain so through life, but they give normal-eyed young when mated with other phenotypic nowhites, or with the genotypic ; and normals or normals and nowhites when mated *inter se.*

The Nowhite variation is not peculiar to this Stock, but has been found in many strains and even in the wild. Throughout the records we have used the letter " **N** " to denote the Nowhite type, as, e.g. **BN.** for Black Nowhite ; **RN.**, Red Nowhite ; **AN.**, Albino Nowhite or " Colourless " as we first named it from its lack of both the white and the coloured pigments (**4**, pp. 330–339). Later, after the appearance of the White-eye, and its White-Nowhite form, **WN.**, to which the description would equally apply, " colourless " as a term was dropped. The letters **HN.** for Half, or One-sided Nowhites, are used to distinguish those animals in which one eye has the interommatidial white pigment present, and the other eye has none.

THE 1922 TEMPERATURE EXPERIMENT.

During the ten years from 1912 to 1922 dredgings were taken at frequent intervals and cultures carried on in the laboratory to the F_2 and F_3 generations, but nothing occurred out of the normal, the retinal colour was always black, the white reticulation was present and " spots " were exceedingly rare.

In 1922, however, it became plain that heterozygosity must exist in what we had hitherto regarded as a homogeneous population, new factors appearing in several of the stocks derived from a dredging brought in on Sept. 12th. The animals were intended mainly for use in studying the influence of temperature on development and reproduction. The young were not examined for eye colour until red-eyes were seen in the F_2 offspring of one of the Incubator pairs. A short résumé of the results may be given here.

TEMPERATURE AND RATE OF DEVELOPMENT.

Ninety-five mated pairs were taken, all with eggs laid on the same date, so as to start on the same level. They were divided into five series, three at temperatures as nearly uniform as possible, viz.: extreme heat, medium, and extreme cold: and two in varying temperatures, viz.: indoors in laboratory conditions, and outdoors in natural conditions. Daily observations were made with maximum and minimum thermometers (Negretti and Zambra).

Table I shows the highest reading of the maximum thermometer, and the lowest reading of the minimum for the month, and the average of daily readings taken at 10 a.m. through the month, for four Series.

Series I. Uniform High Temperature.

For this, an incubator was used, kept as nearly as possible to 20°–21° C. The results were as follows:—

F_1 broods took on the average 10 days in winter (Nov., Dec., and Jan.), from oviposition to the extrusion of the young from the brood-pouch; 8 to 9 days from April to October.

Age of the first F_1 pair to mate and lay eggs was 145 days.

F_2. Eggs laid.	Eggs hatched.
Mar. 1.	Mar. 5. Thrown off.
Mar. 6.	Mar. 10. Thrown off.
Mar. 16.	Mar. 25. Young (3) extruded.
Mar. 25.	Mar. 31. Thrown off.
April 4.	April 13. Young extruded.

and 16 broods since.

F_3. Nearly mature in June.

Series II. Varying Indoor Conditions in a compartment on the north side of the laboratory.

This series was subject to considerable variations of temperature, daily as well as seasonal. The highest summer temperature in the period was 22° C. on June 30, and the lowest winter reading was 8·3° C. on December 11. The daily range occasionally varied in the 24 hours by 2 to as much

TABLE I.

1922 Temperature Experiment.

Date of record.	Maximum temperature for the month. °C	Date of record.	Minimum temperature for the month. °C.	Month.	Average temperature for the month. °C.
Series I : Incubator.					
1922.					
Sept. 21	22·6 °C	Sept. 27	20·5 °C	Sept. 16–30	21·5 °C
Oct. 2	22·2	Oct. 31	18·0	Oct.	20·93
Nov. 13	22·0	Nov. 22	17·5	Nov.	20·15
Dec. 25	21·0	Dec. 25	17·0	Dec.	19·57
1923.		1923.		1923.	
Jan. 16, 19	21·0	Jan. 1, 6, 12	17·5	Jan.	19·77
Feb. 15	21·4	Feb. 23	17·8	Feb.	19·8
March 26	21·9	March 24	17·8	March	20·12
April 30	22·4	April 14	17·2	April	20·0
May 31	22·7	May 14	18·0	May	20·0
June 25	25·2	June 11	19·6	June	21·4
Series II : Varying Indoor Conditions.					
1922.				1922.	
Sept. 28	20·0	Sept. 30	13·0	Sept. 16–30	15·3
Oct. 13	19·3	Oct. 30	9·0	Oct.	13·0
Nov. 11	18·9	Nov. 27	10·0	Nov.	13·8
Dec. 2	16·0	Dec. 11	8·3	Dec.	12·8
1923.		1923.		1923.	
Jan. 31	17·0	Jan. 13	9·3	Jan.	12·3
Feb. 2	19·5	Feb. 23	10·0	Feb.	14·5
March 9, 26	18·0	March 5	11·3	March	14·69
April 30	18·0	April 10	8·9	April	13·76
May 5, 22	20·0	May 11, 12	10·6	May	16·0
June 30	22·0	June 20	13·0	June	16·93
Series III : Uniform Moderate Temperature.					
1922.				1922.	
Sept. 25	15·3	Sept. 16, 18	13·5	Sept. 16–30	14·3
Oct. 2	14·8	Oct. 30, 31	10·0	Oct.	12·45
Nov. 11	12·3	Nov. 1, 6	10·0	Nov.	10·74
Dec. 2, 6, 7, 14, 15, 16	11·2	Dec. 28, 29	9·5	Dec.	10·3
1923.		1923.		1923.	
Jan. 4	10·1	Jan. 13, 15, 22	8·3	Jan.	8·94
Feb. 5	11·2	Feb. 24	8·9	Feb.	9·97
March 28, 29	11·8	March 12	9·3	March	9·96
April 4	11·4	April 10, 11	9·3	April	10·25
May 5	13·1	May 1	10·5	May	11·43
June 28	15·3	June 1	11·8	June	13·22
Series IV : Varying Outdoor Conditions.					
1922.					
Sept. 21	18·4	Sept. 18	8·3	Sept. 16–30	12·55
Oct. 14	16·9	Oct. 30, 31	2·5	Oct.	9·08
Nov. 11	13·0	Nov. 14, 27	−0·5	Nov.	7·22
Dec. 14	11·4	Dec. 11	−1·0	Dec.	7·17
1923.		1923.		1923.	
Jan. 31	11·0	Jan. 13	−0·2	Jan.	6·03
Feb. 1	10·9	Feb. 20	1·9	Feb.	7·75
March 26	15·6	March 5	1·8	March	7·75
April 3	16·0	April 28	2·9	April	8·97
May 4	20·9	May 12	3·1	May	9·78
June 30	22·0	June 5	8·9	June	13·56

as 6 degrees Centigrade. F_1 broods took 15–20 days to hatch from October to January; 12–14 days in the warmer months.

Age of the first pair to mate, at the time of oviposition, was 121 days.

F_2. Eggs laid.	Eggs hatched.
Feb. 12.	Mar. 1. Young (2) extruded.
Mar. 2.	Mar. 17. Young (6) extruded and ♀ eaten.

It is noteworthy that the F_1 of this series, matured *earlier* than the Incubator F_1, 121 days as compared with 145, and that the first batch of eggs hatched and the young F_2 were extruded on the same day that the first batch of eggs was laid in the Incubator.

Series III. Uniform moderate temperature.

The desired conditions were found in the experimental tanks on the north side of the laboratory, which are fed from an underground reservoir, and kept in constant circulation. The temperature was practically steady during the different months, and though it varied with the season, there were no sudden rises and falls as in Series II and IV. The daily range was about 1 degree Centigrade. F_1 broods took on the average 23–30 days to hatch, from November to April; and 16–18 days in June and July.

Age of the first pair to mate, at time of oviposition, was 157 days.

F_2. Eggs laid.	Eggs hatched.
May 2	Thrown off.
May 22	June 12. Young extruded.

The male and female of this pair were hatched on November 26, 1922, but matured earlier than the second pair, which were hatched on November 22. These were 167 days old when their first batch of eggs was laid on May 8; and the young were extruded on May 30.

Series IV. Varying Outdoor Conditions.

The jars were kept on the flat roof of the laboratory in a large weighted packing-case, so that although exposed to the air, they were protected from the force of the wind and from the direct rays of the sun.

The temperature varied from $-1°$ C. in winter to 22° C. in June. The daily range in April was roughly 6–7° C.

F_1 broods took 35–48 days from November to April and 15–20 days from April to June.

The first F_1 pair to mate and lay eggs was 176 days old.

F_2. Eggs laid June 6. Young extruded June 26. The second pair was 191 days old; eggs were laid on June 22; and the young hatched July 5.

Series V. Uniform extreme cold.

Twenty-eight pairs were kept in separate finger-bowls in an ice-chest, all the females having laid eggs the day they were put in. The temperature was fairly steady at 2·5° to 3° C., but a rise of about a degree took place daily for a few minutes when the chest had to be opened for examining the bowls and adding fresh ice.

The females were well-grown, and their batches of eggs of the usual average number for their size, about 40 to a batch. It was found that not only was development greatly retarded by the cold which, of course, was to be expected, but that there was a very high mortality just at the hatching period; this had not been anticipated in view of the fact that broods had survived occasional much lower temperatures in the "varying conditions" experiment.

The results were as follows:—

Out of the 28 bowls, 11 females threw off their eggs within from 1 to 2 months, 8 of them dying at the same time. In 9 females, the eggs were thrown off gradually until only one or two were left in the batch. These were carried until orange (i.e. embryos fully formed, but still enclosed in the egg-capsules) for periods of 68, 71, 74, 75, 81, 84, 90, 94 and 96 days respectively, but none hatched.

In one case, where the eggs had been thrown off, and the male had died, a second male which had been the same time in the ice-chest, 80 days, was put in, mated immediately and a few eggs were laid in 5 days. These gradually disappeared until at 39 days 3 were left macerating.

Only 7 females hatched any young.

No. of Broods.	Hatched in Days.	No. of Young.	New Eggs Laid.
1 brood	71	3	Laid same day, carried 95 days.
1 ,,	72–75	7	Laid 19 days after extrusion.
1 ,,	74	1 and others dead in pouch.	
1 ,,	74	1 and 1 partly developed.	
1 ,,	77	4	1st batch; thrown off at 5 days; 2nd batch laid after 80 days; thrown off at 35 days; 3rd batch laid after 79 days; thrown off at 19 days.
1 ,,	85	4 dead.	Laid 2 days later.
1 ,,	98–99	3	Laid 2 days later and carried 75 days.

In all, 15 batches of eggs were laid in the ice-conditions, and carried in some cases for weeks, but not a single young one was hatched.

A comparison may be made of all the series : broods of eggs laid within a day or two of each other in the first week of November hatched as follows :—

In *Series I* in 9 days ; *II* in 15 days ; *III* in 22 days ; *IV* in 42 days ; and *V* in 77 and 85 days.

By February 26, 1923, the pairs in the different series had given :—

I, 13 broods and eggs not yet hatched.
II, 6 ,, ,, orange.
III, 5 ,, ,, not yet hatched.
IV, 3 ,, ,, ,, ,,
V, 1 brood, no eggs.

As soon as the work reached the F_2 generation it was seen that new recessive factors were present in several of the stocks, affecting the pigmentation of the body as well as of the eyes.

Three of these arose in the Incubator series, viz. two new Red-eye recessives, one in Stock II (r_2) and one in Stock IV (r_4). The third, "White-body" also in Stock II, was connected with the body-pigment. Two more Red-eyes appeared in the second series, "varying indoor conditions," one in Stock III (r_3), and one in the F_2 from another strain which unfortunately did not survive to be tested (2 specimens only, bright red, one normal-eyed and one Nowhite).

A slight reddening of the black pigment was noticed also in the offspring of three other pairs, at or near maturity, but this would appear to be of no significance genetically. It has been observed occasionally in animals freshly brought in as well as in laboratory cultures, but interbreeding with them has never produced anything but the ordinary normal black type.

Stock II.

Stock II (**21**, p. 50 ; **28**, p. 191 ; **31**, p. 313) was of great interest and importance. It contained two new recessive factors, one for Red (r_2) affecting the pigmentation of the eye, and the other, the White-body, inhibiting the normal-coloured pigment of the body and red eye-colour.

The **Stock II Red** gene has reappeared many times in the course of the work, and is still present in the wild population.

On its first appearance in this, the "Main Stock," the colour was a vivid bright clear red, stable throughout the life of the individual, and through the succeeding generations.

But in later years, as new stocks appeared carrying this r_2 gene, a remarkable difference was noted and it was soon recognised that some modifying factor or factors must be present, affecting the deposition of the pigments, the coloured retinal and the interommatidial white.

We found that on taking all these stocks together they fell into two distinct classes, (*a*) *stable eye-colour*, and (*b*) *unstable eye-colour*.

Class a. The " Main Stock II " is the type of Class *a*, *Stable Eye-colour*, in which the full bright red of the eyes on hatching remains unchanged through life. It has remained stable in our cultures whether the animals are reared in the incubator heat or in the cooler laboratory conditions.

Included in this division are :—

Main Stock II.	Incubator, inside mating,	p. 372
MM. LXIX.	,, ,, ,,	p. 390
K. XXXV.	Laboratory, outside,	p. 399
dd. XXXIX.	Incubator, inside,	p. 402 ; **54**, p. 355
dd. XLIII.	,, ,,	p. ,, ,, ,,
dd. LXX.	,, ,,	p. ,, ,, ,,
dd. LXXIII.	,, ,,	p. ,, ,, ,,

Class b. Unstable Eye-colour. In this class, whilst the principal recessive gene is the same as in *a*, some modifying factors are evidently at work causing great fluctuation in the deposition of the pigments, and consequent variations in the intensity of the red coloration.

The range of colour in the retinular pigment is from pale cream or white (i.e. practically no pigment in the retinular cells) through all shades of rose-pink, pale red, orange, bright red and lilac to a dark ruby-red and reddish purple. It has been found impossible to draw a dividing line between the colour tones or to establish any of the shades as a " pure " line, for in many of the animals through life a definite increase or decrease in the amount of pigment deposited takes place. All the intermatings gave Reds, but the variations in the intensity and amount of pigment deposited in the parents' eyes were not necessarily heritable, e.g. Cream × Cream often gave bright full Reds.

The Stocks in this division are :—

S. XIV.	Incubator, inside mating	p. 381
S. XV.	,, ,, ,,	p. 381
M. XVI.	Laboratory ,, ,,	p. 387
M. XVI A.	,, outside	p. 387
M. XL.	,, inside mating	p. 388
M. LII.	,, ,, ,,	p. 389
D. XXXI.	Natural conditions, inside mating	p. 401 ; **54**, p. 340
D. XXXI A.	,, ,, outside mating	p. ,, ,, p. 340
D. XLVI.	,, ,, inside mating	p. ,, ,, p. 341
D. LIII A. & *D. LIII.*	,, ,, outside and inside mating	p. ,, ,, p. 342
D. LXIV A. & *D. LXIV.*	,, ,, outside and inside mating	p. ,, ,, p. 342

The white accessory pigment, also, varied from the normal amount of white reticulation to none, i.e. Nowhite.

The other variation from the normal in Stock II was the "**White-body**," recessive to the pigmented form. In this is found a factor inhibiting the production of the carotinoid pigments, the green of the normal body, and the red of the eyes. The whole animal is pearly white, with not the slightest tinge of colour anywhere in the body, gonads or eggs.

This mutation arose at the same time as the new Red-eye (r_2) (**16**, p. 195), the brood in which they both occurred consisting of Black-eyed animals, some green-bodied, some white-bodied; Red-eyed, green-bodied; and White-eyed, white-bodied (i.e. Reds homozygous for "white-body" and therefore lacking the coloured pigment in body and eyes.)

The white body factor had a remarkable effect on the crosses between the normal form and the mutant. Different results were given according to the sex of the mutant employed; and a new type, the "Changeling," was produced.

For example, in all matings of White-body White-eyed male × Green-body Black-eyed female all the offspring were green-bodied and black-eyed; but the reciprocal cross Green-body Black-eyed male × White-body White-eyed female always gave black-eyed young which were *white-bodied* at birth, but which later developed the green body-pigment—the so-called "**Changeling Blacks**."

Again, in all the Red matings White-body White-eyed male × Green-body Red-eyed female gave all normal green-bodied red-eyed young; but the reciprocal cross Green-body Red-eyed male × White-body White-eyed female produced young, *white-bodied* and *white-eyed* at birth, which later developed pigment in body and eyes and became indistinguishable from the normal green-bodied red-eyed animals. These we called the "**Changeling Whites**."

The following explanation was suggested (**19**, pp. 119–120). Individuals homozygous for the white-body factor cannot lay down body-pigment or red eye-pigment, consequently white-body females lay eggs with no pigment. The developing embryo has therefore no pigment even if the fertilising sperm carries the colour factor. A White individual results which changes to Red as life proceeds, since the dominant normal colour factor introduced by the father is able later to make good the deficit of pigment.

It will be seen that Changeling Whites occur only where Reds would be expected; they always have a White-bodied mother, and always behave genetically as Reds, but are always heterozygous for White-body.

The White eyes are of different kinds. When they first arose in the F_2 they were of the **Flushed-white** type, i.e. eyes in which the ommatidia, quite white at birth, develop the faintest tinge of pink, only visible under a high power, by the time maturity is reached. These, the oldest ommatidia, form the centre of the eye, but the later ommatidia, which increase on the periphery at each growth-stage, remain white always. For a similar happening with coloured eyes see page 393.

The **Quite White** type segregated out in the F_7 generation and are still going. They breed true and have never developed the slightest trace of colour in the eyes or in any part of the body.

Another division of the Whites is known as the **Purple-whites**, from the purple colour which in varying degrees of intensity partially or completely covers the eye. The pigmentation apparently differs in constitution from the granular red and black contents of the retinular cells in the normal eye which retain their colour after dissection and even after preservation, the red for some considerable time, and the black permanently. In the Purple-whites, on the contrary, as soon as the ommatidia are exposed to the air after dissection the colour fades out completely with the greatest rapidity, although later, after some hours or days in the preservative, a faint dull grey tint appears looking like a thin sediment in the bottom of the eye. A chemical analysis has unfortunately not been possible, owing to scarcity of material.

The first Purple-whites appeared amongst the descendants of a cross made with a Flushed White female of this Stock mated to a Red male of Stock I.

They are of several different types, of which the most distinct are :—

Flushed Purple, a White-eye, with the central ommatidia tinged with dark or pale rosy lilac ;

Half Purple, in which the upper half of the eye is white, the lower part lilac-purple ;

Purple-white, in which the whole of the eye is flushed pale or dark purple ; and the

Blackberry-purple, with the pigment in quantity and almost purple-black in tone, the shade of a ripe blackberry.

The White-body mutation has only appeared twice—in this Stock heterozygous for Red and in the F_2 from a homozygous Black pair *M. XXIV* (p. 387) brought in from the wild in 1931. Mated together they proved to be the same genotypically.

It has been frequently noted throughout the work that crosses between the recessives of different strains produce great modifications, and consequent new colour-combinations. For example the cross just mentioned between Stocks I and II gave the new colour Purple-white. Again in the cross between Stocks I and III, the r_1r_1 types were quite distinct from the typical normal clear Reds of the Main Stock—they all showed a darkening in the centre of the eyes (**31**, p. 311). In the cross between Stocks I and V, to take another instance out of many, the r_1r_1 types are quite stable and fully pigmented in the Main Stock, but display in the cross great variation and deficiency of the red pigment (**31**, p. 321).

STOCK III.

Stock III Red (**21**, p. 51 ; **31**, pp. 313–315) differed from the other Reds, r_1, r_2, and r_4, in that it arose gradually. Some of the Black F_1 became reddish, and mating *inter se* gave an F_2 containing Black, Reddish Black, Dark Red, Intermediate and Bright Reds, with a new departure "**Mosaic** Eye" in which some of the ommatidia were black and some bright red (see **33**, Plate III).

STOCK IV.

Stock IV Reds (**16**, p. 195 ; **21**, p. 51 ; and, **31** pp. 315–319) were of different shades, Dark Red sometimes almost black, and Light Red. Dark Reds tended to lighten in colour, but always functioned as Dark Reds and gave Dark and Light offspring. Light Reds consisted of Bright Reds and others slightly deeper in tone ; they gave always Light Red offspring.

THE 1928 H. EXPERIMENT.

Meanwhile, dredgings were still being made in the ditches and thousands of animals examined yearly, but only very slight variations were noted, of little significance compared with the immense numbers of normal. For example, there was sometimes a reduction of the black retinal pigment (reddening) or of the white pigment of the eye (thin reticulation) or a slight increase in the number of the "spotted," but nothing of any importance until the spring of 1928.

During January and February of that year, dredgings were brought in for an incubator-experiment. The ditches were flooded, and overflowing their banks, and it was found that the Gammarus had changed their location and gone farther up the water-courses to the shallower parts. The February collection contained a large preponderance of males.

Thirty-nine pairs were set out at a temperature of 20·8° C., but the heat, gradually raised until it reached 28° C., proved too much for most of the animals. Only four stocks reached the F_3 generation.

"*Reddening*," in varying degree, was of comparatively frequent occurrence, but this, as has been said before, appears to have little or no genetical significance, and may simply be due to an increase in the rate of development, and a consequent inability to produce the black pigment in sufficient quantity to keep pace with growth. As would be expected, it occurs more frequently in the Incubator Stocks than in the laboratory cultures, but seems to have no effect on the offspring.

Only four stocks (three of which died out) gave Red-eyes (**28**, p. 199), viz.:

H. I, in which Reddish Blacks and 12 Intermediate Reds appeared in the F_3 and F_4 generations; only 1 Reddish Black in F_5.

H. VIII, with 13 Reddish Black, 8 Intermediate Red and 6 Bright Red in the F_2; only 3 Reddish Black in the F_3.

H. XXV, with 1 Red, dead at extrusion, in the F_2; and *H. XXXI*, known as Stock V, which proved one of the most difficult of all the stocks with which we have worked.

STOCK V.

Stock V Red (**28**, pp. 194–217; and **31**, pp. 319–335). The account given in the above references describes the experimental work on this stock as far as it had proceeded at the time of publication. It was stated that the conclusions then drawn from it might, and probably would, have to be modified if the results of later investigation should render it necessary and, in fact, certain alterations must be made as shown in the discussion below (see also p. 380).

On its first appearance the new stock was characterised by several striking features:—

1. An extraordinary variety of colour-changes and fluctuation in eye-colour in the individual animals, sometimes in one direction, either darkening or lightening, sometimes, but less often, changing from the one direction to the other and back again, at the growth-stages.

It has been shown that the retinal pigment is first laid down in the embryo (**21**, pp. 45–46) as bright clear red, some time before the black is deposited—the eye-colour changing as the deposit of dark pigment increases first to intermediate red, through dark red and finally to black on hatching. In the typical normal eye this deposit of black keeps pace with growth, masking the red, and the eye-colour is always a dense jet-black, but in the new Stock V the *rate* of the deposition of the two pigments, black and red, appears to fluctuate considerably, and so causes the colour to vary according to which pigment is produced in the greater quantity during any one growth-stage. As we have said before, " any alteration in this balance means a change in eye-coloration " (**28**, p. 216). For example, in the true " Blacks " group, a reduced rate in the deposition will give " dilute black," a thin greyish black tone or purplish black. In the " Reds " group the fluctuations and grades of colour show much more distinctly; with a larger amount of black the Reddish Blacks and Reddish Purples are produced; with a lesser admixture the Dark Reds and Intermediate Reds, whilst a form which we called " New Red " contains only the faintest trace of dark pigment, just enough to deepen the red tint in the eye. The difference between this colour and the normal bright red of Stocks I and II is very slight; but in preserved specimens, where the red pigment is dissolved out, the New Reds show a faint dark deposit, whilst the normal Reds are perfectly clear, and without the slightest trace of it. It must be noted that this extensive range of colour is produced by the admixture in different proportions of two pigments only, black and red.

2. Although the main facts of inheritance were clear (the simple mendelian ratio of 3 : 1), yet, owing to this fluctuation of colour in the recessives, the pigmentation of the parents' eyes was no indicator of the colour of the offsprings' eyes at birth, nor was the colour at any given period a reliable guide to later developments; a Black might lighten to Red, or an Intermediate Red darken to Reddish Black or to almost Black. Nor was it possible to separate the animals by the difference in the rate of pigment-deposition. The rate appeared to vary even in the individual, and instances frequently occurred in which more black than red would be developed at one growth-stage, and more red than black at the next.

3. No pure Bright Reds were known from the Main Stock up to the F_6 generation, at the time of the publication of the results. All the Reds which were produced in the earlier generations had some admixture of dark pigment, and this at first was considered to be a definite character of the new gene (r_5). Even the brightest, the "New Reds," as they were called, contained a trace of it, faint but distinct, though only perceptible to the naked eye as a slight deepening of colour, just a shade deeper than the normal pure bright red of Stocks I and II.

Meanwhile, from a cross (*OH. 745*) which had been made between an F_4 recessive male of the Main Stock, and a Black "Outside" female from the wild, the pure Red had segregated out in the F_2 free from the slightest trace of dark pigment.

It was at first thought that a new gene had made its appearance, and as such it was recorded and named r_6, but the evidence was not considered sufficiently conclusive, and the experimental work was therefore carried further. The later investigation has now definitely proved its identity with the r_5 gene.

These Pure Reds were mated with typical "Stock V Reds" of the same *Cross OH. 745*; and back to Main Stock V recessives; and later with another Stock *MM. XV A.* (a reappearance of r_5), and gave reds, and reds only, with them all (p. 379).

That this deduction was correct as to the identity with r_5 has since been confirmed by the appearance of the Pure Reds in the Main Stock itself, in the F_7 generation. And to put the matter beyond question it was found when a specimen was preserved in alcohol and the red pigment dissolved out that there was no trace of any deposit of dark pigment; the eyes were perfectly colourless.

The history of the *Cross OH. 745* in which the pure normal Red segregated out was as follows :—

A male, *H. 745*, of Main Stock V (F$_2$ of a pair which gave Blacks, Reddish Blacks and Intermediate almost bright Reds) was mated with a Black female freshly brought in from the wild. The male was a recessive, with eyes which looked Black on hatching, but which gradually reddened until, at the time of mating, they were Red to the naked eye; two months after the second brood of the cross was hatched, they developed a purplish tone, and eventually became pale Reddish Purple.

The two broods of the cross consisted of 17 Black (1 spotted) (*OH. 850*) and 30 Black (*OH. 856*). Eleven of the 47 survived to breed, 3 males and 3 females of the first brood, of which 2 males and 1 female had become Reddish Black by maturity; and 2 males and 3 females of the second brood.

Their intermatings gave an F$_2$ of 200 Black* (37 spotted) and 126 Reds (19 spotted), which included 31 Reddish Blacks, 2 Dark Reds, 30 Intermediate Reds of all grades from dark to almost bright red, and 33 of the pure normal Reds.

The following matings were made :

Mixed matings in the brood-bowls : gave 25 B. (2 spd.); 9 RB. (2 spd.); and 1 Int. Red.

B♂$_1$×B♀$_1$ *850* : 32 B. (5 spd.); 8 RB.; and 9 Int. Red (2 spd.).

B♂$_1$×B♀$_3$ *850* : 22 B. (4 spd.); 1 RB.; and 11 Int. Red.

B♀$_1$×B♂ of *856* : 36 B. (6 spd.); 8 RB. (1 spd.); and 16 Int. Red (3 spd.).

B♂$_2$×B♀$_2$ of *850* which had both become Reddish Black, gave 85 B. (20 spd.); 5 RB. (2 spd.); 2 Dark Red; 23 Int. Red (6 spd.); and 33 pure normal Reds (5 spd.).

These "pure Reds" were tested in several ways, first to make sure of their identity with Stock V, and then to prove the distinction of Stock V from the other tested stock reds, such as Stock I and Stock II Reds, and Stock *LVII*, Nowhite Red. From the numerous matings made, the following examples are taken to illustrate the results :—

Ex. 1. *Test Cross 2070.* Mating of a pure Red with a typical Stock V Red, both from the *Cross OH. 745*.

Pure Red ♀ *OH. 2046*×Dark Reddish Purple ♂ *OH. 2070* gave an F$_1$

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* The figures given for the Blacks and Reddish-blacks refer only to the colour at birth and cannot be regarded as giving the correct constitution of the animals. All these young were hatched in the incubator, and it is known that in this Stock, the dark pigment is deposited in greater amount in the higher temperature. The survivors were too few to be of any use in checking the figures.

brood of 15 Light Int. Red, and an F_2 of 1 Dark Red ; 1 Light Int. Red ; and 1 Pure Red.

Ex. 2. *MC. LXV.* The same ♂ *OH. 2070* of Ex. 1 was mated back to a typical " Stock V red," an Int. Red → Red ♀, F_4 *MM XV A.* (p. 391 ; Table II) and gave in the F_1, 12 Dark Red, two of them Half-Nowhite ; in the F_2 6 Dark Red ; and in the F_3* 2 Reddish Black and 2 Dark Red.

Ex. 3. *OH. 1506 b.* 2. Mating of a Pure Red ♂ with a typical " Stock V red " from the same stock, *Cross OH. 745.*

Pure Red ♂ *OH. 1549 c.* × Dark Reddish Purple ♀ *OH. 1506* gave 66 Red offspring, of which 16 were Pure Reds and the others typical Stock V reds with all their characteristics, fluctuation of colour, etc. The same range of colour was repeated in the F_3 and F_4 generations. Intermatings of the F_2 Pure Normal Reds produced, as would be expected, all Pure Normal Reds.

Ex. 4. *Test Cross 1549 c.* The same ♂ *OH. 1549 c.* of Ex. 3 was mated back to a Main Stock V recessive, Int. Red. → almost-Red ♀, *H. 1507* and gave 3 broods of 3 Light Int. Red ; 3 rather darker Int. Red ; and 3 very light Intermediate Reds. The 7 survivors all lightened to the same tint, an almost-Red. Intermatings gave an F_2 of 116 of which 21 were recorded as " Pure Red " at birth. The other 95 ranged from Dark Intermediate to bright, almost Red, but the colour gradations were so close to each other that it was almost impossible to draw a line between them. Two broods examined within a few hours of hatching gave the proportions as 21 Int. Red ; 12 New Red ; and 4 Pure Normal Red. The same variety of colours reappeared in the F_3 and F_4 generations.

These Pure Normal Reds crossed with the Reds of other Stocks, e.g. Stock I Red Nowhite ; Stock II Red ; and Stock *LVII* Nowhite-Red (p. 397) gave all Blacks, the different Reds segregating out in the F_2 in the usual mendelian ratio.

THE 1930 S. EXPERIMENT.

The second statement requiring correction is the association of the " Flesh Red-eye " with a new gene *f* (**31**, p. 335). Later investigation has proved this to be yet another manifestation of the r_2 gene, the " unstable eye-colour " type (p. 373).

The specimens came from a small dredging brought in November, 1930, and kept by Mr. Spooner in the incubator (**32**, published May, 1932). Thirteen pairs were bred from, with recessives appearing in the F_2 generation of three of them :—

In *S. XVI*, the Nowhite variation occurred, and was found to be genotypically the same as the Nowhite of Stock I.

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* Found mature, not examined on hatching.

In *S. XIV*, two recessive forms were described, Flesh Red (gene *f*) and Beet Red (gene *t*), segregating independently of Flesh. Flesh is given as varying from normal Red to almost colourless: and Beet as having red eyes at extrusion but with "an appreciable amount of dark pigment which gives it the appearance of New, Intermediate or Dark Red. During the earlier growth-stages the eye darkens rapidly to a Reddish Black or even Black, the final state varying among individuals."

In *S. XV* also Flesh Reds appeared (F_2, 47 Blacks and 6 Flesh) which when mated with the Flesh Reds of *S. XIV* proved identical.

The Flesh-reds had been tested with Stock I Red and shown to be distinct from it (see also **31**, p. 312, where mention is made of the cross). We know now that Flesh is really Stock II Red, "unstable" division, and that the varying tones of colour compare with other stocks carrying the r_2 recessive.

Other crosses have been made since then, establishing beyond question the identity of the Flesh gene *f* with the r_2 gene of Stock II.

Some of the crosses made were as under:—

Exp. 1. *MC. LIV.* Flesh ♂ × Quite White ♀ *TH. 6064* gave 10 Changelings (1 spd.). These intermated, producing Reds and Whites in the next generation.

Exp. 2 *dd. 681.* One of the F_2 Reds of Exp. 1, a ♀, mated with a Red ♂ *dd. 681* (F_3 *dd. LXXIII*) and gave two broods numbering 28 Reds, all with full red colour when mature.

Exp. 3. *MC. XLV.* Flesh ♀ × Red ♂ (F_3 *MM. LXIX*) gave 32 Reds (5 spd.).

Exp. 4. Flesh ♂ of the bright red type × Nowhite-Red ♀, F_4 Stock *LVII* gave 5 Black (2 spd.).

THE 1931 M. EXPERIMENT.

In 1931 we decided to make as large an experiment as could be conveniently handled to try and get a conclusive ruling on the question of heterozygosity in the wild—as to whether it was inherent in the stock (which seemed certain from the previous evidence), or was caused by the abnormal conditions to which it was subjected in the laboratory, such as extremes of temperature, changes of salinity, depth, food, etc.

For this purpose a dredging was brought in on October 6, 1931, which, in many ways, proved by far the most interesting of any we have ever had. Unfortunately, some bacterial infection was introduced during the later work, probably through the water, which wiped out some stocks, and reduced others to such an extent that we realised that the figures obtained from the work could hardly be called conclusive.

It was evident that another experiment must be instituted to complete

TABLE

1931 M. EXPERIMENT :

FROM OUTSIDE MATING OF ♀.

	F_1	F_2		
Stock No.	Black.	Black.	Red.	
M. I A.	3	57		1 survivor→almost BN.=♀
M. II A.	8	287		3 reddish tinge
M. III A.	8	166		11 reddish tinge (2→B.)
M. IV A.	13	118		F_1 13 dilute ; F_2 16 reddish tinge
M. V A.	10	401		3 reddish tinge
M. VI A.	18	159		
M. VIII A.	3	21		
M. IX A.	8	64		2 reddish tinge
M. XI A.	18	108 & 1 BN.*		*Nowhites* in F_2 and F_3
M. XV A.	38	45		**Red-eye** in F_3
M. XVI A.	20	261	**38.**	**Red-eye** in F_2 (= reappearance of the r_2 gene)
M. XVIII A.	24	238		In brood of 6, 1* Dark Red→RB.→jet B.
M. XXIII A.	14	—		
M. XXIV A.	14	33		Some reddish tinge.
M. XXVIII A.	7	43		
M. XL A.	23	2		
M. LII A.	17	2		

II.

LABORATORY CONDITIONS.

FROM INSIDE MATING OF ♀.

	F_1		F_2				
Stock No.	Black.	Survivors.	Black.	BN.	HN.	Red.	
M. I	101	41	431	3*			*Gradual Nowhite* in F_2 and F_3.
M. II	125	75	746				1 almost Nowhite→B. normal.
M. III	124	62	537	18	9		1 BN. and 2 HN. spotted and irreg. *Phenotypic Nowhite* in F_2.
M. IV	32	6	16				
M. V	92 B. : 1 BN.	51 B : 1 BN	300	1		1 and 17	Clotted-eyes. F_1 1 BN.* ; F_2 some reddish ; variation in white pigment ; irregularity ; new mutation in F_2, **Clotted-eye**.
M. VI	126	74	275				F_1, 1 sl. reddish ♂.
M. VIII	17	11	242				Deficiency of white pigment in many.
M. IX	87	34	436	5	2		Irregularity in 1 BN. and 1 HN. ; deficiency of white pigment in many ; many Gradual Nowhites ; *Phenotypic Nowhites* in F_2 and F_3.
M. XI	—		—				
M. XV	106	9	94				No Reds appeared ; an F_3 of 60 Black which died before they could be tested.
M. XVI	195	22	810	—	—	**97.**	324 B. and 94 R. were from hetero B. matings ; **Red-eye** in F_2 $(=r_2)$.
M. XVIII	126	3	—				
M. XXIII	125	25	125	—	1	—	
M. XXIV	125	13	182				Many of them Whitebody (about 3 : 1 ratio). **White-body** in F_2 (reappearance of genotype).
M. XXVIII	156 & 2* HN.	39	563				F_1 2 HN.*
M. XL	120	5	13	—	—	**2.**	Deficiency of white pigment. **Red-eye** in F_2 (= reappearance of r_2 gene).
M. LII	137	4	4	—	—	**1.**	**Red-eye** in F_2 (= reappearance of r_2 gene); in crosses with St. II Nowhites appeared, and great variation in the white pigment.

TABLE

1931 M. EXPERIMENT:

FROM OUTSIDE MATING OF ♀.

Stock No.	F_1 Black.	Colour variation at maturity.	F_2					
			Black.	RB.	BN.	HN.	Red.	
MM. I A.	8		–					
MM. III A.	18	15 survivors, all reddish tinge	322	–	–	1	–	
MM. VI A.	7		–					
MM. X A.	13		177					
MM. XV A.	11	4 reddish tinge	119	–	–	–	**3**	**Int. R. Red-eye** in F_2 (= reappearance of r_5 gene)
MM. XVII A.	21		129					
MM. XX A.	18	3 reddish tinge	677	–	1	5	66	**Lilac**, new recessive type in F_2; great variation in white pigment.
MM. XXIII A.	20	1 reddish tinge	151	–	–	–		
MM. XXVII A.	20	10 reddish tinge (and 1 with thin retic. also)	53	1*	–	1	–	
MM. XXIX A.	22		14	–	–	1	–	
MM. XXXI A.	18	1 reddish tinge	69	–	–	–	–	
MM. XXXII A.	15		25	–	1	–	–	
MM. XXXIX A.	21	2 reddish tinge	65	–	–	1	–	
MM. XL A.	19		57	4*	1	1	–	
MM. XLV A.	17	5 reddish and 1→RB.	67					
MM. XLVI A.	23	18 = 6 reddish and 12→RB.	96	4*	–	–	–	
MM. L A.	11	10, all reddish tinge	162	–	–	–	–	
MM. LVII A.	12	1 irregular	49	–	–	–	**12**	**Nowhite-Red**: new recessive type in F_2.
MM. LXII A.	14	All reddish tinge	–					
MM. LXVII A.	14	All reddish, 1→RB.	42	6 Dark Red and **1 Int. Red.** **Red-eye** in F_2 not proved				
MM. LXVIII A.	16		3				**1**	**Red, HN.** **Red-eye** in F_2 not proved
MM. LXIX A.	17	Dilute pigment	–					

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* Not examined at birth and therefore not to be accepted as

III.

INCUBATOR CONDITIONS.

FROM INSIDE MATING OF ♀.

	F_1				F_2				
Stock No.	Black.	BN.	HN.	Colour variation.	Black.	RB.	BN.	Red.	
MM. I	47	1 (sp.)	2	31 sl. reddish	158	1*			7 with sl. reddish tinge ; 7 thin retic.
MM. III	37	–	–	23 sl. reddish ; 1 thin retic.	330	–	1	–	
MM. VI	50	–	–	1 sl. reddish	110				
MM. X	39			All sl. reddish ; 7 thin retic.	82	–	1	–	4 sl. reddish.
MM. XV	12	–	–	10 sl. reddish	17	–	–	–	
MM. XVII	2	–	–		–				
MM. XX	38	1 (sp.)	2	and 1 irregular	36	–	–	–	No Lilac.
	–	–	–						
MM. XXVII	10	–	–		29	–	–	–	
MM. XXIX	12			5 sl. reddish	–				
MM. XXXI	8			All sl. reddish	37	–	–	–	7 sl. reddish.
MM. XXXII	7			4 sl. reddish	7	–			
	–								
	–								
MM. L	41	–	1	3→almost BN.	216	11*			1→RB., and 1 very thin retic.
MM. LVII	75			1 irreg., 1→thin retic. and 1 sl. reddish	102				2 ♀♀→sl. reddish and gave **Nowhite-Red** in F_3.
MM. LXII	4				73	–	2	–	4 thin retic.
MM. LXVII	8				5	–	–	–	
MM. LXVIII	–								
MM. LXIX	11				355	–	–	**15**.	**Red-eye** in F_2 (given by one pair, 42 : 15) = reappearance of r_2 gene.

the "birth-colour." All the RB. mated gave Black offspring.

the proof (p. 400), and we have set out the results of this experiment in Tables II and III and the paper which precedes this summary (**54**, pp. 319–356); except for a letter to *Nature* (**38**, pp. 201–202) recording the appearance of certain new recessives, none of the detail of this work has been published till now.

Details of the M. Experiment. Dredgings were taken on October 6 and 19, 1931, and 280 animals were set out, 70 pairs in the heat, and 70 pairs in laboratory conditions. They were all black-eyed, many with a slight reddish tinge, or dilute pigment, and many more with the white reticulation noticeably thin (see p. 398). From the October 19 dredging, for example, 25 out of the 38 pairs set out in laboratory conditions had thin reticulation, both males and females.

The mated pairs which were taken for the work, were those of which the females were ovigerous, i.e. females which had mated previously in the wild and laid eggs fertilised by other mates before pairing with their present mates. These broods, when extruded, were kept for comparison with the broods from the new matings, as we wished to ascertain if any distinction would appear between the young from eggs laid in the wild, and hatched indoors (the "*Outside*" mating), and the young from eggs laid, as well as hatched, indoors (the "*Inside*" mating). This was the first time that such a comparison had been made, but as will be seen in the above table, no distinction could be drawn between them.

The results obtained were unexpected. The highest number of variations, nine, appeared in the offspring from the Inside mating, laboratory conditions, and the lowest number, one, in the Inside mating, Incubator conditions.

The details are :—

LABORATORY CONDITIONS, *Outside* mating, three variations, viz. in the F_2, 1 Red-eye, a reappearance of the r_2 gene; 1 Nowhite, apparently genotypic: in the F_3, 1 Red-eye new ?. *Inside* mating F_2, 3 Red-eyes, reappearances of the r_2 gene; 1 reappearance of White-body; 1 new mutation, the Clotted-eye; and 4 Nowhites, one the Gradual Nowhite, two Phenotypic, and one not proved which appeared only in the crosses made with the stock.

INCUBATOR CONDITIONS, *Outside* mating, five variations, viz. 3 Red-eyes, 1 a reappearance of the r_5 gene, 2 not proved; 2 new mutations, the Nowhite-Red, and the Lilac-eye. *Inside* mating, only one, a Red-eye, a reappearance of the r_2 gene.

The variations fall into three classes, viz.: I. Changes in body-colour; II. Changes in the retinal colour of the eyes; and III. Changes in the white pigment of the eyes.

I. CHANGES IN BODY-COLOUR.

The White-body recessive reappeared in Stock *XXIV* (Inside laboratory) and was proved, by crossing, to be genetically the same as that in Stock II (p. 374).

In the F_1, 125 Black were hatched, but only 13 survived to maturity, and of these one brood alone (*M. 307*) gave healthy offspring.

An F_2 of 182 was produced, divided roughly into green-body Black-eyed, and white-body Black-eyed in the proportion of 3 : 1. Unfortunately, exact figures cannot be given for the body-colours at birth, owing to the difficulty of distinguishing them in the semi-transparent, 1 mm.-long young, and to the great mortality amongst them. The demarcation was clear in the older ones, and as many matings as possible were made, both in the stock, and with Main Stock II White-body.

To give one example: a white-bodied, Black-eyed female (*M. 537 b.*) was mated with a green-bodied male of her own stock, and gave 44 Changeling Blacks, i.e. white-bodied at birth, but developing the full green colour later: next with a white-bodied male of her own stock and gave two broods, 51 white-bodied young: and finally, mated with a Quite White male of Stock II (white-bodied, white-eyed), she had a brood of 19 white-bodied young.

II. CHANGES IN THE RETINAL EYE-COLOUR.

a. Reappearance of the r_2 gene.

The reappearance of the r_2 gene of the Stock II red-eye occurred in no less than 5 of the new stocks, viz.: *M. XVI A., XVI, XL, LII* and *MM. LXIX.*

M. XVI A, Outside, laboratory, and *M. XVI*, Inside, laboratory, may be considered together, their constitution being the same. The Red-eye factor in both was evidently brought in by the female parent, and appeared in the F_2 generation in the simple mendelian ratio 3 : 1. The male parent, *M. XVI*, was tested with two F_2 Red females (giving 24 Black with one, and 9 Black with the other) and proved to be homozygous for Black.

These stocks belong to the (*b*) class of Stock II Red, "unstable eye-colour" (p. 373) and show the great variation in the colour of the red-eye, with a range from cream, through all shades of primrose, rose-pink, pale red, bright red, cherry-red, to dark red and purplish. The fluctuation in colour in some individuals was very marked, cream on hatching developing to full red, and others beginning as red and fading to cream. That these practically White-eyed animals were true Reds was known by their matings, their offspring being hatched bright full red.

An example may be given: a female, hatched bright red, but with the colour fading, was mated with the parent male and gave 9 Black young: then she was put with a Red male, also paling. In both the colour faded to almost white, with only the faintest tinge of pink in two or three ommatidia, exactly like a Flushed White (except that the body was very dark green). They mated, and had a brood of 18 bright Red-eyes. These young, later, also paled.

The *M. XVI A.* brood consisted of 20 Black young of which 19 survived, 2 males and 17 females. Owing to the scarcity of males, it was not possible to test all the females conclusively, but the constitution of 10 animals was definitely proved. Five homozygous, 1 male and 4 females, and 5 heterozygous, 1 male and 4 females.

The homozygous male was mated with 9 females (two known to be homozygous and one heterozygous), and gave an F_2 of 73 Blacks.

The heterozygous male was put with 8 females, and gave an F_2—in mixed broods, 44 Black and 7 bright Red; with 2 heterozygous females (one gave 17 B., 3 R. and the other 33 B. and 19 R.; and a brood between them of 10 B., 4 R.), a total of 60 Black and 26 Reds; with a third heterozygous female, 16 Black and 5 Red; and with two proved homozygous females, 16 Black and 18 Black respectively. The total for the F_2 generation was 261 Blacks to 38 Reds.

Crosses were made between *XVI* and *XVI A.* to prove their identity, and in addition they were tested nine times with stocks carrying the r_2 gene, viz.: 2 matings with Main *Stock II*, 1 with *M. XL*, 1 with *M. LII*, 1 with *MM. LXIX* (stable eye-colour), 1 with *dd. LXXIII*, 2 with *D. LIII* and 1 with *K. XXXV A.* All the shades of Red were tried in these matings, and in every case the young were hatched bright red (193 in all). Two matings with heterozygous Black are given under *M. LII.* Six crosses were made with other mutant Stock Reds, 1 with Stock I Red, 2 with *MMB. LVII* Nowhite-Red and 3 with *M. XV A.*, one being Primrose × Primrose, and gave all Blacks (151).

M. XL. Inside mating, in varying laboratory conditions. This was a very small stock, carrying the r_2 gene, (*b*) division of Stock II, unstable eye-colour.

Only 5 F_1 survived out of 120 hatched. They gave two broods, 7 Black, and 6 Black and 2 Red pale in colour.

One of the latter reached maturity, a male, and was used for testing. It was first tried with a Red female from *MM. LXIX* of the (*a*) division, stable eye-colour; 25 young were hatched with bright red eyes, which *remained unchanged;* then with a Nowhite-Red female, Stock *LVII*, with which it had Black-eyed young. By that time its eyes had faded to almost White, so it was mated with a female of the same type (i.e. Red→ Flushed White) from *M. XVI A.*, and gave 24 young with bright Red eyes.

Only one pair of the Blacks of the same brood had offspring, viz.: 3 broods of 6 Black, 5 of them very spotted, and 3 Half-Nowhite also; 4 Black, all spotted, and 2 of them Half-Nowhite; and 6 Black, 2 spotted. This result was interesting as showing that the white pigment as well as the coloured was affected in this mutating stock. Eleven out of the 16 animals were very spotted, in 5 of these the eye on one side was Nowhite, and in each case the Left eye.

M. LII. Inside mating, in varying laboratory conditions—another small stock belonging to the (*b*) division Stock II Red.

The Black-eyed F_1 numbered 137, but only the last brood survived and intermated in the brood-bowl. One F_2 brood was produced, of which 3 Black males, 1 Black female and 1 Pale Red male came to maturity. Matings in the bowl gave one F_3 brood, consisting of 5 Black and 2 Dark Primrose, i.e. Reds with so little colour that only the faintest tinge of primrose could be seen in the centres of the eyes. These two died without offspring, but one F_4 brood was given by the Blacks, viz. 2 Jet-black and 1 Dark Red—and with these the main stock came to an end.

Three males, 2 Black and the Pale Red, were left of the F_2 and crosses were made with these to prove the identity of this stock with Stock II.

The Pale Red male was tested with four different females:

MC. I. First with a female from *M. XVI A*, known to carry the r_2 factor, with eyes the same dilute shade of red; the young produced numbered 33, all with full bright red pigment.

MC. II. with a Red female from *MM. LXIX* (the (*a*) division, stable colour); offspring 13 bright Red.

MC. XII. with a Quite White female of Main Stock II; 89 Changeling Whites were hatched, 86 normal-eyed (13 spotted), 1 White-Nowhite spotted, and 2 White Half-Nowhites spotted; and finally with a Nowhite-Red female of the *LVII* Stock. This mating produced 42 Black young (4 spotted).

One of the surviving F_2 Black males was mated with a Red female from the *XVI A.* strain (r_2), and gave 58 Blacks and 64 Reds in four broods. The difference in the colour of the Reds on hatching was noticeable.

In the first brood, 2 Red were hatched, rather pale red. They matured, male and female, and gave a brood of 10 young with pale pink eyes, much paler than the parents.

The second brood consisted of 9 Reds, of a full clear colour. These came to maturity with the bright red pigment unchanged.

The third brood gave 19 Reds, of which 8 survived. In them all the colour had faded almost completely, leaving only a faint orange shade in the centres, like the Flushed White type.

In the fourth brood 34 Reds were hatched all pale in colour, but none survived.

The second surviving F_2 Black male was crossed with a Red female of *M. XVI* (r_2 factor), in which the colour faded gradually until the eyes became almost white with only a pinkish-purple flush in the centres. Four broods were produced consisting of 73 Black (11 spotted) and 61 Red (13 spotted).

In the 9 Reds of the first brood the colour lessened as they grew; the 19 Reds (12 spd.) of the second brood were hatched full clear red and remained unchanged; the 18 (1 spd.) of the third brood were Dark Primrose at birth, but the colour increased to pale red; and in the fourth the 15 were hatched pale pink, unspotted.

The second brood of this cross gave unusual figures, showing a great variation in the white pigment from Spots, "excess," to Nowhite, its complete absence. The numbers at birth were: as to coloured pigment, 15 Black to 19 Red; as to presence of white pigment in the normal white reticulation, to complete or partial absence, 25 : 9; and as to Spotted compared with unspotted, 20 : 14. They were divided as follows: 12 Black normal-eyed (5 spotted); 1 spotted BN.; 1 spotted Black HN. with irregular Nowhite right eye; 1 spotted Black, with the right eye almost Nowhite; 13 bright Red normal-eyed (6 spd.); 4 spotted RN., two also irregular-eyed; and 2 spotted Red HN. Unfortunately none survived to breed, and no information could be obtained as to inheritance.

It is known that the deposition of the white pigment is totally inhibited in the Genotypic Nowhites, but these Spotted Nowhites would appear to be in a different category. Judging from external evidence only, it looks as if the white pigment might have been deposited in normal quantity and released or set free from the cells in which it was formed, by some tension or pressure of the covering cuticle. We know that the white pigment, unlike the coloured granular retinular pigment, is fluid and flows easily together (cf. Clotted-eye, p. 399) into "spots" or "curd-like masses"; and that these spots frequently change position and shape at the moults; that they are found apart from the eye in certain definite positions (**6**, p. 353); and that the spots on the Spotted One-sided Nowhites are always very large on the nowhite side.

MM. LXIX. Inside mating, Incubator conditions. Only one F_1 brood was produced in the "Inside" stock of which eleven came to maturity, seven males and four females. Ten matings were made using different males and females, the offspring totalling 355 Black and 15 Red; 42 Black and the 15 Red came from one pair.

The Red was tested and found to belong to the (*a*) division of Stock II Reds, normal stable bright red colour. This type has persisted all through the generations, and is still to be seen in the F_8.

Crosses have been made with four other stocks carrying the r_2 factor, viz. *M. LII*, *M. XVI A.*, *M. XL* and the "*Flesh-red*," with all of which Red-eyed offspring were produced. Tested with Reds from Stock V (r_5) and *MM. XV A.* (r_5); *M. XV A.*; *MM. XX A.* the "Lilac" Stock; and *MM. LVII A.* Nowhite-Red, Black-eyed young were given in each case.

b. Reappearance of the r_5 gene.

The reappearance of the r_5 gene, showing the same wide range of colour and fluctuation of colour in the individual, as in Stock V, occurred as follows :—

MM. XV A. Incubator conditions, "Outside" mating of ♀ *MM. XV.* The brood consisted of 11 Black, of which 10 survived to maturity, 7 males and 3 females.

The 3 females were paired, with the following results :—

Pair 1 had 3 broods of 8 Black; 1 Black, slight reddish tinge; and a brood not examined till six weeks old when 7 survivors were found, 4 Black and 3 Dark Intermediate Reds. Only 3 of the first brood survived to maturity; Black male and female, and Black male→Reddish Purple →Int. Red. This male was mated with a Black female from the *Pair 2* mating. They had 44 Black offspring which, mated *inter se*, gave in the F_3 97 Black, 5 Deep Red and 17 Intermediate Red. Ten of these 17 became mature, with changes of eye-colour from Red to Dark Reddish Purple; bred together, their offspring numbered 54, viz. 4 Reddish Purple, 13 Intermediate Red and 37 Light Int. Red. One of the females an Int. Red→Red was mated with a Stock II Red and a Stock V Dark Reddish Purple; with the first male she had 38 Black young, and with the second she gave 12 Dark Red (2 HN. spotted).

Pair 2 gave 80 Black in 8 broods—the female was then tried with the heterozygous male of *Pair 1*, and proved homozygous, giving 20 Blacks. Presumably male *2* was also homozygous, the 47 F_3 offspring of chance matings in the bowl, and the 35 F_4 were all Black-eyed.

Pair 3 had no offspring.

c. Description of Three other Red-eyes.

Three other Red-eyes which appeared in this experiment will now be described. Unfortunately the numbers were small and could not be sufficiently tested.

Details are as follows :—

M. XV A. Laboratory conditions, from the female's Outside mating.

The brood numbered 38 Black, but very few came to maturity. Interbreeding in the bowl gave an F_2 of 45 Blacks (in very small broods, 3 : 1 : 3 : 2 : 4 : 3 : 6 : 17 ; and 6). Three animals reached maturity in the seventh brood, 2 males and I female, all heterozygous Black ; mated together an F_3 was produced of 79 Black (17 spd.) and 22 Red (3 spd.) (with one male, 27 B. (2 spd.)) and 2 Red ; with the second male 36 B. (11 spd.) and 14 Red (3 spd.) ; and " mixed," not known which male, 16 B. (4 spd.) and 6 Red.

Of the 22 Red F_3, 15 were bright red at birth and 7 pale red, but in all the colour lightened to a greater or less degree, e.g. in 4 of the bright Reds the eyes became almost White with only 2 or 3 flushed purple ommatidia in the centres. Of the Pale Reds, 1 lightened and 4 others remained unchanged. All died without offspring, except two " Pale Reds " which mated and gave 7 Pale Red young.

The change in the intensity of colour was reminiscent of Stock II, but crosses made with this stock and also with Stock V showed that neither the r_2 nor the r_5 gene was present.

Eleven crosses were made : one with a Quite White female of *Stock II* gave 24 Black Changelings ; three with *M. XVI* (r_2), Pale Red × Pale Red, and Primrose × Primrose, gave all Black ; as also did two crosses with *MM. LXIX* (r_2) and one with *Stock V* Red (r_5). A cross with *Stock I* failed.

The Inside mating of the female *M. XV* produced 106 in the F_1, but only 4 of these had any offspring, all Black. One F_1 male was crossed with a Red female *M. XVI A.* (r_2) and gave 32 Black (5 spd.) ; and one F_2 female was tried with a heterozygous F_2 male of *M. XV A.* and gave 20 Black (6 spd). No others survived to mate.

MM. LXVII A. Incubator conditions, " Outside " mating. Of the 16 Black of the brood, 14 survived, 6 males and 8 females. In none of these at maturity was the eye-colour of the normal jet-black intensity—4, 2 males and 2 females, showed a reddish tinge, one male more so than the others.

42 Blacks and 7 Reds were produced in the F_2 generation. The Reds consisted of 1 Intermediate Red, from chance matings in the bowl containing the 2 " reddish " Black males ; and 6 Dark Red in a brood of 18 given by a Black pair.

This was unfortunately one of those mutating stocks which die out rapidly owing to the difficulties in breeding them, either through feebleness, e.g. the Int. Red which became a female, but did not survive to mate, or through infertility, e.g. a Black female which mated and had eggs eight times, but hatched none ; or through cannibalism.

It was impossible to test the Reds, for only two survived, both females.

In one, the Inter. Red, the colour darkened slightly with growth, but not as far as the " dark red " shade. In the other, the only survivor of the 6 Dark Reds, an interesting development was noticed in the eye-colour. The Dark Red tone of the central and oldest ommatidia persisted, but all the later ommatidia as they formed on the periphery at the growth-stages were a clear pale orange-red, with no trace of the dark pigment. When preserved the orange-red dissolved out, leaving those ommatidia colourless, but the central ones showed a dark deposit.

This female was mated with a Nowhite-Red of Stock *LVII*, and was eaten by her mate after extruding a brood of 7 Black.

MM. LXVIII. Incubator conditions, Outside mating. This is another of the unsatisfactory small stocks referred to above.

The F_1 brood consisted of 16 Black-eyed, but the matings in the brood-bowls produced only 2 F_2 broods, 2 Black; and 2 Black and 1 Red Half-Nowhite.

Three survived, Black male and female, and the Red, a female. The male mated with the Black female, and had 30 Black young; and with the Red female, 3 Black. None of the young lived to maturity.

d. Appearance of Two New Mutant Characters.

There also appeared two quite new recessive types, the **Lilac**-eye, Stock *XX*, and the **Nowhite-Red**, Stock *LVII* (**38**, p. 27).

STOCK XX. LILAC.

The Lilac mutation arose in the Incubator conditions from the " Outside " mating of female *MM. XX* and evidently derived from the Outside male parent. The female herself was homozygous for Black, as shown by the fact that only Black-eyed were produced in the four generations resulting from her mating with the male *XX* (" Inside " mating).

F_1 generation.

The brood of 18 normal Black-eyed young hatched on October 10, 1931, five days after being placed in the incubator. Sixteen survived, 7 males and 9 females. Mated together as far as it was possible, to test them, 1 ♂ and 1 ♀ (*Pair 6*) proved heterozygous for the new mutation Lilac-eye.

The results of the matings are as follows :—

	F_2		F_3		F_4	
Pair No.	Black.	Lilac.	Black.	Lilac.	Black.	Lilac.
Pair 1	36	—	14	—	—	—
Pair 2 hetero ♂ 6, ♀ 2	14	—	76	4	113	23
Pair 3	10	—	—	—	—	—
Pair 4	11 (1 BN.)	—	30 (1 HN.)	—	—	—
Pair 5	130 (1 HN.)	—	87 (1 BN.)	—	51	—
Pair 6 hetero ♂, ♀	190	66	189 (1 BN., 1 HN.)	170 (5 LN.)	104	56
Pair 7	5	—	—	—	—	—
Pair 8, ♂ 3, ♀ 7	38	—	9	—	3	—
Pair 10, ♂ 10, hetero ♀ 6	20	—	—	—	—	—
Pair 11, ♂ 5, ♀ 3	19	—	—	—	—	—
Pair 12=hetero ♂ 6, ♀ 5	120 (2 HN.)	—	—	—	—	—
Pair 13, ♂ 10, ♀ 5	23	—	—	—	—	—
Mixed Matings	61 (2 HN. sp.)	—	98 (1 HN., 1 irreg.)	—	23	—

This stock was an exceedingly difficult one to work with : the individual broods were usually of small numbers, many of the young died before maturity, and matings among the survivors were frequently infertile even with the normal-eyed animals. With regard to this infertility, a curious occurrence was noted in later generations (F_3 and F_4). Females, sometimes of the same brood, all with the normal body- and eye-colour, and gonads of the usual blackish green tone, gave different coloured eggs. Some laid the normal dark green fertile eggs, while others had their pouches full of opaque *white eggs*, very noticeable in life, the pearly whiteness of the egg mass showing up in startling contrast with the green body colour. Altogether 26 batches of white eggs were produced, but no young were ever hatched.

The details are as follows :—

In the F_2 one B. ♀→BN. gave 1 batch of white-eggs.

One F_2 pair (♂ 20×♀ 4·2) mated 7 times, five times with no results and twice with white-eggs.

A second F_2 pair (♂ 20×♀ 20) had 4 normal broods, but in the next generation one F_3 pair gave 6 white-egg broods.

A third F_2 pair (♂ 20·2×♀ 4·1) had 4 broods of normal eggs, but the white-eggs appeared in the next generation : one F_3 ♀ mated with 3 different males of her own brood, with one she had 7 broods of white-eggs, with the other two males she mated and separated without eggs. In another F_3 mating, two females and one male were left in the bowls, and 10 batches of white-eggs were laid.

Instability of the white pigment was another characteristic of the stock, ranging from excess of pigment, such as heavy reticulation and "spotting" to the deficiency or absence shown in partial or complete Nowhites.

In the F_2, besides the cases of "thin reticulation," 6 instances were found, 1 complete Black Nowhite, and 5 Half-Nowhites (2 spotted). The BN. became a female, and, mated with a Black male of its own brood, gave 29 Black and 1 Half-Nowhite; none survived. None of the HN. lived to maturity, but from the *Pair 5* brood in which the HN. appeared, 1 BN. was produced in the F_3, and 2 more BN. in the F_5. These, a male and female, had 4 offspring, which, when examined about a month after birth, were found to be 3 BN. with rather dilute pigment and 1 Black almost Nowhite, but with flecks of white reticulation.

From this result it would appear that the Nowhite is not the genotypic, but belongs to the class which we have named "phenotypic Nowhite" (see p. 367).

The instability of the white pigment was very marked in the Lilacs; in the later generations the white was sometimes lacking altogether, sometimes present only as streaks or flecks instead of the normal even reticulation.

The **Lilac-eyes**. The first of these appeared in the second, third and fourth broods of *Pair 6* and were on hatching a pale clear lilac colour with heavy white reticulation. The female parent was tried with another male for about six weeks, and then put back with male 6. In the broods following it was noted that the clear definite lilac tint of the three previous broods had changed or intensified in some way and that the tone on hatching was now more reddish and more clouded, an indefinable shade which we called "**Reddish Lilac**." This, however, was not the permanent condition as was soon made manifest in both the coloured and the white pigments, and to illustrate the full extent of the change and the rapidity with which it was accomplished, an instance may be taken from the development of the Lilacs in the seventh brood.

In three weeks after hatching the white reticulation had vanished, and the coloured pigment had cleared from the centres of the ommatidia and risen to form as a dense rim round the circumference of each, so that the eye looked like rings of dark pigment enclosing circles of a warm cream colour. It had the remarkable appearance of a dark brownish black reticulation on a light ground, instead of the usual white reticulation on a dark ground. This is the typical "**Creamy Lilac**" form, found in all the F_2 survivors.

Further changes in the Lilac colour were observed in the F_3 generation. The eyes of the F_3 young at birth looked exactly the same shade of lilac as those of the newly hatched F_2, but as they grew a difference soon became evident. The two forms, Reddish and Creamy, instead of merging one into the other, segregated out sharply into two distinct types, one, the *Creamy Lilac* as described above, and the other a *Reddish Lilac* in which the lilac colour remains in the retinular cells but develops and retains a much redder tone. The white reticulation in this type is not completely lost, usually some flecks or streaks of white persist, especially towards the periphery of the eye.

The **Lilac Nowhites**, also, first arose in the F_3. They are quite distinct from, and darker in colour than the Lilacs, a very dark plum colour, so concentrated as to look almost Black.

Details of the matings in the different colour groups will be found in Appendix II, p. 409.

STOCK LVII. NOWHITE-REDS.

This is another remarkable stock and the only one of the type found in the whole course of the work. In it, the recessive, Nowhite-Red, has the two mutant characters—red pigment instead of black, and no white-pigment instead of the normal white reticulation—always combined, and never segregating out one from the other.

The Red is a vivid bright cherry red, with all the retinular cells densely pigmented. It is distinct from the Reds of all our other stocks, and the Nowhite too is different. It seems that the factor which inhibits the appearance of the white only operates when combined with the *red* of this mutation. Mated with the genotypic Nowhites of other stocks, the normal white reticulation appears in the young. We have called this the " **Nowhite-Red** " to distinguish it from the other types of Nowhite.

The Stock arose from the " Outside " mating of the female *LVII*. The eggs were orange when brought in and were extruded next day in the incubator—12 Black-eyed young being produced.

Five survived to maturity, 2 males and 3 females, but only two can be said to have been thoroughly tested, the male and female of *Pair 1*—which gave 33 Blacks and 9 Nowhite-Reds. *Pair 2* had only 4 Black young; and the third female mated with male 1, had one brood of 8 Black. A brood was found just extruded by female 1 in the brood bowl before the pairs were separated, 4 Black and 3 Nowhite-Reds.

As will be seen, the Blacks and Nowhite-Reds appeared in the 3 : 1 ratio, and this has been maintained through the generations till now, when the F_6 are maturing.

Of the 49 Black and 12 Nowhite-Red in the F_2, only 13 survived to mate; 2 males and 8 females of the Black, and 1 male and 2 females of the Nowhite-Reds.

The results of the F_2 matings were as follows :—

Black×*Black*	Pair 1.	47 B.
	Pair 2.	9 B, 3 NR.
	Pair 4.	13 B, 7 NR.
and Mixed matings		21 B.
		48 B, 5 NR.
Nowhite Red×*Nowhite-Red*		94 NR.

The survivors of the 138 Black, 9 males and 13 females, and of the 109 Nowhite-Reds, 37 males and 33 females, produced a large F_3, the colours appearing according to expectation in the offspring.

Eighteen crosses were made between the Nowhite-Reds of this and the following generations, and the Reds and Red Nowhites of other stocks, viz. 3 with stocks carrying the r_1 gene ; 9 with others carrying the r_2 gene ; 2 with Stock V Intermediate Red, and Bright Red (39 young) ; 1 with a Red Nowhite male from a cross $r_1 \times r_5$ (22 young) ; 2 with the Lilac-eye ; and 1 with the Dark Red of *MM. LXVII A.* (7 young). The Nowhite-Red was proved to be definitely distinct from them all ; the offspring without exception were normal-eyed, with black pigment in the retinular cells and the white accessory pigment in an even reticulation over the eyes. The mortality was very high, and most of them died without breeding, but the results in those which reached the F_2 were as follows :—

With stock carrying the r_1 gene ; 3 crosses of Red Nowhite males of Stock I with Nowhite-Red females of *LVII* gave 30 normal-eyed Black young. The only survivor, a male (*M.C. 548*), was first mated back to a Main stock *LVII* Nowhite-Red female, and gave an F_1 of 24 Black ; 25 R.B. ; and* 47 Red nowhites.

Two matings of the F_1 *Blacks* gave 12 B., 3 RN. ; and 14 B., 3 RN. respectively. Matings of the *RB* gave 5 B., 1 Red ; 29 B., 3 RN. ; 22 B., 4 Red, 8 RN. ; and 6 B. *RN* by *RN* mixed matings were 22 RN. and 1 Red almost HN. ; 7 RN. ; and 13 RN. ; whilst two matings of *B*×*RN*, and *RN*×*B*, gave 43 B., 25 Red, 31 RN. ; and 25 B., 16 RN., respectively.

The same male *M.C. 548* was then crossed back to a Stock I Red Nowhite female, and produced an F_1 of 9 B. ; 3 BN. ; 5 Red ; and 6 RN.

With stocks carrying the r_2 gene ; 9 crosses, including two with Main Stock II white-body white-eggs, one cross with *XL*, one with *LII*, one with *S. XIV*, two with *LXIX*, one with *XVI A*, and one with *XVI*. In all, 249 black-eyed young were produced. The 13 offspring of the last cross (with *XVI*) mated *inter se* gave 50 B. ; 22 Red ; and 23 RN.

With the Lilac Stock ; 2 crosses, one of which with three matings gave only 4 young, all normal-eyed Black, and all spotted. Of these a male

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* It is impossible to distinguish at birth between the Red Nowhites of the two stocks.

and two females reached maturity and gave in 4 broods, 8 Black, 2 Dark Red, and 4 Red nowhites (from one female, 4 B., 1 DR.; and 1 B., 3 RN.: from the other 1 B.; and 2 B. (1 spd.), 1 DR., and 1 RN.).

III. CHANGES IN THE INTEROMMATIDIAL WHITE PIGMENT.

At the time of the commencement of the Gammarus work, the eye-pigments, coloured and white, were characterised by stability and only very rarely was anything noted as out of the normal. In 1928 (H. expt.) this statement no longer held good, and by 1931, the date of the M. experiment, the character of the wild population had completely changed in this respect. There was much instability, and reduction of the pigments throughout the stock, particularly in the interommatidial white. More animals were found with thin reticulation (i.e. with the white lines so faint and thread-like as to appear almost nowhite) than with the normal amount in the eyes.

In fact all the variations, genetic and non-genetic, that have yet appeared in the distribution of the white accessory pigment were seen in this experiment, some for the first time. The range was from *excess* of the white, " heavy reticulation " to *deficiency* of the pigment, " thin reticulation "; and from the evenly spaced distinct white reticulation, the *normal*, to its complete absence, the *Nowhite*. (See Table II, *M. VIII*, *IX*, *XL* and *MM. XX*, *A.*)

The *Gradual Nowhite* appeared in several stocks with its characteristic feature of the steady reduction of the white pigment to its complete loss, a " Nowhite " form indistinguishable at sight from the genotypic Nowhite, but functioning as a normal (**48**, p. 693). An example may be given from *M I* stock—an F_2 BN. ♂ of this type mated with a B. normal ♀ which had gradually lost nearly all the white pigment. The offspring produced were 5 Black, normal reticulation; 5 Black, very thin retic.; and 2 BN. When they were examined two months later, it was found that of the 5 B., " very thin retic.," three had become completely Nowhite, one almost nowhite, and one half-nowhite; whilst the " 2 BN." had developed flecks of white.

To another variation, apparent for the first time in the work, we have given the name of " *Phenotypic* " Nowhite, in distinction from the " Genotypic." The two forms cannot be separated by appearance either at birth, or at any period of their lives, but the white factor is present in the one, the phenotypic, and absent in the other, the genotypic, and consequently when mated together, only normal-eyed young are produced.

The Nowhite of the Nowhite-Reds, *Stock LVII* (where the black and the white pigments are both inhibited) is also phenotypic.

Examples may also be seen in *M. III*; *M. IX*; *MM. XX A.* and others. In *M. III*, out of an F_2 of 564, 18 were phenotypic BN. (one spotted and irregular) and 9 HN. (two spotted and irregular). A mating BN×BN gave 8 BN. Another of BN×HN produced 22 Black; 6 BN.; and 1 HN. One of the BN., a female, was tested with a genotypic BN. male, and gave Black normal-eyes.

In the F_2 of *M. IX*, there were 5 phenotypic BN. (one irregular) and 2 HN. (one irregular). From a mating BN×BN, 7 BN. were hatched; and one of the BN. tested with a genotypic nowhite gave 5 Black normal-eyed young.

The most remarkable of the new developments was the *Clotted-eye* which arose in the F_2 of the *M. V* stock (**48**, p. 695–698, Fig. 1, *b* and *c*). The young were all hatched with perfectly normal eyes, but only a few remained normal. The irregularity showed itself soon after birth, but the rate of progression was very rapid. At first the eye looked as if the pigments had liquefied, and run together forming white patches above, and black patches below. Later on, the white pigment collected in clotted masses, with an occasional ommatidium showing—while in the final stages the white pigment increased so much in volume as to obscure the outline of the margin and the ommatidial cones completely. Sometimes the shape of the ommateum was broken and in consequence it became irregular and flattened out, sometimes it was unaffected under the bulging masses of white, as could be seen when the white pigment was dissolved out later. In extreme cases all the cells, retinular as well as interommatidial, appeared to have burst, the white pigment rising to the surface in clotted lumps, and the coloured sinking to the bottom, and collecting in little pools or patches of colour deep down.

Taking all the evidence into consideration, from this stock and from the reappearance of the form in the D. experiment and in later dredgings from the wild (p. 402), we came to the conclusion that the irregularity was in all probability caused by a thinness or weakness of the cell-walls, which sooner or later break down and liberate the cell-contents.

THE 1932 K. EXPERIMENT.

A dredging was brought in on Sept. 12, 1932, and several pairs were set out, but owing to adverse conditions the experiment was given up and is only mentioned here because two of the pairs gave some red-eyed offspring in the F_2 generation from their Outside matings.

One pair, *K. XXV*, 3 days after being brought in to the laboratory, extruded a brood of 12 Blacks. Intermatings in the brood bowl (7♂ and 3 ♀) gave 9 Black (2 spotted and 1 HN. spd.); 2 Dark Reds, and 3 Bright Reds. None survived.

Another pair, *K. XXXV*, extruded 7 days after capture a brood numbering 9 Blacks. One male and seven females reached maturity, and gave offspring as follows :—8 Black ; 8 Black and 1 Red ; 6 Black and 1 Red ; and 7 Black, 1 RB. and 3 Red.

In this stock, the eye-colour of the recessives was a full bright clear red, stable through life. Proof that it was indeed another appearance or the r_2 gene was obtained by crossing one of the F_3 females with a Red ♂ of *M. XVI* (r_2) when 18 young were hatched all with bright red eyes. These young also, intermating, produced similar red-eyed offspring.

THE 1933 D. EXPERIMENT.

The 1931 M. Experiment to test the wild stock for heterozygosity having failed through an outbreak of disease, another experiment on similar lines was started in February and March 1933 (**54**).

Half the animals were kept in an incubator at an air-temperature of 21° C., and half in an outside shed in or as near natural conditions as possible. As in the M. Experiment, the young hatched from eggs laid and fertilised in the wild, " Outside mating," were kept for comparison with the young from eggs laid and fertilised in the indoor conditions, " Inside mating."

Natural Conditions.

I. *Effects of extreme cold.*

The temperature of the shed in which the Gammarus were kept ranged from 25·8° C. to −1·5° C. For the first time the effect of continued low temperatures on the developing embryo was noted, as causing a delay or retardation of the rate of deposition of the black pigment. In many cases the action was completely inhibited till after extrusion, the young hatching out with bright red eyes, but in all the instances recorded the eyes darkened to black, usually before the first moult took place.

Another unexpected result of extreme cold during the embryonic growth was dwarfing, or retardation of body-development. The animals affected took months to reach maturity, and only a few gave offspring, partly owing to the difficulty of finding suitable mates, but partly also to the exhaustion of oviposition. The females were exceedingly small, and although the eggs were only one or two in number, yet they were normal-sized ; the young also which hatched from them were the usual size and developed normally.

II. *Changes in the interommatidial and retinal pigments.*

The variations in the white pigment included in addition to the numerous sporadic cases of partial or complete Nowhites, the two definite

Nowhite types, Genotypic and Phenotypic. Gradual Nowhites, with the steady reduction of white pigment from normal to Nowhite, also occurred, as well as Gradual Normals, with the steady increase of white from Nowhite to normal. But perhaps the most interesting observation was the effect of the low temperature on the embryonic eyes. The laying-down of the white occurs normally in the later stages of development, but during the extreme cold the rate of its deposition was slowed down to such an extent that the young were born with no trace of white in their eyes, the same effect as with the melanic pigment. In the same manner the rate accelerates after extrusion so that the deficiency is made good, in a longer or shorter time, according to the temperature.

Changes in the *retinal pigment*. The r_2 gene reappeared in four stocks, *D. XXXI*, *XLVI*, *LIII*, and *LXIV*.

In *XXXI* and *LXIV* it was carried by the female and segregated out in the F_2 of both Outside and Inside matings ; in *XLVI*, also in the F_2, probably carried by the male.

In *LIII A.*, for the first time in the work, Red-eyed recessives appeared *in the F_1 from the wild*, proving both parents to have been heterozygous for Red. The female was mated with 3 other males, homozygous for Black, and the recessives appeared in the F_2 from all these matings.

Three other stocks gave Reds, *D. VIII*, *XVIII*, and *XXIX* ; the first was shown to be distinct from Stocks I and II ; the others died out untested.

A new variant type, *D. X*, arose, which bears a great resemblance to the Lilac Stock *XX* (p. 393) ; in the eye-colour of the recessives, the two types, Creamy and Reddish Lilacs, being represented ; in the pale pigmentation of the gonads and eggs ; and in the variations of the white pigment from excess to complete absence. This stock is still breeding, but the recessives are so few and so delicate that no knowledge of its genetic position has yet been obtained.

INCUBATOR CONDITIONS.

I. *Effect of high temperature.*

The incubator was kept at approximately 21° C., but while the Gammarus will live and breed at this temperature for a time it is evidently too high, the stocks tending to die out within a generation or two.

The time of incubation from oviposition to extrusion from the pouch was practically constant for the F_2 as well as for the F_1, 9 days being the usual period. The time taken in development, however, was surprisingly long, as long in fact as in the " Natural Conditions " part of the experiment. The F_2 broods were small and feeble, with few survivors, but when, later, the remnants of the heterozygous stocks were removed to laboratory temperature, they recovered strength and became established.

II. *Changes in the interommatidial and retinal pigments.*

Changes in the interommatidial pigment were very frequent, with the tendency always towards reduction of the white pigment. Nowhites, Half Nowhites and Spotted occurred in many stocks, and Clotted-eyes were found.

The changes in the retinal pigment numbered 4, all reappearances of the r_2 gene in *dd. XXXIX*, *XLIII*, *LXX* and *LXXIII*.

One of these stocks, *dd. LXXIII*, is noteworthy as being the second instance of a recessive appearing *in the* F_1 *from the wild*. Out of 43 F_1 young hatched, one had the right eye bright red and the left eye black. It functioned as a heterozygous Black, giving 102 Black to 34 Red when mated with heterozygous Black females, and 23 Black to 41 Red in three matings with F_2 Red females.

FIRST APPEARANCE OF CLOTTED-EYES IN THE WILD

The Clotted-eyes arose in Stock V of the M. Experiment (p. 399). The character proved heritable, and still is, in the Laboratory cultures. There seems, however, to be another form of this variation, indistinguishable from the type in appearance, but genetically different. Similar instances have been recorded from time to time ; for example, the heritable White-eye of Stock II compared with the non-heritable White-eye of Stock I (**4**, p. 287) ; the two forms of Nowhite, Genotypic and Phenotypic ; and now this case of the heritable and non-heritable Clotted-eye.

The dredging in which the latter type was first found in numbers was brought in from the wild on January 15, 1935. Out of a total of 301, 220 were normal-eyed and 81 clotted. A second dredging taken on February 12 gave 189 normal to 35 clotted.

The F_1 and F_2 generations from matings of normal-eye × clotted, and clotted × clotted, consisted of normal-eyed young only. Experiments are still being carried on, but so far no sign of the clotted variation has shown itself in any of the offspring.

FIRST APPEARANCE OF RED-EYE RECESSIVE IN THE WILD

Though heterozygosity had now been definitely proved to exist in the wild population, yet all the known homozygous recessive animals came from cultures in the laboratory : none had yet been taken in the wild.

In 1935, however, the final proof was obtained, and a Red-eyed recessive was brought in from the ditches (**47**, p. 836).

It became a male, and was found to carry the r_2 gene (stable colour). Mated with a Red female from Main Stock II it gave a brood of 9 Red. It was next tried with a Red Nowhite female of Stock I, the result being 20 Black young. Mated again with a female from *MM. LXIX* Stock (r_2 stable colour) it gave 14 R.

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* Printer's error on page 202, line 24. The line should read: "white II ♀ mated with red ♂ gave white young."

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APPENDIX I.

Professor Huxley in *Problems of Relative Growth*, 1932, p. 40, referring to the work on the Moulting and Growth-stages of Gammarus, 1924, says: "Sexton states that sexual maturity occurs at the seventh instar, that proportions continue to change for two further instars in the male, one in the female, but after this no further proportion-changes occur (though the males at least may increase about 40 per cent in length). That this statement is not accurate is shown by Kunkel and Robertson, whose graphs demonstrate a change in the proportions of several organs up to the largest sizes found."

A reference to the paper mentioned (**12**, pp. 352 and 387) will show that what I actually stated was that sexual maturity was reached at the eighth growth-stage; that the differentiation of the characters (not "proportion-changes") stopped there in the female, but continued in the male for two more growth-stages before the "definitive adult male" form was reached; that sexual maturity was attained at about half-growth and that thereafter the characters now assumed by both sexes were preserved unchanged through the rest of their lives, except for the increase in size till the maximum growth of the species was reached. And finally that there is a fixed definitive form, and a definite maximum size.

Professor Kunkel (**22**, p. 657) says of his work that "it is fully realised that the method of studying growth of an organism by measuring a population, as has been done in the present study, is open to certain objections from which the study of successive ecdyses of the same individual is free." I agree with him, especially when the method is applied to an animal like *G. chevreuxi* in which growth is accomplished in a series of definite stages, with each stage essentially constant in its characters, and differing in the relative proportions of the parts from the other stages.

The great objection, of course, as he points out, to studying the question by means of the successive ecdyses of one animal is the difficulty of obtaining complete series through the "tendency of the animal to eat its own skin very shortly after shedding it," added to which is the impossibility of mounting the fragile sloughed-off moults without distortion. On the other hand, the results of the second method, measuring a population collected at random cannot be considered free of error. Besides the different line of development of the male and female at sexual maturity, and the variation in the proportions of the different parts during that period, there are other difficulties likely to confuse the issue. Take, e.g., the secondary sexual characters the antennæ, the first and second gnathopods, the first peræopod, and the third uropod, which are used in specific distinction. Certain of these, such as the antennal flagella, and the rami of the third uropods in particular are very liable to injury, and are frequently broken, and are then replaced by regeneration, but though the regenerated part is eventually restored to normal size the process is a gradual one and requires time to bring it into line with the rest of the organism.

An instance of how misleading this can prove in an argument may be quoted from Professor Kunkel's paper (p. 658).

He says that "in general sexual differences could not be determined at as early a stage as Mrs. Sexton has shown in the careful drawings of successive moults of the same individual. The characteristic curved hairs of the second antenna of the male do not appear generally as early as she has figured them," and then he cites the measurements of the smallest individuals whose sex he could be sure of, "the flagellum of the first antenna in one case had only ten segments which would correspond with Mrs. Sexton's fourth stage"—whereas the true explanation is, that if a sexually mature individual had only ten joints in the flagellum it would mean, *not* that it was at the fourth growth-stage, but that at some previous stage the flagellum had been broken and was regenerating, but had not yet caught up to the other parts—that it had in fact only arrived at the fourth stage, with several stages still to go before reaching normality.

My paper was mainly concerned with figuring and describing the development of male and female from birth to the attainment of sexual maturity at half growth, and though measurements were taken of the older stages, they were not included. If, however, the aim had been to study the relative growth of certain parts through life an equally accurate but much easier method could have been employed. By preserving one or two specimens at each stage a complete developmental series could have been formed, from birth to sexual maturity, from maturity to maximum growth and then to maximum length of life. The measurements of such a developmental series of male and female apply to all males and females of the species. The striking point which emerged from the work on the moults was the *constancy* of each stage of development; size might vary according to the temperature during growth, but the relative proportions of the body and appendages at each stage remained unchanged.

The two series of drawings referred to (ten of the male, and eight of the female), while taken as far as possible from two individuals, are in fact representative of a very large number of animals. Each moult, as it was cast, was compared detail by detail with the same stage in all the series kept under daily observation. Three thousand moults were examined in this way, and drawings made, of which over three hundred are still in the laboratory, in addition to those figured in the paper.

E. W. S.

APPENDIX II.

DETAILS OF THE DIFFERENT MATINGS IN THE LILAC STOCK *XX*, 1931 M. EXPT., (see p. 396).

The **Lilacs** were all derived from two heterozygous animals, the male and female of *Pair 6*. The male was mated also with two homozygous females, with one of which he gave 120 Black (2 Half-Nowhite), no survivors, and with the other, 14 Black, from which the Lilac reappeared in the F_3 (p. 394).

Details of the Pair 6 mating. The offspring of this pair consisted of Black and Lilac in a 3 : 1 ratio, viz. 190 Black to 66 Lilac. The first brood was 3 Black. The next three consisted of Blacks and Lilacs, 7 B. to 4 L., 8 B. to 2 L., and 4* B. to 3 L. The 9 Lilacs were characterised by pale clear lilac colour and heavy white reticulation. None of them survived to maturity, but it was noted that the amount of white pigment lessened with growth.

Male 6 having been put with 2 females to test them for heterozygosity, female 6 was mated with another male, and had 20 Black-eyed young, all of which died immature.

After an interval of six weeks, the pair were put together again, and had 10 more broods, 168 Blacks and 57 Lilacs (viz. *6 B. to 1 L., *12 B. to 7 L., *13 B. to 7 L., 12 B. to 5 L., *16 B. to 5 L., 16 B. to 3 L., 17 B. to 4 L., 30 B. to 7 L., 20 B. to 9 L. and 26 B. to 9 L.).

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* Broods examined directly after extrusion; the others within two or three days.

The different F_2 *matings* and their offspring are as follows :

Group 1. Black × Black which gave all Black offspring. There were 12 matings in this group, 6 mixed matings in the brood-bowls and 6 of separate pairs. The numbers in the broods were small, viz. 11, 1, 9, 16, 2, 2, 2, 14, 4, 8, 19, 1 and 4, 7, 8, 6, 12, 5. The last 6 broods came from one pair, the eggs were normal dark green in 3 broods, but in the 3 following the colour was a pale eau-de-nil shade.

Group 2. Black × Black matings which gave Black and Lilac offspring. Mixed matings from three brood-bowls, 28 Black to 6 Lilac. From *174 a bowl*, *3 Black and 1 Lilac (*314*†) ; two of the Black survived and mated. The female laid one batch of the *white-eggs* mentioned above, but none hatched. Reduction of the white pigment took place in both, in the female as far as the complete nowhite condition. The Lilac became a ♀ and was mated but with no results.

From *236 a bowl*, 11 Black and 1 Lilac (*289*). The Blacks in mixed matings gave 11 Black (*352*), which in the next generation produced 28 Black (13 spotted), none of which survived.

The Lilac →♂ was tried with 1 Lilac, and 1 Black female, with no results ; with a second B. ♀ it had 26 Black (3 spd.), none survived.

From *299 a bowl*, 14 Black and 4 Lilac (*426*). None survived.

Group 3. Black × Black matings which gave Black, Lilac and Nowhites.

From *174 a bowl*, 9 Black normal, 1 Black Nowhite, 1 Black Half-Nowhite, 4 Lilac and 1 Lilac Nowhite spotted (*395*). This is the first record of the Dark Lilac Nowhites, only 5 of which were found in the stock, this one and the 4 mentioned below (*see 414*). It was left in the bowl with the Lilacs ; later, 1 young Lilac was found (*517*).

Group 4. Black × Lilac matings which gave Black and Lilac. Two matings.

From one pair (*194 a and b*) Black ♂ × Lilac ♀, 7 Black and 3 Lilac (*396*).

From another (*194 b. 2*) Lilac ♂ × Black ♀, 2 Black and 2 Lilac (*430*). None survived.

Group 5. Lilac × Lilac matings which gave Lilac and Lilac Nowhite. There were 6 mixed matings in the different brood-bowls, 5 giving 103 Lilac, and 1 giving 19 Lilac and 4 Lilac Nowhite, and 2 matings of separate pairs, giving 28 Lilac young.

In these matings, the distinction between Creamy and Reddish Lilacs was first noted.

The details of the F_3 *broods* are as follows, with the colour of the survivors and their offspring if any. Numerous matings were made with no results.

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* See footnote on p. 409.

† The brood numbers italicised and in brackets refer to the records of the stock kept in the Laboratory.

From *194 b. bowl.* 35 Lilac (8 spotted) (*328*). Only 2 survived to maturity, both females, one the typical Creamy Lilac, the other, Reddish Lilac. No results.

From *221 b. bowl* there were several broods and one from a separate pair. The first brood consisted of 6 Lilac (*281*); three survivors, 2 males and 1 female, all the Creamy Lilac colour. No results. The second brood of 18 Lilac (*325*); the 5 survivors were 1 female Creamy Lilac, 2 males and 2 females Reddish Lilac. The mixed matings in the brood-bowl gave an interesting result: 25 young were found of various sizes, consisting of 11 Creamy (3 spd.) and 14 Reddish (7 spd.). The third brood, 14 Lilac (*338*), had no survivors. The fourth brood, 2 males, spotted, mature when found (*530*), one, Creamy Lilac, with a little of the white reticulation still remaining, and one very Reddish Lilac. No results.

From *221, Pair 1.* Eleven Lilac (4 spd.) (*326*); 4 survivors, all the Creamy Lilac shade. Later, in one of these, a male, the Creamy tint became slightly flushed with pink.

From *236 b. bowl* 14 Lilac (3 spd.) (*312*); ten survivors, Creamy Lilac (one later developed the pink flush). Two females were mated with the Lilac Nowhite ♂ *414 b.* (see below), and had 2 broods, 3 Lilac (1 spd.) (*522*); and 8 Lilac (4 spd.) (*523*.) One brood, mixed mating, 2 Lilac spd. (*378*).

From *253 Pair 3.* 17 Lilac (*348*) Dead.

From *266 b. bowl.* 7 Lilac (*340*) Dead.

From *317 b. bowl.* 7 Lilac (*440*) Dead.

Group 6. Lilac × Lilac mating which gave Lilac and Dark Lilac Nowhite.

From *236 b* ♂ × *282 b* (3♀♀) 19 Lilac and 4 Lilac Nowhite (*414*). Survivors were—Lilacs, 3 males (2 spd.); 2 females spotted; and 7 nearly mature (4 spd.) all still lilac; two others, males, had changed colour, one spotted was now a Dark Lilac Nowhite, and the other Dark Lilac Half-Nowhite. One brood was hatched from mixed matings, of 14 Lilac (4 spd.) (*481*) from which a mating was made with Stock *XX* (Inside mating), p. 413.

Two of the four Lilac Nowhites survived, but one was eaten by its mate; the other was mated with two Lilac females and gave offspring. (See above, under *236 b*).

F_4 *Matings.*

From *Group 3* Black×Black giving Black, Lilac and Nowhite. One brood (*517*) of 1 Lilac→♀ Reddish Lilac with flecks of white reticulation. Eaten by mate.

From *Group 5* Lilac×Lilac giving Lilac. Two broods (*507*) and (*559*) 11 Creamy Lilac (3 spd.) and 14 Reddish Lilac (7 spd.) of various sizes from mixed matings of Creamy × Reddish. They were divided into 5 bowls according to size and colour, as follows :—(*a*) 5 larger Creamy Lilac→three males and two females. All developed a slight "pink flush" later. Mixed matings gave one brood (*545*) of 3 Lilac with colour rather redder than usual. (One survived for a short time→Creamy Lilac with a pink flush and some white reticulation.) In (*b*) bowl, 5 smaller Creamy Lilac, with the pink flush. Two survived, ♂ and ♀, both with reddish flush and some white reticulation. No results. In (*c*) bowl, 9 larger Reddish Lilac→6 males and 3 females. Mixed matings gave 4 Reddish Lilac (*564*) very small (three showing white reticulation, and the fourth with hardly any white, but with the dark rings around the ommatidia). Later, the one survivor, a spotted ♂, still Reddish Lilac, was tried with three females. No results : females eaten. In (*d*) bowl, 5 smaller Reddish Lilac. One survivor, a female, still Reddish Lilac with more white than usual. No results. One brood in (*e*) bowl (*559*), 1 Creamy Lilac→♂. No results.

From the same *Group 5* 1 brood (*378*) of 2 Lilac spotted was hatched. One survivor ♀, Creamy Lilac shade. No results.

And 2 broods, from Lilac females × Lilac Nowhite ♂ (*414*), viz. (*522*) of 3 Lilac (1 spd.)→Reddish Lilac in four to five weeks ; and (*523*) 8 Lilac (4 spd.). All died.

From *Group 6* Lilac × Lilac giving Lilac and Lilac Nowhite. One brood from mixed matings of the Lilacs, 14 Lilacs (4 spd.) (*481*). From this brood a Creamy Lilac ♂ was taken for a mating with Stock XX (i.e. the stock from ♀ XX's Inside mating) which had given nothing but Blacks (see p. 413).

Details of the Pair 2 Mating. This was a mating of the heterozygous ♂ 6 with the homozygous ♀ 2 which gave an F_2 of 14 Black.

The F_3 consisted of 76 Black and 4 Lilac—viz, 6 broods of Black, 11 : 4 : 12 : 4 : 8 : 6 and 4 broods from one pair, of 31 Black and 4 Lilac.

Only 2 of the Lilac survived, ♂ and ♀ Creamy Lilac. No results ; the male ate this female, and two Black ones with which it was being tested.

In the F_4 generation mixed matings gave 53 Black and 10 Lilac, including 2 Dark Lilac ; and matings of 3 pairs gave 60 Black and 13 Lilac.

The Lilac-eyes in the descendants of *Pair 2* were of exactly the same types as in those of *Pair 6*.

Crosses made with Stock I Red Nowhite, Stock II Red and Stock *LVII* Nowhite-Red :—

With Stock I. A Creamy Lilac F_4 ♂ with pink flush was mated with a Red Nowhite ♀ of Stock I, and gave one brood of 7 Black (3 spd.). These, mated *inter se*, gave 10 young in the next generation, viz. 3 Black and 1 Black Nowhite, 2 Reddish Black, 3 Red and 1 Lilac of the Reddish Lilac type with more white reticulation than usual.

The ten were mated together as much as possible, and produced an F_3 as follows :—

Black ♂ by Black ♀ had 7 Black (3 spd.) and 7 BN. offspring ; the same Black ♂ mated with the BN. ♀ gave 12 Black and 12 BN. ; one Red ♂ mated with this same BN. ♀ and produced 36 young (of various sizes when examined), 3 Black, 11 BN., 5 Dark Reddish Lilac Nowhites, 3 Dark Red and 14 RN.

The 2 Reddish Blacks became Black with only a slight reddish tinge and gave Black and Red in the proportion of 3 : 1.

The 1 Reddish Lilac became a ♀ and mated with one of the Red males and had a brood (*604*) of which only 2 survived, 1 B. spd.→♂ and 1 Red spd. (not a clear red, but more of the Inter. Red shade)→♀.

The (*604*) ♂ and ♀ mated and 4 broods were produced, viz. 2 Black (1 spd.) ; 2 Black (1 spd.) and 2 RN. ; 3 Black (1 spd.), 1 Dark Reddish Lilac and 2 Red spotted ; 1 Black, 3 BN. and 3 Red (survivor→Int. Red).

It is noteworthy that the Creamy Lilacs have not yet appeared in this cross stock.

With Stock II Red. A Creamy Lilac F_4 ♂ was mated with a Red ♀ F_3 *MM. LXIX* Stock (r_2 gene) and had 46 Black offspring (4 spd.). None survived.

With Stock LVII Nowhite-Red. A Creamy Lilac F_4 ♂ was mated with two Nowhite-Red F_4 Stock *LVII*. They mated several times but only one brood was produced by each female, viz. 1 Black spotted, died ; and 4 Black, all spotted.

The 3 Black survivors were a male, and 2 females which mated, one ♀ giving 5 Black, 1 Dark Red and 3 nowhite Red young ; the other, 3 Black (1 spd.), 1 Dark Red and 1 nowhite Red, all of which died before maturity was reached.

Cross between the XX Stocks. " Outside " and " Inside." A cross was made with a Creamy Lilac ♂, F_4 of the Outside Stock, mated with an F_3 Black ♀ from the Inside Stock (of the same parent female *XX*), which had given only Black-eyed normals and an occasional Black Nowhite.

One brood was hatched, consisting of 13 Black (2 spd.). These interbred, but from their matings only 7 young were produced, 6 Black (2 spd.) and 1 BN., a number too small to be conclusive.

The next generation, F_3, consisted of 16 Black and 4 Reddish Black, two of these almost dark red in colour.

The F_3 Blacks were mated together, giving only the normal black-eyed in the F_4, but in the F_5 the Lilac appeared, one brood consisting of 4 Black and 3 of the " Reddish-Lilac " type with partial white reticulation. These died without offspring.

The F_3 Reddish Blacks became darker, i.e. black with a slight tinge of red. All survived, two males and two females, and gave a large F_4

containing a great variety of colour, Black, Reddish Black, Dark Red, Dark Red Nowhite, Dull Red, Lilac Nowhite, Reddish Lilac, Intermediate Red, and deep Red, but no Creamy Lilac appeared in this, or in the following generations as far as the work has gone.

The changes in colour in the F_4 and F_5 generations covered an equally wide range :—**B**. remained unchanged ; B→slightly reddish ; B→DR ; B. lightened to Int. Red ;

RB unchanged ; RB→B ; RB→R. Purple→Purple Black ;

DR unchanged ; DR→P.B→B ; DR→R.P.→P.B. ; DR→Dark Lilac : DR→Reddish Lilac with white reticulation :

DR Nowhite→Dark Lilac Nowhite :

Dull Red→Reddish Lilac :

Reddish Lilac→some with broken white reticulation, some with perfect reticulation, and others, a new combination, with the white reticulation but with the dark rings also, round the ommatidial cones, characteristic of the Creamy type in the Main Stock :

Int. Red→Reddish Lilac ; and the **Deep Red**→Reddish Lilac.