Experiments on the Mendelian Inheritance of Eye-colour in the Amphipod *Gammarus chevreuxi*.

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With Plate I.

CONTENTS.

THE Amphipod which was used in the following experiments was described in 1913 (*Journ. M.B.A.*, Vol. IX, pp. 542–545) under the name of *Gammarus chevreuxi*, and its life history was then worked out (see Sexton and Matthews, *l.c.*, pp. 546–556).

The usual colour of the eye in this species, as in the other species of the genus *Gammarus*, is black, but several females and young with red eyes were observed while the above work was in progress. Beyond recording

the fact, however (*l.c.*, pp. 543 and 552), no special attention was given to the matter.

The descendants of two original pairs taken in June, 1912, were kept under observation for moults, etc., in the Laboratory until August, 1913, when the present writers undertook to investigate the variation in eye-colour, with a view to determining first if it were a sex-limited character, and secondly, if it conformed to the Mendelian law of inheritance.

In the course of these investigations we have received constant assistance and advice from the Director, Dr. E. J. Allen, F.R.S., at whose suggestion they were first undertaken, and we wish here to acknowledge our great indebtedness to him.

GENERAL CONDITIONS.

Before entering into the detailed results of the experiments it will be necessary to give a brief description of the habits of the species as well as of the conditions under which the animals were kept in the Laboratory.

Gammarus chevreuxi is an ideal species for experimental work. It is very hardy, quickly reaches maturity, and breeds all the year round. The young are extruded from the marsupial pouch and another batch of eggs laid generally within 24 hours of the time of hatching. During the summer season a brood takes from 12 to 14 days to hatch, and the period of sexual activity is reached at the age of 36 days; in winter in natural conditions a brood takes 30 days to hatch, and does not become sexually mature for at least 3 months, low temperature, as would be expected, retarding development. In the Laboratory, however, which is heated in the winter, there is practically no difference in the seasons, and it therefore becomes possible to obtain several generations in the year.

It may be well to state here that in this species of *Gammarus* the female never lays eggs unless a male is present, and also that it is absolutely impossible for a male to fertilise two broods of eggs with one deposition of sperm. The male generally takes the female a few days before the eggs hatch, and carries it until the young are extruded from the pouch. The female then moults, assisted by the male, as described in the paper referred to above (*l.c.*, p. 550). The aperture of the oviduct is opened by the removal of the old cuticle, and the male deposits the sperm in the pouch around it, but unless the eggs are laid within a few hours they cannot be laid at all. The cuticle hardens rapidly, and a plug of the glutinous lubricatory matter which accompanies moulting and oviposition closes the aperture and hardens to the consistency of the cuticle, effectually

blocking the oviduct, until another moult takes place and the plug is sloughed with the old cuticle.

Another point must be mentioned in regard to the suitability of this species for laboratory work, and that is the ease with which it adapts itself to artificial conditions. This is probably due to the fact that it comes from brackish water ditches where it is habituated to great variations of temperature, salinity, pressure due to depth of water, etc. The density, for example, varies to an extraordinary degree according to the season, tides, excessive rainfall or drought, ranging from 1 to 1·028.

In the experimental work, it has been necessary to keep the water as nearly as possible at the same salinity, as any sudden change of conditions always affects the animals' growth and breeding. A mixture of one part of sea-water to six parts of fresh water gives the same density (1·004) as that found in the ditches when the animals were taken, and such water we have generally used.

The best results have been obtained by keeping the animals in finger-bowls, generally one pair in a bowl. Each bowl contained about 200 c.cs. of water, and was covered with a glass plate to check evaporation and exclude dust. No aerating apparatus was used, the animals obtaining sufficient air for their needs from the surface of the water exposed in the bowls. In the same amount of water but with a smaller surface exposed to the air they did not flourish at all, as was found later when using jam jars and honey jars for the broods ; only a very small proportion of the young reached maturity.

For food dry leaves of all kinds were used, after they had been allowed to rot in water. It was found that the animals preferred the soft tissues of the leaves of elm, hazel, and sycamore, rather than the harder leaves of oak, beech, etc. A fine delicate Ulva from the ditches they ate freely, but when the supply failed and the harder marine variety (*Ulva latissima*) was given, they did not eat it until it macerated. They flourish better and are much healthier with some of the mud from the ditches in the bowls, but in all these experiments we were obliged to keep the water clear, in order to watch the animals without disturbing them unnecessarily. The young are so minute—about 1 mm. in length when hatched—that they completely escape observation in the mud, clinging as they do to any particles of dirt or weed.

The bowls were kept in ordinary diffused light, strong sunlight being avoided.

A word of explanation is necessary as to the terms employed in this paper. The Black eye-colour is dominant to the Red, and therefore Red is referred to as Recessive (R.). Black divides into Pure (P.) and Impure, but instead of the term " impure " the word Hybrid (H.) is used.

THE PIGMENTATION OF THE EYE.

The structure of the eye of *Gammarus* has been well described and figured by Parker (" The Compound Eyes in Crustaceans," *Bull. Mus. Comp. Zool.*, Harvard, Vol. XXI, Plate I), the species investigated by him being the *Gammarus ornatus* of Milne-Edwards (=*Gammarus locusta*, Linn., Stebbing, *Das Tierreich*, V. 21, p. 476).

Sections of the eye of *Gammarus chevreuxi* show precisely the same internal structure. The eye in this species is reniform in the adult, oval in the young animal, much raised and rounded. The superficial aspect presents a reticulation of opaque white pigment, with the ommatidia appearing as coloured spots, black or red, in the spaces of the network.

The black pigment of the retinular cells of the ommatidia of the *Black* eye appears to be produced by a combination of black and red, even in the so-called " Pure Black " animals (Fig. 1), with a larger admixture of the red in the " Hybrid Blacks " (Figs. 2 and 4).

The pigment of the *Red* eye is pure red, with no alloy of the black (Figs. 3 and 5).

Sometimes the retinular cells are unpigmented and the white reticulation shows up in a very striking way, giving the effect of chalk-white eyes — the " *All-white* " eye referred to in the paper (Figs. 8, 9, and 10).

Occasionally again, some of the ommatidia are pigmented and some not ; this variation is called the " *Part-white* " eye (Fig. 7).

The white opaque pigment is subject to great variation, sometimes showing as faint thread-like lines, sometimes broken up and irregular, sometimes present in excess, obscuring the ommatidia, and sometimes it is entirely lacking, the " *No-white* " eye (Fig. 6). Animals are often found with one or both eyes affected. The defect can be transmitted by normal-eyed animals to both black and red eyed offspring.

THE FIRST APPEARANCE OF THE RED EYES.

Two pairs of *Gammarus chevreuxi* were taken in June, 1912, those referred to in the previous paper as Pair I and Pair II. All four animals were black-eyed.

All the broods from Pair II were black-eyed. This stock (which is referred to as the " Pure Black " stock) has been kept under observation from June, 1912, till now, December, 1915, fresh black-eyed material from the ditches being added from time to time. Not a single red-eyed animal has appeared in it. The strain was tested by mating also, to make absolutely certain of its purity before using it in the experiments.

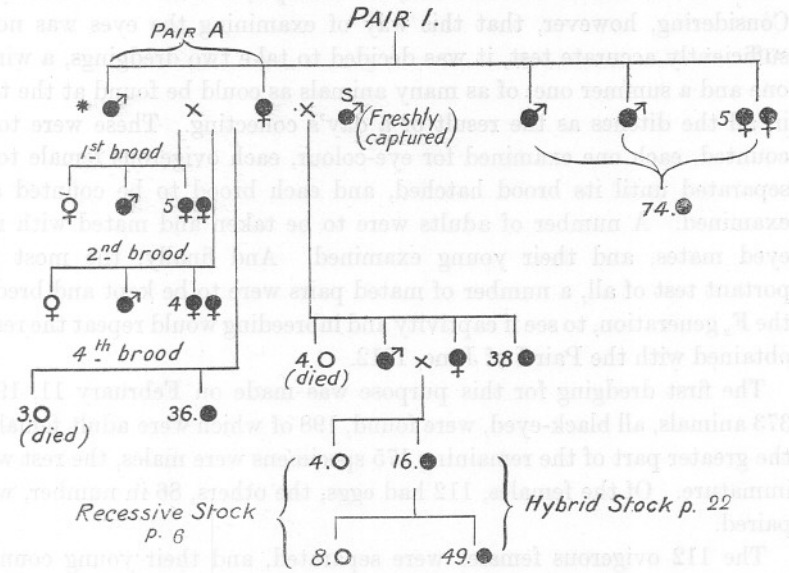
The first brood from Pair I were all black-eyed: 9 young ones coming to maturity, 3 males and 6 females. The first pair to mate (Pair A) were evidently the strongest, and had the largest broods, although owing to unfavourable conditions only a few in each brood survived. The first brood from this Pair A numbered 18; the young were counted on hatching, but not examined for eye-colour; of these 1 male and 6 females came to maturity, one of the females with red eyes. The second brood was 28 in number, 1 male and 5 females surviving, and again one female had red eyes. For the third mating of the female A a different male was used (male S), one which was freshly captured. The ensuing brood was extruded on October 26, 1912, 44 in number, and as these were being separated into finger-bowls for observation of moults 4 red-eyed ones were found. Male A was then put back with the female A, for the fourth mating; the brood numbered 39, 3 with red eyes.

It will be seen that this female, mated with two different black-eyed males, produced some red-eyed young in each brood. No red eyes were observed in any of the other offspring of Pair I nor in their progeny.

The four red-eyed young of the third brood died before reaching maturity, and the remainder of the brood were kept separate, each in a finger-bowl, until they were 66 days old. On December 31 they were put together in a bell-jar, and some mated at once. The first female to mate of this brood was separated from the others, and from her and her offspring all the red-eyed stock has descended.

By August 7, 1913, this female and its mate were dead, and only 20 young were found in the jar, 16 black-eyed and 4 red-eyed. The four were removed, and the black-eyed left together for three months longer; when again examined on November 5 the numbers were 65 black-eyed and 8 red, and with these work was commenced.

The following scheme shows the origin of this red-eyed stock, clear circles indicating the red-eyed, black circles the black-eyed animals :—



* The male of Pair A mated with two of the other females of the same brood ; the resulting young numbered 27, all black-eyed.

It cannot, of course, be stated as an absolute fact that red-eyed specimens never occur in the ditches in natural conditions, but so far not a single one has been found, although thousands of specimens brought in at different seasons of the year have been examined. The red strain has only shown itself in the one female, female A and her progeny. It seemed possible at first that the red-eyed strain could be accounted for on Mendelian lines. If the original Pair I had been a Pure Black mated with a Hybrid Black all the offspring would have been black-eyed, half the number Pure Blacks and half Hybrid Blacks ; and if in their matings a Hybrid should mate with a Hybrid the red-eye strain should have appeared. But, as far as can be seen, only the female A had the red strain ; both her mates, male A and male S, when mated with other females (some from the same brood as female A and some from other stocks) had only black-eyed offspring, and, moreover, in none of the other members of the brood nor in their offspring has the red strain appeared. It might have been that male A was a Hybrid Black and that female A was the only Hybrid Black female in the brood, but it seems pushing coincidence too far to suggest that male S captured some months later and taken at random from a large dredging should be a Hybrid and

the only Hybrid in it. None of the others captured then or at any other time have shown the red strain.

One series of 5000 was counted, for example, and all were black-eyed. Considering, however, that this way of examining the eyes was not a sufficiently accurate test, it was decided to take two dredgings, a winter one and a summer one, of as many animals as could be found at the time in all the ditches as the result of a day's collecting. These were to be counted, each one examined for eye-colour, each ovigerous female to be separated until its brood hatched, and each brood to be counted and examined. A number of adults were to be taken and mated with red-eyed mates, and their young examined. And finally, the most important test of all, a number of mated pairs were to be kept and bred to the F_2 generation, to see if captivity and inbreeding would repeat the result obtained with the Pair I of June, 1912.

The first dredging for this purpose was made on February 11, 1915. 373 animals, all black-eyed, were found, 198 of which were adult females; the greater part of the remaining 175 specimens were males, the rest were immature. Of the females, 112 had eggs, the others, 86 in number, were paired.

The 112 ovigerous females were separated, and their young counted on extrusion. The broods in many cases were small, many of the animals having apparently not long reached maturity; e.g. the first broods to hatch were in number as follows: 11, 3, 15, 4, 9, 11. In all, the number of young extruded was 641, all of them black-eyed.

Forty-six adults were mated with Red mates; all produced black-eyed offspring, proving beyond doubt that they were Pure Black and not Hybrid. The number of broods counted was 62, the number of young 853.

Twelve of the 86 black-eyed pairs brought in from the ditches were taken to breed to the second generation of offspring. Of these Pairs II to XII * were kept for two or three broods each, and then returned to the rest of the dredging. The total number of young in the first generation of offspring from these broods was 473, all black-eyed.

For the second generation, one brood from each of the Pairs II to XII was taken, the first to mature in each case. This was done because the red eyes appeared in the first brood of the first F_1 pair of the original stock (p. 22).

When these broods reached maturity some of the mated pairs were separated and the others allowed to mate in the brood-bowl, the young in all cases being removed as soon as possible and examined for eye-colour.

* Pair I produced no young, the female throwing off the eggs; the male was then paired with three other females, two Black from the dredging, and one Red from the old stock; all laid eggs, but no young were hatched, and the male died.

215 young were counted, all black-eyed ; some of these have commenced breeding.

This generation is very interesting from the fact that in it, as in the same generation of the original stock, a deviation from the normal occurred. In the case of the original stock the black pigment was absent, and the result was a red eye with the superficial network of opaque white pigment unaltered. In this second case the black pigment was present in every instance, but in the broods of Pair V the white pigment was affected in greater or less degree. The female of this pair had less white than is usual in the eye—the reticulation was perfect, but the lines of white were very thin and thread-like. The eyes of the young in the first generation were the same, but in the young from the first pair of these that mated there was considerable variation. One brood of 13 contained 2 young with " no-white " eyes on both sides (Fig. 6), 5 others with the white reticulation very faintly marked, and 6 with eyes like the male parent and female grandparent. This brood is being kept separate to see if the defect follows the Mendelian lines of inheritance of characters.

No individuals of the third generation from these pairs have been hatched yet (Nov. 19, 1915), but 60 which have been examined from the General Stock bowl all have normal black eyes.

The summer dredging was taken on July 6, 1915. 372 living animals were examined, all black-eyed. A good many more were brought in, but did not survive overnight, owing to the heat and overcrowding of the pots and consequent fouling of the water.

Twenty-two adults were mated with Red mates ; 31 broods were counted, containing 348 young, all black-eyed.

Thirteen black-eyed pairs of those paired in the ditches were separated from the others, and placed in finger-bowls to breed to the second generation. By November 19, 1915, there were 127 of the first generation and one brood, 5 in number, of the second generation, all with black eyes and all normal except the offspring of one pair. In this bowl, four of the first generation were left, two females with normal eyes, and two from a younger brood, one with normal eyes, and the other with a white patch on each eye at the upper end caused by three or four of the ommatidia being unpigmented, the " part-white " eye (cf. Fig. 7 for an example of this in the Recessives). This is the first occurrence recorded in the course of the work of a variation appearing in the *first* generation from animals brought in from the ditches.*

* Only one specimen has been recorded from freshly captured animals, a male, with the left eye affected.

EXPERIMENTAL WORK.

The first question to be decided was whether the red eye-colour was a sex-limited character or not—the only adult specimens previously observed having been females.

In order to settle this point the 8 red-eyed young found on November 5 (p. 22) were placed in a bowl by themselves to come to maturity. It is impossible to distinguish males from females until the animals reach sexual maturity, which occurs when they are about half-grown, the males being then easily distinguishable by the fine coiled hairs of the lower antennæ, and by the larger gnathopods. The 4 red-eyed young of August 7 were kept in the Laboratory, only two coming to maturity, both female. The 8 red-eyed young of November 5 were kept in another room, not heated, with a temperature ranging from 4°–10° C., and were in consequence much slower in maturing; but in three months' time both males and females were seen—thus settling the question of the red eye-colour being a sex-limited character. In February three pairs mated. These were kept separate, each pair in a finger-bowl to itself, and the others, which were females, with the two August 7 females, were paired with males from the " Pure Black " stock. Males and females paired were also taken from this stock, and thus we had Recessive mated with Recessive (R.×R.), Pure Black mated with Recessive (P.×R.), and Pure Black mated with Pure Black (P.×P.). We started daily observations and records on this generation, calling it the Parent Generation, and counting from it the F_1, F_2, etc.

Our aim now was to discover if the Mendelian laws of inheritance of characters were applicable to the results of these crosses, and the experiments to this end will be given in detail under the different divisions —I Recessives and II Dominants.

I. THE RECESSIVES.

We commenced work in February, 1914, with the three pairs just referred to, adding in May seven pairs taken from the 42 red-eyed animals hatched since November 5, 1913, in the Hybrid Stock (see p. 22). Each pair and its offspring have been kept separate, the broods on hatching removed from the parents' finger-bowl and examined for eye-colour, each brood being numbered and set aside to come to maturity. In every case in which both parents were red-eyed all the offspring have been red-eyed.

The red-eyed animals appear to be more delicate than the black-eyed, shorter-lived, and less fertile. They are quite as large and as active, and

in many cases observed, reached maturity *before* the black-eyed in the same brood. Yet, if left to breed together with no admixture of the black-eyed strain, they gradually diminish in numbers, throwing off the eggs sometimes soon after deposition, or dying after having had only one or two broods. Seven of the ten stocks have failed in this way.

The results of the Experiments with the *Parent Generation* are given below in detail, and are typical of all the experiments with inbreeding Recessives. They are as follows :—

Exp. 1. Two broods, 10 and 12 respectively ; female died.
,, 2. Mated, but no eggs laid.
,, 3. One brood ; only 1 young hatched.
,, 4. One brood of 20 young.
,, 5. First brood, only 1 young hatched ; second brood, 3 young. Thinking the small numbers might be due to some defect or unhealthiness in this male, it was taken away and another added. Third brood, only 1 young hatched. Male again changed. Fourth brood, 14 young; fifth brood, eggs thrown off before hatching. Male again changed. Sixth brood, 17 young.
,, 6. One brood, 19 young. A second brood was laid, but the female died before the eggs were hatched.
,, 7. Mated, eggs laid, but thrown off before hatching, probably unfertilised.
,, 8. Mated, eggs thrown off as in Exp. 7.
,, 9. One brood, 10 young. A second brood was laid, but the eggs were thrown off before hatching.
,, 10. Mated, eggs thrown off as in Exps. 7 and 8.
,, 11. Mated, eggs thrown off as in Exps. 7 and 8.

The total results for six months for the Parent Generation were : One pair mated, no eggs laid ; seven broods not hatched ; eleven broods hatched, the young numbering in all 108 ; average per brood 9·8. Only about half of these survived to maturity.

In the next generation, the F_1, a rather different system was followed. In some cases records of separate pairs were kept, in other cases the whole brood was left together in a finger-bowl, each female removed after oviposition, kept separate until the eggs were hatched, and then returned to the brood-bowl to mate again.

The total results in the twelve months from July, 1914, to July, 1915, for the breeding of the F_1 generation are : 2 pairs mated with no results ; 80 broods from the other pairs, 20 of these not hatched, 60 hatched,

numbering 422 young (these are the F_2 generation), average 7 per brood, a smaller average than in the preceding generation.

In the *F_2 generation* the same system was followed as in the F_1, the ovigerous females being removed from the brood-bowl till they had extruded their young, and then returned to it. But in one or two cases where only males or only females were left of a brood, mates from a different brood, but of the same family and the same generation, were added. These records were kept separately. The females of the first category laid 26 broods between September, 1914, and October, 1915, and hatched 207 young. One pair mated twice with no results, and two other pairs also mated with no results. In the second case, where male and female came from different broods, only two broods, of 7 and 8 young respectively, were hatched, the male in each case dying soon after, but these broods appear stronger than the others.

Several of this F_2 generation are still breeding (Nov. 3, 1915), but the numbers already obtained are sufficient for proof and record.

Of the *F_3 generation* 105 have survived (Nov. 19, 1915), many of them not yet mature. In several broods all the individuals have very pale eyes, with hardly any of the red pigment showing. The results for this generation are unsatisfactory, only a few young being hatched. Ten pairs have mated so far, as follows :—

From the first category (individuals of the same brood paired in their brood-bowl) 2 pairs mated, no eggs laid ; 1 pair mated, eggs thrown off ; 3 pairs with 18 young in 4 broods.

From the second category, which appears to yield a stronger stock (the two F_3 broods of the two F_2 pairs in which male and female came from different parents), 4 survive of the first brood of 7, not yet mature ; the second brood of 8 matured, and 6 matings have taken place : 4 young (all dead now) ; eggs thrown off ; no eggs ; 9 young (all dead) ; 7 young (1 left) ; and 5 young (3 left, mature females).

The total number for the *F_4 generation* thus far is only 31. Some individuals of one brood, the 9 young referred to above, came to maturity, and 2 broods of *F_5* were hatched, numbering 12 young. Of these 9 survived, and are now nearly ready to breed.

II. THE DOMINANTS.

The Dominants are divided into *Pure Black* and *Hybrid Black*, which will be dealt with under separate divisions.

According to the Mendelian laws of inheritance of characters, the

matings of the Dominants with other Dominants and with Recessives should show the following results :—

(*a*) P.×P. : mating of Pure Black with Pure Black should give all black-eyed offspring, Pure Black, which should breed true through all succeeding generations.

(*b*) P.×H. : matings of Pure Black with Hybrid Black should give all black-eyed offspring, half Pure Black and half Hybrid Black.

(*c*) P.×R. : mating of Pure Black with Recessives should give all black-eyed offspring, Hybrid Black, which when bred together should show the red-eyed strain in the next generation.

(*d*) H.×H. : matings of Hybrid Black with Hybrid Black should give three black-eyed offspring to one red-eyed, i.e. in the proportion of one Pure Black and two Hybrid Black to one Recessive.

(*e*) H.×R. : matings of Hybrid Black with Recessive should give offspring half of which would be Hybrid Black and half Recessive.

The Pure Blacks.

Only a short note is necessary under this heading.

The Pure Black stock (p. 22) has been kept and interbred for over three years in a large jar. Observations have been made on it at different seasons of the year, all the animals being taken out and examined for eye-colour. Different pairs also have been kept separate from time to time and their progeny recorded to the third and fourth generations, but in all the cases not a single red-eyed one has been found.

With other dredgings brought in at intervals since June, 1912, the same results have been obtained. The last dredgings examined were those described on p. 25.

With regard to the 194 young from the P.×P. matings mentioned on p. 41, the record of the number of their offspring has not been kept, it having been thought sufficient to examine the eye-colour of all the animals in the different bowls from time to time to make sure that no red-eyed one appears.

In the P.×H. matings which have been tried, the young were all black-eyed. The difficulty with these has been in bringing a sufficient number of any one brood to maturity in order to test them for P. and H. characters. Only one case succeeded well enough to be recorded, the Brood 1 of Experiment 118 referred to on p. 41. Twenty-two young were hatched, and twenty-one reached maturity—seven males, thirteen females, and one abnormal one. Each of these was mated with a red-eyed mate except

in the two instances noted, when a proved Hybrid mate was used, with the following results :—

P.♀	13	young.	Black.
H.♂	120	,,	67 Black and 53 Red.
? ♀	4	mates, 2 P. and 2 R.	5 broods laid, none hatched.
P.♂	64	young.	Black.
H.♀	14	,,	8 Black and 6 Red.
P.♀	6	,,	Black.
P.♀	71	,,	Black.
P.♀	165	,,	Black. The eye of this female is figured. See Fig. 1.
P.♀	56	,,	Black.
H.♀	8	,,	5 Black and 3 Red.
? ♀	1	,,	Black. Female eaten.
H.♀	15	,,	11 Black and 4 Red.
H.♀	4	,,	3 Black and 1 Red. This female was tested with a Hybrid Black mate.
H.♂	14	,,	10 Black and 4 Red. Also with a Hybrid Black mate.
? ♀			Eaten by mate.
H.♂	57	,,	31 Black and 26 Red.
H.♀	20	,,	12 Black and 8 Red.
P.♂	37	,,	Black.
H.♂	24	,,	12 Black and 12 Red.
P.♂	8	,,	Black.
? ?			This is the Abnormal one mentioned above.

In the third cross (*c*) P. × R., the matings have always produced black-eyed offspring, all Hybrid Black. The figures obtained in the F_2 generation may be quoted here—1563 young (see p. 39), as well as those of the Parent generation given in the next paragraph.

The Hybrids.

Parent generation.

In the *Parent generation* 33 experiments were made, starting in November, 1913, Pure Black males being mated with Recessive females, and Recessive males with Pure Black females, 16 experiments with the first cross, and 17 with the second. There were 18 broods hatched from the first cross P.♂ × R.♀, numbering in all 323 young; and 21 broods from the second cross R.♂ × P.♀ with 313 young. In all these experiments without exception, the young had black eyes. In the first cross some paired without results, the others had from one to six broods each, the largest number in a single brood being 38. In the

second cross, all but one of the broods were hatched, the largest number being 49.

F_1 generation.

All the young of this generation were black-eyed, as was to be expected, in accordance with the Mendelian law that the offspring of Dominant mated with Recessive resemble the dominant parent in character. The further development of the law, that though the offspring are dominant in appearance, yet in constitution they are hybrid, could not be determined until the next generation, the F_2, appeared, the eye-colour alone not being a sufficiently accurate guide in distinguishing Pure Blacks from Hybrid Blacks.

In order to make sure of each individual F_1 and to keep its history clear, all the F_1 broods were kept in separate bowls till mature, and then as each pair mated it was removed and records kept of all the matings, the young being counted and examined for eye-colour immediately after extrusion from the pouch. All the F_1 that reached maturity were tested and all proved Hybrid Blacks.

The results for the eye-colour in the F_2 generation are given below.

F_2 generation.

The first idea was to take the F_2 broods in order as they hatched to the number of 1000 young, and to find if the proportions held good—three black to one red. Seventy-four broods were taken in this way, the young numbering 586, 437 of which were black-eyed and 149 red-eyed, the reds therefore being very slightly in excess of the theoretical figure. These broods appeared during the summer months, when the animals mature more rapidly and have a much quicker succession of broods than in the lower temperature. As the numbers in the broods were decreasing, the adults dying off, and the whole of the stock looking unhealthy, it was thought well to strengthen it before continuing the experiment. A change of food was given, and plenty of mud from the ditches.

It was then decided to pick out the three largest and strongest of the F_1 broods, and to count all the F_2 progeny produced by them. K, M, and N broods, which had matured under the healthier conditions, were chosen—K brood consisted of five males and nineteen females, M of nine males and six females, and N of fifteen males and seven females.

The first 72 broods from these three families (from Oct. 22, 1914, to March 2, 1915) contained 1004 young, 753 black-eyed and 251 red-eyed, in the exact proportion, as will be seen, of 3 to 1. K family was represented by 43 broods, total number of young hatched 655, of which 487 were Black and 168 Red ; M family by 19 broods, 271 young, 204

Black and 67 Red; and N family by 10 broods, 78 young, 62 Black and 16 Red.

By this time the animals were beginning to die out. N family was finished by June 16, 1915; M family on that date had only 1 male and 4 females left (this male died on July 22); while K family still had 3 males and 16 females.

The next 65 broods (to May 24, 1915) brought the number of young extruded to 2000=1505 Black and 495 Red: K family with 1228, 924 Black and 304 Red; M family with 582, 438 Black and 144 Red; and N family with 190, 143 Black and 47 Red.

The number 3001 was reached on July 24, 1915, total number of Black 2270, and of Red 731; K family with 1540 Black and 490 Red; M family with 552 Black and 181 Red; and N family with 178 Black and 60 Red.

The figures therefore for the second and third thousand give to the Blacks a slight excess over the theoretical figure. In the first thousand (1004) the proportions, three Black to one Red, were exact; in the 2000 they were very nearly right; but in the 3000 the Black rather predominated, the fact that the Black is the hardier strain probably accounting for this.

This same slight but steady increase can be seen on a small scale in the detailed Brood-records of the Hybrid crosses H.×R. In the first broods of each pair the proportions are nearly always exact, half Black and half Red, but the total results for all the broods show a preponderance of Black (see lists, p. 35); compare also for an example of a single brood Exp. 85, p. 38.

In Exp. 85 Brood 1 numbered	24	=	12	Black,	12 Red.
,, 2 ,,	25	=	13	,,	12 ,,
,, 3 ,,	23	=	12	,,	11 ,,
,, 4 ,,	28	=	14	,,	14 ,,
,, 5 ,,	20	=	9	,,	11 ,,
,, 6 ,,	32	=	20	,,	12 ,,
,, 7 ,,	17	=	8	,,	9 ,,
,, 8 ,,	21	=	13	,,	8 ,,
,, 9 ,,	14	=	8	,,	6 ,,
,, 10 ,,	26	=	15	,,	11 ,,
,, 11 ,,	34	=	15	,,	19 ,,
,, 12 ,,	42	=	25	,,	17 ,,
,, 13 ,,	26	=	12	,,	14 ,,
Total . 13 broods.	332 young.		176	Black.	156 Red.

The records of the individual families are very interesting. In K family, in which females preponderate, breeding commenced October 25, 1914, the first thousand was reached on April 20, 1915: 63 broods hatched out numbering in all 1007 young, of which 756 were Black and 251 were Red, exact proportions. In the second thousand there were 59 broods hatched containing 1003 young, 767 Black as against 236 Red, the Black therefore in excess. 23 more broods were laid with 431 young, 336 Black and 95 Red. As will be seen, the proportion of Black is again higher. The last male died on September 21, 1915, on which date the records were perforce brought to a conclusion.

These figures prove conclusively that in the F_2 generation the proportions are 3 black-eyed to 1 red-eyed. The next step was the testing the black-eyed F_2 to get the proportion of Pure Black to Hybrid Black, but the results of this work are not exact and naturally cannot be. It is easy enough to separate the colours, black from red, immediately on hatching, but impossible to determine the question of the constitution of the black-eyed until they breed. Owing to various causes a high rate of mortality has to be allowed for, and the results therefore can only be given on the *survivors*.

The animals undergo many ecdyses, the young every few days, the adults at longer intervals, the males again at much longer intervals than the females. The moulting period is always critical even to the strong ones. It is absolutely fatal to the weakly ones in a brood, the others attacking them in their feeble condition and devouring them. With the adults the mortality is higher among the females. The reason is that the male carrying the female for some days prior to the extrusion of a brood, and assisting it through the moult which immediately precedes the deposition of a fresh brood, very frequently ends by eating it directly after. A great many females have been lost in this way in the course of the work.

But the principal cause of the high death rate is the development of injurious bacteria in the bowls. At first it was thought that the bacteria had been introduced with the rotting leaves given as food, and many methods of sterilising the leaves were tried. After a while it was noticed that all the broods set out on a certain date had perished, and on comparing this result with a similar one in Mr. Crawshay's experiments, he discovered that the same sea-water had been used in all, and that this water was infected, although taken as far out as the Eddystone for the sake of avoiding shore contamination.

Several kinds of bacteria have been observed, some fatal within a day or two, some after several weeks, and others which, except for retarding development, do not injure the animal. One of this last-mentioned kind turns the water a milky colour, and forms dense slimy masses all round

the bowl, and over the food, and even clings to the amphipods themselves. With a lens it is easy to see long streamers of this slime trailing behind the little creatures as they swim.

Proportions of Pure Black to Hybrid Black in the F_2 generation.

The experiments to find the proportions of Pure Black and Hybrid Black were made with the surviving F_2 progeny of the first F_1 brood, Brood A, that came to maturity. The following table shows the parentage with the number of young hatched, 210 in all, 153 black-eyed and 57 red-eyed.

P. ♂ × R. ♀ *Parent generation.*

(From Pure Black stock.) | (One of the 8 Reds, p. 26.)

First Brood—Brood A = 22 young. Hybrid. *F_1 generation.* extruded 16.3.1914.

A.F_1 pair. 4 broods.	B.F_1 pair. 4 broods.	C.F_1 pair. 1 brood.	D.F_1 pair. 2 broods.	E.F_1 pair. 3 broods.	F.F_1 pair. 2 broods.	G.F_1. 1 brood.
55 young.	48 young.	7 young.	20 young.	43 young.	27 young.	10 young.
						F_2 *generation.*
Hatched :—						
38 Black.	37 Black.	6 Black.	15 Black.	30 Black.	21 Black.	6 Black.
17 Red.	11 Red.	1 Red.	5 Red.	13 Red.	6 Red.	4 Red.
Survived :—						
23 Black.	15 Black.	4 Black.	13 Black.	18 Black.	15 Black.	5 Black.
10 Red.	7 Red.	0 Red.	3 Red.	7 Red.	4 Red.	3 Red.

Only 127 reached maturity, 93 black-eyed and 34 red. Of the Black 44 were males and 49 females; of the Red 20 were males and 14 females.

The testing was done with red-eyed mates, the Blacks being separated into finger-bowls and each given a Red mate. The resulting broods would at once show the P. or H. character, for if the black-eyed animal were a Pure Black the offspring would be all black-eyed; if a Hybrid Black, half the young would be black-eyed and half red-eyed. Later, when the constitution of each had been determined, the survivors were mated together: P. × P.; P. × H.; H. × H.; and H. × P.

It sometimes happens that the individuals of the first brood of Hybrid × Recessive, if few in number, are all of one eye-colour, not the normal proportions, half red and half black. This occurred six times in the course of these experiments; in four broods the young were all black-eyed; in two, all red; the highest number in any of these broods was four. To avoid error each pair was kept for at least three broods to make quite sure of the constitution; in some cases the black-eyed were mated with two or three different red-eyed mates.

In all 141 experiments were made with the 93 Black-eyed animals. Thirteen, 7 males and 6 females, died without proof, probably through

some inherent weakness; in one or two cases broods of eggs were laid but not hatched, in others the stronger mate ate the weaker one.

Of the 80 that survived, 22 proved Pure Black, 8 males and 14 females, and 58 proved Hybrid Black, 29 males and 29 females. Ten experiments were made with P. males, 24 with P. females, 38 with H. males, and 56 with H. females.

The details of the experiments are as follows :—

TABLE

showing the details of the experiments made with the black-eyed F_2 progeny of one brood of the F_1 generation of Hybrids, from September, 1914, to September, 1915, in order to find the proportion of Pure Black to Hybrid Black. Theoretically it should be 1 P. : 2 H., but, as has been already explained, the results recorded here cannot be considered exact owing to the high mortality amongst the immature.

Experiment Number.	Black. ♂	Black. ♀	Number of Young hatched.	Eye-colour. Black.	Eye-colour. Red.	Number of Broods.	Pure Black or Hybrid Black.
Exp. 1	—	♀	—	—	—	—	No results : ♀ eaten.
,, 2	—	♀	—	—	—	—	No results : ♀ died.
,, 3	—	♀	—	—	—	—	No results : ♀ died.
,, 4*a*	♂	—	—	—	—	—	No results.
,, 4*b*	,,	—	33	All	—	4	P.
,, 5	♂	—	31	16	15	3	H.
,, 6	—	♀	25	15	10	2	H.
,, 8*a*	♂	—	—	—	—	—	No results.
,, 8*b*	,,	—	35	21	14	3	H.
,, 9*a*	—	♀	21	12	9	1	H.
,, 9*b*	—	,,	—	—	—	—	No results.
,, 10	—	♀	—	—	—	—	No results : ♀ died.
,, 11	♂	—	56	34	22	4	H.
,, 14*a*	♂	—	2	—	2	1	H.
,, 14*b*	,,	—	26	13	13	2	,,
,, 16	♂	—	49	21	28	4	H.
,, 17*a*	♂	—	21	11	10	2	H.
,, 17*b*	,,	—	—	—	—	—	Eggs laid, not hatched.
,, 19	♂	—	28	All	—	3	P.
,, 20*a*	♂	—	—	—	—	—	No results.
,, 20*b*	,,	—	9	4	5	1	H.
,, 20*c*	,,	—	44	22	22	1	,,
,, 21	♂	—	—	—	—	—	No results : ♂ died.
,, 22	—	♀	—	—	—	—	No results : ♀ eaten.

Experiment Number.	Black. ♂	Black. ♀	Number of Young hatched.	Eye-colour. Black.	Eye-colour. Red.	Number of Broods.	Pure Black or Hybrid Black.
Exp. 23*a*	—	♀	—	—	—	—	No results.
,, 23*b*	—	,,	3	2	1	1	H.
,, 23*c*	—	,,	22	10	12	2	,,
,, 24*a*	—	♀	21	12	9	3	H.
,, 24*b*	—	,,	6	1	5	1	,,
,, 25	—	♀	11	6	5	1	H.
,, 26*a*	—	♀	—	—	—	—	No results.
,, 26*b*	—	,,	70	31	39	4	H.
,, 26*c*	—	,,	—	—	—	—	No results.
,, 27	—	♀	11	7	4	1	H.
,, 28*a*	♂	—	—	—	—	—	No results: ate its mate.
,, 28*b*	,,	—	116	54	62	4	H.
,, 29*a*	—	♀	5	3	2	1	H.
,, 29*b*	—	,,	36	19	17	3	,,
,, 30	—	♀	5	4	1	1	H.
,, 31	—	♀	23	11	12	1	H.
,, 32*a*	—	♀	—	—	—	—	No results.
,, 32*b*	—	,,	50	25	25	2	H.
,, 33*a*	—	♀	—	—	—	—	No results.
,, 33*b*	—	,,	46	All	—	2	P.
,, 34*a*	—	♀	8	All	—	2	P.
,, 34*b*	—	,,	14	,,	—	2	,,
,, 34*c*	—	,,	67	,,	—	5	,,
,, 35*a*	—	♀	13	3	10	1	H.
,, 35*b*	—	,,	10	8	2	1	,,
,, 35*c*	—	,,	55	23	32	2	,,
,, 35*d*	—	,,	34	20	14	2	,,
,, 36	—	♀	58	All	—	4	P.
,, 37*a*	—	♀	35	16	19	2	H.
,, 37*b*	—	,,	76	43	33	2	,,
,, 37*c*	—	,,	5	4	1	1	,,
,, 38*a*	—	♀	71	All	—	4	P.
,, 38*b*	—	,,	13	,,	—	..	,,
,, 39*b*	—	♀	—	—	—	—	No results.
,, 39*c*	—	,,	—	—	—	—	,,
,, 39*d*	—	,,	35	26	9	3	H.
,, 39*e*	—	,,	—	—	—	—	Eggs laid : ♀ eaten.
,, 40	—	♀	25	All	—	2	P.
,, 41	—	♀	81	42	39	4	H.
,, 42*a*	—	♀	13	All	—	3	P.
,, 42*b*	—	,,	11	,,	—	1	,,

some inherent weakness; in one or two cases broods of eggs were laid but not hatched, in others the stronger mate ate the weaker one.

Of the 80 that survived, 22 proved Pure Black, 8 males and 14 females, and 58 proved Hybrid Black, 29 males and 29 females. Ten experiments were made with P. males, 24 with P. females, 38 with H. males, and 56 with H. females.

The details of the experiments are as follows :—

TABLE

showing the details of the experiments made with the black-eyed F_2 progeny of one brood of the F_1 generation of Hybrids, from September, 1914, to September, 1915, in order to find the proportion of Pure Black to Hybrid Black. Theoretically it should be 1 P. : 2 H., but, as has been already explained, the results recorded here cannot be considered exact owing to the high mortality amongst the immature.

Experiment Number.	Black. ♂	Black. ♀	Number of Young hatched.	Eye-colour. Black.	Eye-colour. Red.	Number of Broods.	Pure Black or Hybrid Black.
Exp. 1	—	♀	—	—	—	—	No results : ♀ eaten.
,, 2	—	♀	—	—	—	—	No results : ♀ died.
,, 3	—	♀	—	—	—	—	No results : ♀ died.
,, 4*a*	♂	—	—	—	—	—	No results.
,, 4*b*	,,	—	33	All	—	4	P.
,, 5	♂	—	31	16	15	3	H.
,, 6	—	♀	25	15	10	2	H.
,, 8*a*	♂	—	—	—	—	—	No results.
,, 8*b*	,,	—	35	21	14	3	H.
,, 9*a*	—	♀	21	12	9	1	H.
,, 9*b*	—	,,	—	—	—	—	No results.
,, 10	—	♀	—	—	—	—	No results : ♀ died.
,, 11	♂	—	56	34	22	4	H.
,, 14*a*	♂	—	2	—	2	1	H.
,, 14*b*	,,	—	26	13	13	2	,,
,, 16	♂	—	49	21	28	4	H.
,, 17*a*	♂	—	21	11	10	2	H.
,, 17*b*	,,	—	—	—	—	—	Eggs laid, not hatched.
,, 19	♂	—	28	All	—	3	P.
,, 20*a*	♂	—	—	—	—	—	No results.
,, 20*b*	,,	—	9	4	5	1	H.
,, 20*c*	,,	—	44	22	22	1	,,
,, 21	♂	—	—	—	—	—	No results : ♂ died.
,, 22	—	♀	—	—	—	—	No results : ♀ eaten.

Experiment Number.	Black. ♂	Black. ♀	Number of Young hatched.	Eye-colour. Black.	Eye-colour. Red.	Number of Broods.	Pure Black or Hybrid Black.
Exp. 23*a*	—	♀	—	—	—	—	No results.
,, 23*b*	—	,,	3	2	1	1	H.
,, 23*c*	—	,,	22	10	12	2	,,
,, 24*a*	—	♀	21	12	9	3	H.
,, 24*b*	—	,,	6	1	5	1	,,
,, 25	—	♀	11	6	5	1	H.
,, 26*a*	—	♀	—	—	—	—	No results.
,, 26*b*	—	,,	70	31	39	4	H.
,, 26*c*	—	,,	—	—	—	—	No results.
,, 27	—	♀	11	7	4	1	H.
,, 28*a*	♂	—	—	—	—	—	No results: ate its mate.
,, 28*b*	,,	—	116	54	62	4	H.
,, 29*a*	—	♀	5	3	2	1	H.
,, 29*b*	—	,,	36	19	17	3	,,
,, 30	—	♀	5	4	1	1	H.
,, 31	—	♀	23	11	12	1	H.
,, 32*a*	—	♀	—	—	—	—	No results.
,, 32*b*	—	,,	50	25	25	2	H.
,, 33*a*	—	♀	—	—	—	—	No results.
,, 33*b*	—	,,	46	All	—	2	P.
,, 34*a*	—	♀	8	All	—	2	P.
,, 34*b*	—	,,	14	,,	—	2	,,
,, 34*c*	—	,,	67	,,	—	5	,,
,, 35*a*	—	♀	13	3	10	1	H.
,, 35*b*	—	,,	10	8	2	1	,,
,, 35*c*	—	,,	55	23	32	2	,,
,, 35*d*	—	,,	34	20	14	2	,,
,, 36	—	♀	58	All	—	4	P.
,, 37*a*	—	♀	35	16	19	2	H.
,, 37*b*	—	,,	76	43	33	2	,,
,, 37*c*	—	,,	5	4	1	1	,,
,, 38*a*	—	♀	71	All	—	4	P.
,, 38*b*	—	,,	13	,,	—	—	,,
,, 39*b*	—	♀	—	—	—	—	No results.
,, 39*c*	—	,,	—	—	—	—	,,
,, 39*d*	—	,,	35	26	9	3	H.
,, 39*e*	—	,,	—	—	—	—	Eggs laid : ♀ eaten.
,, 40	—	♀	25	All	—	2	P.
,, 41	—	♀	81	42	39	4	H.
,, 42*a*	—	♀	13	All	—	3	P.
,, 42*b*	—	,,	11	,,	—	1	,,

Experiment Number.	Black. ♂	Black. ♀	Number of Young hatched.	Eye-colour. Black.	Eye-colour. Red.	Number of Broods.	Pure Black or Hybrid Black.
Exp. 43	♂	—	—	—	—	—	No results: ♂ killed by
,, 44	—	♀	35	16	19	2	H. [mate.
,, 45*a*	—	♀	9	All	—	1	P.
,, 45*b*	—	,,	50	,,	—	2	,,
,, 46	♂	—	10	6	4	2	H.
,, 47*a*	♂	—	29	12	17	3	H.
,, 47*b*	,,	—	—	—	—	—	No results: ♂ died.
,, 48*a*	—	♀	19	All	—	2	P.
,, 48*b*	—	,,	14	,,	—	1	,,
,, 49*a*	—	♀	30	16	14	2	H.
,, 49*b*	—	,,	18	13	5	1	,,
,, 50	—	♀	26	10	16	3	H.
,, 51	—	♀	84	32	52	3	H.
,, 52	♂	—	41	All	—	2	P.
,, 53	—	♀	82	All	—	4	P.
,, 54(1)	♂	—	—	—	—	—	No results.
,, 54(2)	♂	—	—	—	—	—	,,
,, 54(3)	♂	—	—	—	—	—	,,
,, 55	—	♀	108	All	—	5	P.
,, 56	—	♀	—	—	—	—	No results.
,, 57*a*	—	♀	30	14	16	2	H.
,, 57*b*	—	,,	63	36	27	2	,,
,, 57*c*	—	,,	135	65	70	5	,,
,, 59*a*	♂	—	—	—	—	—	No results: ♀ eaten.
,, 59*b*	,,	—	56	All	—	3	P.
,, 60	♂	—	117	63	54	4	H.
,, 61*a*	—	♀	58	34	24	3	H.
,, 61*b*	—	,,	15	11	4	1	,,
,, 62*a*	—	♀	18	6	12	3	H.
,, 62*b*	—	,,	—	—	—	—	No results: eggs thrown off: ♀ died.
,, 63	—	♀	81	All	—	4	P.
,, 64	♂	—	—	—	—	—	No results: ate ♀: died
,, 65	—	♀	63	All	—	4	P. [in moulting.
,, 66*a*	—	♀	12	All	—	1	P.
,, 66*b*	—	,,	145	,,	—	10	,,
,, 66*c*	—	,,	131	,,	—	3	,,
,, 67	♂	—	12	3	9	2	H.
,, 68	♂	—	125	62	63	4	H.
,, 69	—	♀	56	27	29	3	H.

Experiment Number.	Black. ♂	Black. ♀	Number of Young hatched.	Eye-colour. Black.	Eye-colour. Red.	Number of Broods.	Pure Black or Hybrid Black.
Exp. 70*a*	—	♀	—	—	—	—	No results : too young ?
,, 70*b*	—	,,	135	All	—	7	P.
,, 71*a*	—	♀	118	63	55	4	H.
,, 71*b*	—	,,	—	—	—	—	No result : ♀ eaten.
,, 72*a*	—	♀	9	7	2	1	H.
,, 72*b*	—	,,	33	16	17	1	,,
,, 72*c*	—	,,	27	11	16	1	,,
,, 73	♂	—	19	5	14	1	H.
,, 74*a*	—	♀	26	11	15	3	H.
,, 74*b*	—	,,	7	3	4	1	,,
,, 75*a*	—	♀	64	33	31	3	H.
,, 75*b*	—	,,	16	8	8	1	,,
,, 76	♂	—	47	23	24	4	H.
,, 77	♂	—	30	16	14	4	H.
,, 78	♂	—	9	All	—	2	P.
,, 79	♂	—	28	14	14	2	H.
,, 80	♂	—	1	1	—	1	No results of value :
,, 81	♂	—	76	All	—	4	P. [♂ eaten.
,, 82*a*	♂	—	9	6	3	4	H.
,, 82*b*	,,	—	26	11	15	1	,,
,, 85	♂	—	332	176	156	13	H.
,, 87	♂	—	191	95	96	10	H.
,, 89*a*	♂	—	—	—	—	—	No results : ate ♀.
,, 89*b*	,,	—	4	3	1	1	H.
,, 90	♂	—	59	All	—	2	P.
,, 91	♂	—	57	31	26	5	H.
,, 92	♂	—	183	95	88	10	H.
,, 93*a*	—	♀	32	15	17	1	H.
,, 93*b*	—	,,	—	—	—	—	No results : eggs thrown off.
,, 94	♂	—	53	21	32	4	H.
,, 95	—	♀	146	68	78	6	H.
,, 96	♂	—	94	54	40	6	H.
,, 97	♂	—	2	1	1	2	H.
,, 98	♂	—	209	95	114	12	H.
,, 99	♂	—	86	All	—	7	P.
,, 100	♂	—	10	6	4	1	H.
,, 104	♂	—	256	140	116	10	H.
Total 141 Exps.	37 ♂	43 ♀	5494 Young.	3555 Black.	1939 Red.	323 Broods.	P.=8 ♂ and 14 ♀. H.=29 ♂ and 29 ♀.

The total number of young hatched was 5494. Of this number 388 were the black-eyed offspring of P. ♂ × R. ♀ and 1175 of P. ♀ × R. ♂. The number of young from the other cross, H. × R., was 3931—1992 Black-eyed and 1939 Red-eyed—1134 Black and 1098 Red in the mating H. ♂ × R. ♀, and 858 Black and 841 Red in the mating H. ♀ × R. ♂. As will be seen, the proportion is not quite exact, the Blacks being rather in excess. It appears to vary a good deal with the individual, though perhaps the number of offspring from a single pair is not sufficiently large to eliminate mere chance variation. Some animals have a succession of broods fairly evenly divided into Black and Red, while others have a preponderance of one colour or the other, others, again, having first one brood unevenly divided, the next restoring the balance, and so on. Examples of the first will be found in Exps. 87 and 57.

In Exp. 87 (♂ H., with the same mate for all the broods)—

Brood	1	numbered	27	=	14	Black and	13 Red.
,,	2	,,	14	=	5	,,	9 ,,
,,	3	,,	14	=	9	,,	5 ,,
,,	4	,,	13	=	7	,,	6 ,,
,,	5	,,	10	=	5	,,	5 ,,
,,	6	,,	13	=	8	,,	5 ,,
,,	7	,,	20	=	10	,,	10 ,,
,,	8	,,	24	=	11	,,	13 ,,
,,	9	,,	32	=	14	,,	18 ,,
,,	10	,,	24	=	12	,,	12 ,,
			191	=	95	,,	96 ,,

Exp. 57 (♀ H., with 3 different mates)—

Brood	1	numbered	8	=	3	Black and	5 Red.
,,	2	,,	22	=	11	,,	11 ,,
,,	3	,,	30	=	16	,,	14 ,,
,,	4	,,	33	=	20	,,	13 ,,
,,	5	,,	21	=	11	,,	10 ,,
,,	6	,,	32	=	13	,,	19 ,,
,,	7	,,	20	=	8	,,	12 ,,
,,	8	,,	38	=	20	,,	18 ,,
,,	9	,,	24	=	13	,,	11 ,,
			228	=	115	,,	113 ,,

For examples of the preponderance of one colour Exps. 60, 61, 71, and 95 will serve ; 60 is a ♂ H. and 71 a ♀ H., both had 4 broods each, with 117 and 118 young respectively.

In Exp. 60—

Brood 1 numbered	11	=	7	Black and	4	Red.
,, 2 ,,	28	=	12	,,	16	,,
,, 3 ,,	47	=	28	,,	19	,,
,, 4 ,,	31	=	16	,,	15	,,
	117	=	63	,,	54	,,

In Exp. 71—

Brood 1 numbered	12	=	6	Black and	6	Red.
,, 2 ,,	24	=	17	,,	7	,,
,, 3 ,,	41	=	22	,,	19	,,
,, 4 ,,	41	=	18	,,	23	,,
	118	=	63	,,	55	,,

In Exp. 61 the Black are in excess.

Brood 1 numbered	8	=	4	Black and	4	Red.
,, 2 ,,	21	=	12	,,	9	,,
,, 3 ,,	29	=	18	,,	11	,,
,, 4 ,,	15	=	11	,,	4	,,
	73	=	45	,,	28	,,

In Exp. 95 the Red preponderate (cf. Exp. 51).

Brood 1 numbered	15	=	7	Black and	8	Red.
,, 2 ,,	14	=	5	,,	9	,,
,, 3 ,,	24	=	11	,,	13	,,
,, 4 ,,	33	=	15	,,	18	,,
,, 5 ,,	27	=	15	,,	12	,,
,, 6 ,,	33	=	15	,,	18	,,
	146	=	68	,,	78	,,

For illustrations of broods unevenly divided the first broods in Exps. 32 and 72 may be given.

Exp 32. Brood 1 with	23	=	10	Black and	13	Red.
,, 2 ,,	27	=	15	,,	12	,,
	50	=	25	,,	25	,,

Exp. 72. Brood 1 with	9	=	7	Black and	2	Red.
,, 2 ,,	33	=	16	,,	17	,,
,, 3 ,,	27	=	11	,,	16	,,
	69	=	34	,,	35	,,

The H. ♀ of Exp. 75 had two broods of 21—in one case 13 Black and 8 Red, in the other 13 Red and 8 Black.

The number of young in a brood varies with the individual, but the numbers in the broods of a single pair do not vary much as a rule; to take an example, Exp. 99 had seven broods as follows: 11, 15, 13, 10, 11, 15, 11.

Exp. 35 is an interesting one, showing the varying proportions of Black and Red with different mates. This female was mated with four males with the following results: with male *a*, one brood of 13 young, 3 Black, 10 Red; with male *b*, one brood of 10, 8 Black, 2 Red; with male *c*, two broods of 28 and 27, with 12 Black and 16 Red, and 11 Black and 16 Red respectively; and with male *d*, two broods of 11 and 23, with 6 Black and 5 Red, and 14 Black and 9 Red respectively.

Many of the F_2 animals had died by the time these experiments were finished, only a few remained to be mated together. Two matings of P.×P. were made, the other P. animals being paired with H. mates.

P.×P.	Exp. 83*a*	5 broods	133 Black-eyed young.		♂ died.
,,	,, 83*b*	2 ,,	61 ,,	,,	♀ died.
P.×H.	,, 84	1 brood	Eggs thrown off.		♂ died.
P.×H.	,, 110*b*	1 ,,	6 Black-eyed young.		♂ eaten.
P.×H.	,, 118	2 broods	48 ,,	,,	♂ died.
P.×H.	,, 119	1 brood	3 ,,	,,	♂ died.
H.×P.	,, 102	1 ,,	43 ,,	,,	♂ died.
H.×P.	,, 105*a*	3 broods	85 ,,	,,	♂ died.
$H.F_3 \times P.F_2$	,, 105*b*	4 ,,	113 ,,	,,	♂ eaten.
H.×P.	,, 107	—	—		♀ died.
$H.F_3 \times P.F_2$	,, 115	1 brood	5 ,,	,,	♂ died.
H.×P.	,, 117	—	—		♂ died.
Total . .	In 21 broods		497 Black-eyed young.		

The matings of H.×H. are as follows:—

H.×H.	Exp. 86	2 broods	60 young	= 46 Black	14 Red.	♀ eaten.
H.×H.	,, 103	1 brood	22 ,,	= 16 ,,	6 ,,	
	and another brood					
	(♂ unhealthy)		1 ,,	= 1 ,,	—	♂ died.
H.×H.	Exp. 106*a*	1 brood	52 ,,	= 48 ,,	4 ,,	♂ died.
,,	106*b*	—	—	= —	—	♀ died.
H.×H.	,, 109	4 broods	62 ,,	= 43 ,,	19 ,,	♂ died.
H.×H.	,, 110*a*	1 brood	38 ,,	= 30 ,,	8 ,,	♂ died.
H.×H.	,, 112	2 broods	40 ,,	= 29 ,,	11 ,,	Both died.
H.×H.	,, 116	1 brood	11 ,,	= 7 ,,	4 ,,	♂ died.
Total .	In 12 broods		286 young	= 220 Black	66 Red.	

The proportions of Black to Red in these H. × H. experiments are about right if Exp. 106*a* is not counted.

The H. male in this experiment had been previously mated with a Red female, and had had one brood of 9 young, 1 Black and 8 Red—a preponderance of Red.

The H. female had also been previously mated with a Red male, and had had 84 young in 3 broods, 32 Black to 52 Red, a preponderance of Red. When mated together the one brood of 52 was the result, 48 Black to 4 Red—a preponderance of Black. (The Red male with which the H. female mated was tried with another H. female which had already had 30 young, 16 Black and 14 Red—with the result, one brood of 18 young hatched, 13 Black and 5 Red.)

A great many of the F_3 animals from these experiments were mated in order to see if the results would repeat those already obtained in the P. F_1 and F_2 generations. They were examined regularly, but only a few records were kept, the fact that in all cases they bred true being considered sufficient evidence of accordance with the Mendelian law.

SEX.

All the broods were kept in separate bowls to come to maturity, in the hope of finding the number of males and females hatched, and if the number varied in the different crosses. Owing to unfavourable conditions, bacteria, etc., it was found impossible to get good results. The broods, therefore, are taken in which over fifty per cent of the young came to maturity, and the numbers are given below, together with the broods in which less than fifty per cent survived, and the number of broods which failed entirely.

As a rule, all or nearly all the animals in the small broods survived, probably because they were stronger, as they were certainly larger than the others on hatching. Almost all the very large broods failed in spite of repeated efforts to save them by separating them into several bowls so as to avoid overcrowding, etc.

Of the broods that failed entirely 5 were in the P. × R. cross, 11 in the R. × P., 29 in the H. × R., and 23 in the R. × H., 68 in all.

Broods in which 50 % *and over survived to maturity.*

Black and Red Crosses. ♂ ♀	Number of Broods.	Number Hatched.	Number Survived.	Black. Males.	Black. Females.	Black. Hatched.	Black. Survived.	Red. Males.	Red. Females.	Red. Hatched.	Red. Survived.
P. × R.	10	106	83	50	33						
R. × P.	25	299	240	129	111						
H. × R.	43	551	387	95	101	282	196	90	101	269	191
R. × H.	37	510	351	92	95	269	187	72	92	241	164
	Broods in which less than 50 % *survived.*										
P. × R.	10	142	31	16	15						
R. × P.	26	492	86	45	41						
H. × R.	43	889	174	46	43	438	89	36	49	451	85
R. × H.	40	860	176	44	36	432	80	55	41	428	96
Black Crosses.											
P. × P.	7	194	79	40	39						
P. × H.	5	57	28	10	18						
H. × P.	9	169	40	17	23						
H. × H.	13	286	30	7	15	220	22	6	2	66	8

RECORDS OF ABNORMAL EYES: "NO-WHITE," "PART-WHITE," AND "ALL-WHITE."

Attempts at breeding the animals together to ascertain if the "no-white," "part-white," and "all-white" variations follow the Mendelian lines of inheritance have so far not succeeded, although these abnormalities seem to run in certain families and not in others. For example, in the F_1 Hybrids (p. 31) a great number of cases occurred in Families A and K, only one case in M, and none in the other families.

Some instances may be given in illustration of the proportions and degree per brood of the "*no-white*" variation. In K family at least six normal-eyed females transmitted this strain, some more than others, and the proportion of "no-white"-eyed in their broods was much higher than in A family. These females mated in the brood-bowl and were removed to extrude their young, and then returned to the bowl to mate again. During the month of June, 1915, 20 broods were extruded by the different K females (some of them having two broods each in the month), and

in 10 of the broods "no-white"-eyed individuals were found, as follows:—

14 young:	11	Black,	3	Red;	amongst them	2	Black	"no-white."
9 ,,	8	,,	1	,,	,,	4	,,	,,
19 ,,	15	,,	4	,,	,,	3	,,	,,
						2	Red	,,
3 ,,	3	,,	0	,,	,,	1	Black	,,
15 ,,	11	,,	4	,,	,,	6	,,	,,
28 ,,	23	,,	5	,,	,,	1	,,	,,
						1	Red	,,
30 (2 broods together)	23	,,	7	,,	,,	5	Black	,,
						2	Red	,,
17 young:	14	,,	3	,,	,,	4	Black	,,
15 ,,	14	,,	1	,,	,,	5	,,	,,

In the A family 7 animals out of the 93 black-eyed (p. 34) produced some "no-white"-eyed young, 1 male and 2 females from Af_2, 1 male and 1 female from Df_2, 1 male from Ef_2, and 1 male from Ff_2. The details are as follows:—

In Exp. 28. Brood III. 1 Black "no-white" on one side, and 1 Red "no-white" both sides. The Black was a female, the Red died before maturity. The ♂ of this experiment mated with the ♀ in Exp. 27 and had 1 Black abnormal ♀ in Brood III.

,, 59. ,, I. 2 "no-white" Black—both died.
,, II. 1 ,, ,, preserved.
,, III. 2 ,, ,, one died. The other was a female "no-white" on right side.

,, 60. ,, II. 1 "no-white" Red male, one side only.
,, IV. Contained 31 young, the eyes in all with very imperfect and broken reticulation.

,, 66. ,, VI. 1 "no-white" Black, female, left side.

,, 93. One brood of 12 young. 1 "no-white" Black, died. All the others with the reticulation imperfect. One brood of 7 young, 5 with imperfect reticulation. One brood of 32 young, all with imperfect reticulation.

,, 112. The male was from Af_2, the female from Df_2.
Brood I. 38 young. 1 "no-white" Black, died. Many with reticulation imperfect. Several Red, very pale colour.
,, II. 2 young. 1 Black "no-white" both sides—died.

Another instance is that of Exp. 99 (p. 38), a Pure Black male from Bf_2 mated with a Recessive female, both with normal eyes. All their young, 86 in number, were also normal. In the F_2 generation the " no-white " and the " part-white " strains appeared in the offspring of a pair from Brood 7. This pair had three broods, the first brood of 6 died young. Of the second brood, 21 in all, 13 Black and 8 Red, only 7 survived, 3 Black males, one with the left eye affected (in this case 2 or 3 of the ommatidia formed a little cluster apart from the ommateum). 4 Red survived, two normal and two " part-white " as figured (Fig. 7). (In the first brood from these Reds 3 out of the 4 young (F_4) had normal eyes, the fourth had eyes like the male figured.) The third brood numbered 30, 20 Black and 10 Red, of these 6 survived, 4 Black and 2 Red, only one normal-eyed amongst them, a Black. Two of the other Blacks were " no-white " on both sides, and the remaining one was normal on the right side, but had a small cluster of ommatidia apart on the left side. Of the two Reds, one was " no-white " on the left side, the other had the white reticulation partly lacking, i.e. partly " no-white."

Many cases have occurred in which the white pigment (instead of being diminished or lacking) is present in excess. This appears to be always accompanied by a diminution of the coloured pigment of the retinular cells, the red, e.g., being hardly perceptible, even with a strong lens showing only as a very pale pink tint, instead of the vivid blood-red of the normal red eye. This variation has been noticed especially in the later generations of the inbred Recessives, and it is possible that another generation or two of inbreeding may produce the " *all-white* " eye.

Only a few " *all-whites* " have been recorded so far (Nov. 19, 1915). Two, a male hatched April 2, 1915, and a female hatched April 14, have appeared in the Pure Red Stock amongst the young from Brood 4, Exp. 5, Recessives (p. 27) (descendants of the fourth brood of female A of the original experiments, p. 22). The female came to maturity but died without mating. The male (Fig. 8) was mated with female B (Figs. 9 and 10), referred to below (a Hybrid with degenerate " white " eyes), and proved pure Red—the 5 young being normal-eyed, 2 Black and 3 Red. It died in moulting, November 19, 1915, without mating again. Two other females from the same brood as the " all-white " female extruded their young in the brood-bowl, 6 in number, all with very pale pink, almost " white " eyes.

A curious instance of the " all-white " accompanied by degeneration of the eye was noted in the forty-second brood of M family, F_1 Hybrids (p. 31). This brood was extruded on June 1, 1915, and numbered 12 young, 7 Black, 1 Red, and 4 " all-white " eyes. These four proved to be 1 male and 3 females. The male mated with one of the females, eggs were

laid but thrown off, then the male died and was eaten. A Pure Black male was put in, mated with one of the females, but ate it after mating.

By September 26 only the two females B and C were left. The "all-white" male from the Pure Red Stock with unpigmented perfectly formed eyes (Fig. 8) was put with them and mated at once with female B (Figs. 9 and 10), eggs were laid, and 5 young were extruded, on October 16, all with *normal* eyes, 2 Black and 3 Red. This result proved beyond doubt that female B was a true Hybrid and the male a true Recessive.

The second female, C, was left with the same male, but as no mating had taken place by October 28, a Red male was added, mated, and the eggs were laid on November 4.

The figures given of the eyes and eye-colours are all taken from living animals, for the colours alter so rapidly after death, that notes made on the colour in dead or preserved specimens are not of the slightest value. For instance, the white pigment disappears within an hour or two of death, and the red also fades out completely, though much more gradually.

GENERAL NOTES.

Breeding different generations together.—Eight experiments were made with males of the F_3 generation and females of the F_2: one with R. $\times$ H.; two with H. $\times$ R.; two with H. $\times$ H.; and three with H. $\times$ P. In the first, the male was rather small, mated three times, and carried the female for 7, 6, and 6 days respectively with no results; female disappeared. In 2nd Exp. the female was eaten; 3rd Exp., one brood of 26 young was hatched, 11 Black and 15 Red, the male died; 4th Exp., one brood of 6 young; 5th Exp., eggs were laid, not hatched, male died; 6th Exp., same male as in the second experiment, one brood of 5 young, male died; 7th Exp., female laid eggs but died before they hatched; 8th Exp., four broods of 36, 17, 30, 30; male eaten. The results are not satisfactory, probably because of the difference in size. The females were large, and the males had only just reached maturity. When the animals are about the same size there is nothing to distinguish their matings from those of animals of the same generation.

Fertility.—A great variation in fertility has been noticed, not only in individuals, but often in all the members of any one brood.

Some instances may be given in illustration from broods of the F_1 generation of the Recessives. As an example of infertility Brood 1 of Exp. 9, p. 27, may be taken. Ten young were hatched, and most of them reached maturity, but after six months' breeding they all perished without leaving a single descendant. Brood after brood of eggs were laid, but not a single young one was hatched.

Another brood, Exp. 5, Brood 4, kept under the same conditions as the one just mentioned and breeding during the same time had 132 young. In the following four months, March to July, 1915, 91 young were hatched.

The next brood of the same experiment, Exp. 5, Brood 5, shows a curious variation. There were the same number of individuals as in the last, the same conditions, etc. After six months' breeding only 2 young were hatched from all the eggs laid, but in the following three months, April to July, 1915, 90 young were hatched.

For instances of fertility and infertility in individuals some of the F_2 animals may be taken. Sometimes an animal will mate several times with no results; the most striking case of this was the H. female of Exp. 39 (p. 36). Mated with a Black male, eggs were laid on September 12, 1914, carried for six days and then thrown off; eggs again on September 25 and again thrown off before hatching. The male was taken away, and another Black male put in: eggs laid on October 9 and thrown off; eggs again on October 23 and again thrown off. Then the female was left for a period without a male. On November 29 the male was put back, and eggs were laid, a large number, thrown off some days later; eggs laid on December 14, a large number, thrown off; eggs laid on January 3, 1915, all there on January 12, but on the 14th they were all thrown off except two, these were carried a day or two longer but not hatched. The male was taken away, and a Red male put in, which died on January 26 without any mating taking place. Then two more Red males were added —one disappeared on February 8. The female laid eggs, very few, these were thrown off on February 11. The male was again changed. On February 17 eggs were laid, and from these 4 young were hatched on March 8. Eggs were laid on March 10 and 12 young hatched on April 3. A fresh brood laid on April 3, hatched out on April 23, 19 young. The male disappeared and another was put in. Eggs laid on June 1, very few in number. On June 9 the male ate the female. The seven males used in this experiment, 2 Black and 5 Red, were all healthy animals, which had already fertilised the eggs of other females.

Numbers in Broods.—As a rule it is found that an exceptionally large brood of young is followed by a very small brood, or by the omission of one period of sexual activity, but in several cases the animals had a series of large broods, the highest numbers recorded in two succeeding broods being: In Exp. 11 (R. ♀ mated with H. ♂), 42 in the brood and (mated directly after with another H. ♂, Exp. 20) 44 in the next; in Exp. 51 (H. ♀ × R. ♂), 40 and (mated then with H. ♂, Exp. 106) 52, the largest number in a brood yet recorded; Exps. 60, 68, and 104 (H. ♂ × R. ♀) had 47 and 31, 30 and 48, and 40 and 43 respectively, and Exps. 70 and

71 (P. ♀ × R. ♂, and H. ♀ × R. ♂) had 43 and 23, and 41 and 41 respectively, all except Exp. 70 being Hybrid Black and Recessive matings.

Different rate of development.—There is often a marked difference in the rate of development of individuals in the same brood, and also of broods from the same pair. For example, in Exp. 85 (p. 38) some members of a brood hatched on January 6, 1915, were mature in March, the others not till June. Many instances like this were noted.

In Exp. 99, Brood V took four months to reach maturity; Brood VI, seven months; while Brood VII was mature in two months, and the animals were then much larger than many of the broods hatched three months earlier.

It was found that Bacteria greatly retarded growth; in one case a female took eight months to become mature, and was then only about half the normal size.

SUMMARY.

1. Twenty-one thousand, five hundred and fourteen (21,514) amphipods of the species *Gammarus chevreuxi* Sexton have been examined for eye-colour, 21,302 referred to in this paper, and 212 in other experiments, not included.

2. The normal eye-colour of this species is black, with a superficial reticulation of opaque white pigment.

3. The pigmentation of the eye is very variable within limits. Eyes have been observed either partially or entirely lacking in the coloured pigment of the retinular cells, or with either a partial or entire lack, or else an excess of the opaque white pigment.

4. The red strain appears to have arisen as a "sport" in the second generation of offspring of the first animals captured. No red-eyed animals have yet been found in natural conditions, although many thousands have been brought in from time to time and examined. Those counted for the purpose while the work for this paper was in progress numbered 8697, but this figure does not include the many thousands previously observed. Experiments have been made repeatedly with a view of getting the Red strain again from the Pure Black, but with no success.

5. The Red eye-colour is not a sex-limited character; about as many males as females come to maturity. 4248 red-eyed animals have been examined, 4175 referred to in the paper, and 73 in control experiments.

6. The inheritance of the coloured pigment of the eye follows the Mendelian law—Black is dominant and Red recessive. The dominants are divided into Pure Black and Impure or Hybrid Black.

7. The Pure Dominants and the Recessives breed true through all generations.

8. The crosses which have been made and the young hatched from them are as follows :—

Pure Black × *Recessive.*—3779 black-eyed young ; 3746 in paper, 33 in control experiments.

Hybrid Black × *Recessive.*—4255 young, of which 2176 were black-eyed and 2079 red-eyed. Those referred to in the paper numbered 4189, 2138 Black and 2051 Red, the others came from other experiments in the F_4 generation—not included.

Pure Black × *Pure Black.*—All black-eyed young, 1715 in number.

Pure Black × *Hybrid Black.*—All black-eyed young, 379 in number.

Hybrid Black × *Hybrid Black.*—4393 young, of which 3327 were black-eyed and 1066 red-eyed. Those referred to in the paper numbered 4302, 3259 Black and 1043 Red—the other 91, being from the F_4 experiments, not included here.

9. The absence or diminution of the white pigment seems peculiar to some broods. The "*no-white*" eye appeared in the second generation of offspring of Pure Black animals brought in from the ditches. The individuals affected in this way are more difficult to rear than the others, and, so far, attempts to breed them have not been successful.

10. The absence of the coloured pigment and degeneration of the eye occurred also in the F_2 generation—in this case from Hybrid Black animals.

11. The absence of the coloured pigment in perfectly formed eyes, the "*all-white*" eye, occurred in the Recessives. A great diminution of the red pigment has also been observed, particularly in the F_4 generation of the inbred Recessives.

12. The absence of the coloured pigment in part of the eye, the "*part-white*" eye, was observed in the first generation of offspring of Pure Blacks brought in from the ditches. It has been noted several times in both black and red eyes of specimens bred in the Laboratory, but only once in fresh-captured material. This case was a male, with one eye affected.

13. About as many males as females survive to maturity.

14. The breeding together of animals from different generations gives the same results as regards proportions of colours as the breeding together in the same generation.

EXPLANATION OF PLATE I.

FIG. 1.—Pure Black eye. Female from Brood 1 of Exp. 118 (p. 41). Extruded June 9. Mated with Red male, first brood hatched Aug. 26 numbering 14; five more broods, 19, 31, 24, 41, and 36 respectively, all black-eyed. Figured Oct. 29, 1915, a few hours before moulting. × 58.

FIG. 2.—Hybrid Black eye. Female from Brood 7 of Exp. 105*b* (p. 41). Extruded April 29, figured Nov. 24, 1915. Mated with H. male, one brood of 13, 10 Black and 3 Red. × 58.

FIG. 3.—Red eye. Large male from Recessive stock. Figured Nov. 5, 1915, two days before moulting; examined after moulting but no increase of ommatidia seen. × 58.

FIG. 4.—Right eye of young Hybrid from H.×R. cross. Extruded Oct. 22, figured Oct. 25, 1915; the white pigment was then much more solid in appearance than when newly hatched. × 75.

FIG. 5.—Right eye of young Red from Recessive stock. Extruded Oct. 21, 1915, and figured three hours after extrusion. × 75.

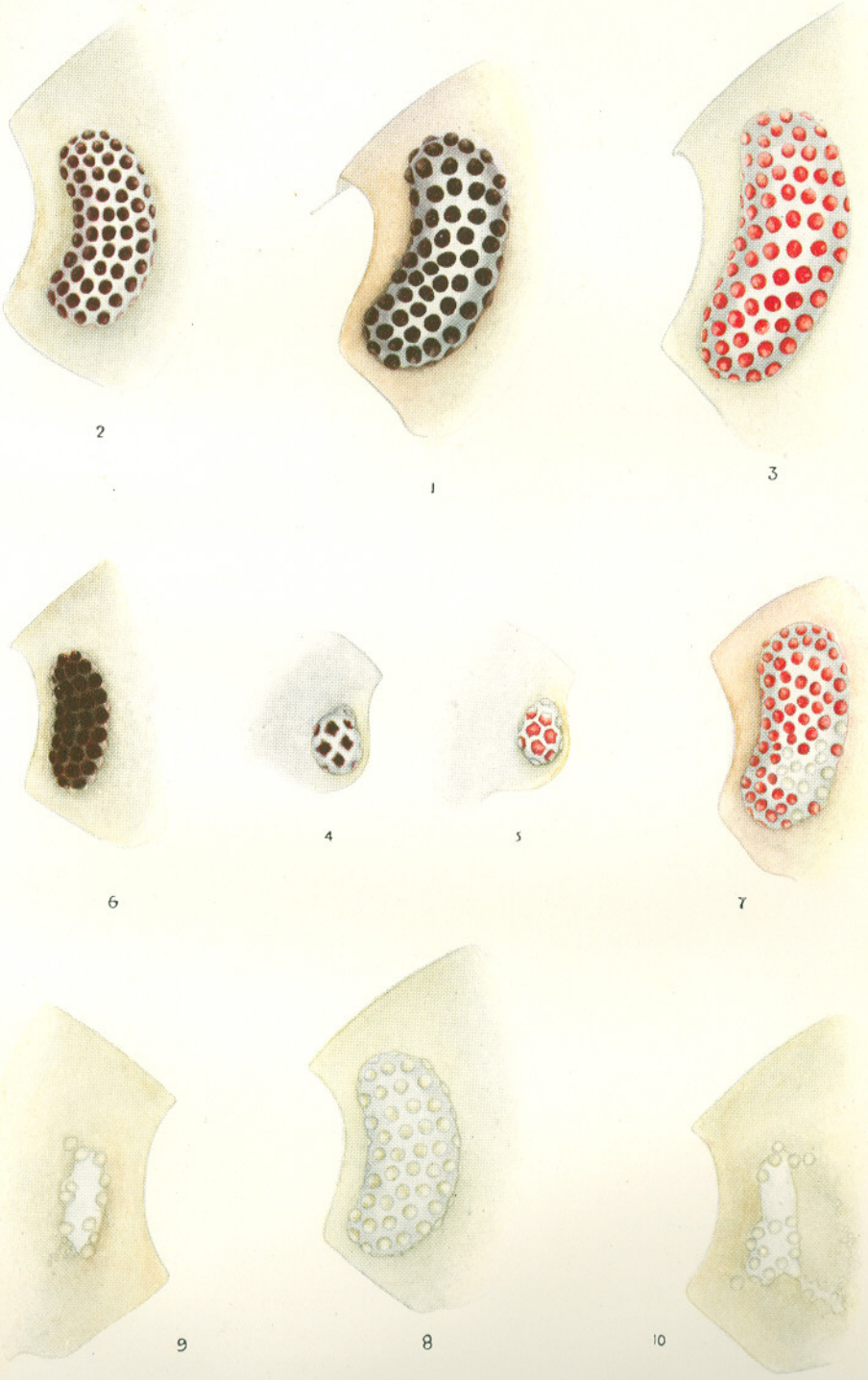
FIG. 6.—"No-white" eye. Young male from the second generation of Pure Blacks (p. 25). Figured Nov. 23, 1915. × 58.

FIG. 7.—"Part-white" eye. Male. F_2 generation from P.×R. cross (see p. 45). Extruded June 15. Figured Nov. 2, 1915. × 58.

FIG. 8.—"All-white" eye. Male from inbred Recessive stock (see p. 45). Extruded April 2, died in moulting and figured Nov. 19, 1915. × 58.

FIG. 9.—"All-white" degenerate eye, right side. Female B. F_2 generation from P.×R. cross (see p. 46). Extruded June 1, figured Nov. 16, 1915. × 58.

FIG. 10.—"All-white" degenerate eye, left side, from Female B. Figured Nov. 16. × 58.



E. W. Sexton del.