Variations in the White Pigment of the Eye in *Gammarus chevreuxi* Sexton, with a Description of a New Genetic Type, the "Clotted eye."

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With 1 Figure in the Text.

A GREAT number of variations from the normal type have occurred in *Gammarus chevreuxi* during the course of the work carried out from 1912 until now.

In reviewing these variations it is convenient to class them according to the parts affected :

(1) Pigmentation of the body (**3**, p. 194).
(2) The Red retinal pigment of the eye (**4**, pp. 45–52).
(3) The Black retinal pigment of the eye.
(4) The white accessory pigment of the eye (**2**, p. 350).
(5) The structure of the eye, involving also the structure of the head (**1**, p. 274 ; **6**, pp. 356–369).

The two last classes are dealt with in this paper.

(4). VARIATIONS AFFECTING THE WHITE PIGMENT OF THE EYE.

In the normal type (Fig. 1, *a*) the eye is of compound structure, sessile, non-facetted, convex, reniform in shape with the margin entire and clearly defined. It is composed of rows of ommatidia each consisting of a two-celled cone, and five retinal cells arranged round a central axis, the rhabdome. The coloured pigment is contained in the retinal cells. The interstices between the ommatidia are filled with the so-called "accessory" cells containing the opaque* white-pigment which gives the effect, in the living animal, of a regularly spaced, chalky white reticulation spread over the eye, with the ommatidia showing through the meshes as shining black spots.

* The two kinds of pigment, retinal and inter-ommatidial, differ in origin and in constitution. The eyes are usually described in terms of retinal colours, such as Black, White, Red, Purple, or Lilac, so, in order to avoid any confusion, particularly with the White Eye, the inter-ommatidial white accessory pigment is designated in this paper by the hyphenated term "white-pigment."

The principal genetic variations from the normal type are the No-whites ; but there occur others covering almost the whole range between the two extremes, both the quantity and distribution of the white pigment in the inter-ommatidial cells being affected.

Even in the *normal* eye, in which the chalky white lines are regularly spaced and defined, the lines may vary in thickness from " very thin," i.e., threadlike in appearance (*deficiency* of white), to " heavy," i.e., about twice or three times the usual thickness (*excess* of white), the effect given being, in the one case, of ommatidia larger than normal, and in the other, smaller.

The *Sub-white* is a variation occasionally met with, more frequently in the Albino stock (especially when it carries the No-white factor), in which only the proximal inter-ommatidial cells appear to be pigmented, and the white-pigment therefore instead of the usual chalky white reticulation shows as a faintly marked, milky looking deposit deep down in the eye.

The *No-white* eye in which the white-pigment is completely inhibited was first found in 1914 in Stock I (**2**, p. 350), and has appeared in many stocks since. It behaved as a simple Mendelian recessive.

This is the type to which we have given the name of Genotypic No-white, to distinguish it from a second No-white type of exactly the same appearance which we have recently investigated, and called Phenotypic No-white. The two types may be defined thus :

No-whites, *genotype*, hatch no-white (may be coloured, Black (BN), or Red (RN) ; or White (WN) ; or Albino (AN)*) ; remain no-white throughout life ; give no-white when mated *inter se* or with other genotypic no-white stocks ; and an F_2 ratio of 3:1 when mated with normals.

No-whites, *phenotype*, hatch no-white ; and remain no-white all through life like the genotypic, but give normals when mated with genotypic no-white stocks ; and normals, or normals and no-whites when mated *inter se*.

The Phenotypic No-whites were first noted in 1931, in three of the stocks in the M experiment† ; and again in 1933 in five stocks of the D experiment.†

The genetic No-white forms belong to the type of inheritance in which the characters segregate out sharply one from the other, but there is another type of inheritance amongst the No-whites in which every member of the particular strain where the variation occurs is affected to a greater or less degree.

In this type, practically all the young in each generation start from

* When first found, the No-white form of the Albino was described as the " Colourless eye," but since the appearance of the White No-white to which the description of " colourless " could equally apply, we have dropped this use of the term entirely.

† Records of these experiments have not yet been published, but a paper is being prepared for a future number of this Journal.

normal (in appearance), diverging steadily from normality until at or about maturity they show the characters of the variation. Besides these, the "Gradual No-whites," two other types may be included in this division, viz., the "Irregular Coloured eye," in which the structure of the ommateum is affected, and the new "Clotted eye" in which both the structure and the pigment-cells, coloured as well as white, are modified.

In the *Gradual No-whites*, the characteristic feature is the steady and gradual *reduction* of the white-pigment till it vanishes completely

The first appearance of this type was recorded in 1917 (**1**, p. 329; **2**, p. 351), and as it is typical of all the others found later, the description of it will suffice for all. From a Black pair (male thin reticulation, and female a spotted Half No-white) 311 young were hatched in four generations, 309 perfectly normal-eyed, and 2 Half No-whites, i.e., with one eye normal and one eye no-white. Not only had they all the usual amount of pigment in the reticulation of the eye, but many showed an excess of white in the form of "heavy" reticulation, and 170 had numerous spots of white apart from the eye. By the time they were half-grown the white reticulation had become thin and threadlike, and by the time maturity was reached it had disappeared altogether. Not a single specimen in the four generations reached full growth with the reticulation normal. Animals which had become fully No-white were mated together, and also tested with genotypic No-whites; in all cases Black normal-eyed young were produced.

There have been many instances of this *gradual reduction* of white-pigment, especially of late years, but an even more remarkable genetic variation was the *gradual increase* of the white-pigment, which arose in the D experiment in several stocks.

The effect of the "increase" factor is the steady development of the white-pigment from no-white to normality—the *Gradual Normal*, as opposed to the effect of the "reduction" factor from normality to no-white—the *Gradual No-white*.

As far as we know now, the inheritance does not run on the same lines. In a stock carrying the reduction factor, as has been pointed out, *all* the members are affected, but in a stock containing the increase factor, it would appear that only a certain proportion are. It is difficult to judge, for few of the stocks survived beyond the F_2 generation, and in those which did produce an F_3 the figures were too small to be of value—but it looks as if there may have been a 3 to 1 ratio.

To give examples of the figures: in Stock *D LXV* an F_2 of 254 Black young was hatched, consisting of 186 normal-eyed at birth, and 68 with the white-pigment affected. Of these 68, 45 had very thin, threadlike reticulation; 17 were practically no-white, with only one or two flecks or spots of white in the eye; and 6 were completely Black No-white in

appearance with no trace of the white-pigment visible under a high power. All the 68 became perfectly normal-eyed.

In another stock, *D XXXVIII* (2) in one brood-bowl containing one male and five females the offspring numbered 68 normal-eyed and 28 others of which 20 were Black No-whites (in appearance), 1 practically BN, and 7 had very thin reticulation. All these developed the white-pigment later.

An interesting fact is that a stock carrying the genotypic No-white factor can also carry the factor for Increase of white-pigment.

In one stock, *MMB LVII*, Red-with-Nowhite, a new recessive segregated out (**7**); mated *inter se*, Red-with-Nowhites were given; but in three crosses with proved genotypic Red No-whites of Stock I (double recessives r_1r_1 *ww*), all the young were normal-eyed.

(5) Variations affecting the Structure of the Eye involving also the Structure of the Head.

These are of very rare occurrence, and in fact only two such have been recorded up till now.

In all the mutating stocks described above, the eye or ommateum is regular in structure, with the margin clearly defined, increase taking place on the periphery at each growth-stage.

At the other extreme is the degenerate eye-structure, of which the *Albino* is the type, in which the ommateum is broken up, the retinal cells are completely lacking, and only a small proportion of the ommatidial cones, and the inter-ommatidial cells remain. No two eyes are alike, not even in the same animal. The white-pigment forms curd-like scattered masses (with the cones lying loosely in them), instead of the even reticulation seen in the normal-eye; and spots of white apart from the eye are of frequent occurrence.

This is a sharply defined and heritable variation behaving as a simple Mendelian recessive which has only appeared once in our cultures, in Stock I (**1**, p. 274) in 1915.

The second appearance of defective structure, in this case associated with malformation of the head and brain, was the *Irregular Coloured eye* (**6**, p. 355) which arose in 1918 from a mating of a dorsally spotted Albino female with a Black No-white male, heterozygous for red.

In this the range of variation extended from the perfect normal-eye, through all stages of degeneracy to the loss of the eye. In the extreme cases, the breaking-up of the ommateum was as marked as in the Albino, with this important difference, that the coloured retinal cells were always present. The white-pigment assumed many shapes, from regular reticulation to superficial clotted masses in the eye, and spots on the head.

The tracing of the inheritance was frequently obscured by the gradual onset of the irregularity common through the strain.

The "Clotted eye."

The same delayed action appears in this new variant and there is also a similar wide range of expression from the perfectly normal through degrees of Clotted, to an extreme which could be easily mistaken for Albino.

The character occupies a somewhat intermediate position between the normal and the aberrant types, Albino and Irregular-eye, and may perhaps throw light on some of the causes which produce the defects in eye-structure.

It seems to us that the Clotted effect is in all probability caused by a thinness or weakness of the cell-walls both retinal and inter-ommatidial, and that these cells breaking down sooner or later, liberate the pigments. The white inter-ommatidial pigment rises to the surface (cf. the same white-pigment action after death, as the cells disintegrate) to form curd-like clotted masses, while the coloured pigment from the retinal cells sinks to the bottom of the eye, collecting there as patches of colour.

All the young are hatched with perfectly normal eyes, but only a few remain normal. Unfortunately it is not possible to give the proportion of unchanging normals to changing. Their matings could not be controlled, owing to their scarcity and the difficulty of finding suitable mates for the survivors.

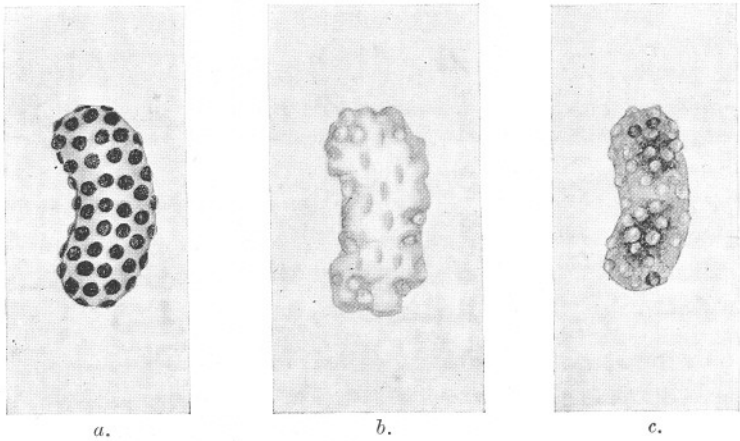
The irregularity makes its appearance shortly after birth, in only a few days, about the time of the first moult. The rate of progression is very rapid, although it seems to vary even in individuals of the same brood (see *MC* 131, for example, described below). When the change begins, the eye looks as if the white reticulation were made of highly glazed china, as if the pigments had liquefied here and there and run together to form little white patches on the surface, and black patches below. At this stage, some of the reticulation is still regular, and many of the ommatidia still intact; the others look as if the coloured pigment had collected like a blot deep down in the eye quite apart from the cones above.

As the animal grows older and the irregularity increases, the white-pigment collects in clotted lumps and masses, with only an occasional coloured ommatidium still intact, or uncoloured cone visible, or with small spots, specks, bars or streaks of black scattered over the eye.

Later, in the final stages, the white-pigment increases so much in

quantity (or changes its consistency) that the masses coalesce, and cover the ommatidial cones completely, obliterating also the shape of the margin.

Sometimes, the shape of the ommateum is affected, and becomes more or less irregular in outline. The eye loses its convexity with the breaking of the margin and tends to flatten out, thus increasing the irregularity. But in other instances, where the outline appears irregular, the shape of the margin remains unchanged under the obscuring white-pigment, as has been proved by experiment. A specimen (*MC* 131*b*) was taken, which, hatched perfectly normal, had changed to the extreme type of Clotted



a. *b*. *c*.

FIG. 1.—(*a*) Normal-eye type. Young male, 3 months old. × 65.

(*b*) Clotted-eye type. Young male, the same age as (*a*). Figured from life, Aug. 12, 1935. × 65.

(*c*) The same animal, figured Aug. 14 after the white pigment had been dissolved out, showing that, in this case, the ommateum margin was unbroken. × 65.

before it reached maturity (Fig. 1, *b*). It was preserved in 70% alcohol and re-examined as soon as the white-pigment dissolved out. It was then seen that the shape was quite regular, and that numbers of cones representing ommatidia were present, although only one or two remained intact, the black pigment from the others having collected in a mass at the bottom of the eye (Fig 1, *c*).

The Clotted eye appeared in the F_2 generation from a pair (*MV*) (male dilute Black, female jet Black) brought in on October 5, 1931, and set out in laboratory conditions.

The F_1 consisted of 91 Black normal, and 1 Black No-white, in 6 broods, of which 52 survived. These gave an F_2 of 300 Black normal, 1 Black No-white, 1 Black Half No-white and 17 Clotted and irregular eyes.

There was also a certain amount of variation both in the black pigment and the white.

The first three F_1 broods died without offspring. In the fourth (*M* 150) 15 survived, and mated *inter se*, producing the Clotted types referred to.

The fifth matured and gave 1 brood of 5 Black normals and 1 BN.

The sixth and last (*M* 350) was not examined till mature when 21 Black normal (5 males and 16 females) and 1 BN female were found, mature. The latter mated with a male from her own offspring, and gave 64 Black, two of them irregular-eyed.

In *M* 150, the brood which produced the Clotted eyes, 15 animals reached maturity, 7 males and 8 females, and were set out in pairs, giving offspring as follows :

Mixed matings in the brood-bowl. 28 Black (3 spotted) ;

Pair 1, 57 Black, including 1 spotted, and 1 with very thin reticulation ;

Pair 2, 15 Black, including 1 Half No-white spotted, and 2 with very thin reticulation (1 spotted) ;

Pair 4 (male's eyes reddish), 13 Black ;

Pair 6, 12 Black ;

Pair 7, 7 Black, including 2 spotted, and 2 reddish.

Pair 5. The matings of Pair 5 female, first in the brood-bowl, and then with male 5, gave 47 Black including 17 clotted, many irregular (*M* 768). Two of her brood with the first (unknown) male in the bowl also mated with male 5, and gave 6 Black normal and 1 clotted.

This was the first time the Clotted eye had been seen in numbers. It is true that it had occurred sporadically though very rarely, in different stocks, but never more than one specimen at a time.

The normals in Brood *M* 768 died without offspring. The clotted, many of them irregular, interbred and produced many young. The F_6 generation of this stock is now maturing.

One of the extreme examples, a female, was crossed with a Black Clotted male (heterozygous for red) from Stock II (*M. Cross LV*). One brood was hatched of which four Black normal survived, 2 males and 2 females.

These interbred, and gave an F_2 of 61 young in several broods, 29 Black normal, 9 Black clotted, 19 Black very irregular and clotted and 4 Reds, three clotted.

Six only of the normals survived, 1 male and 5 females, four with reduction of the white-pigment, and one reddish.

The 19 very clotted intermated in the brood-bowl, and gave 25 all clotted. One pair kept in a separate bowl produced 121 young ; all hatched normal, and became clotted. To give an example of the rapidity of the change, one of the broods *MC* 131, may be described. It was hatched

on May 7, 1935, and consisted of 15 quite normal. By July 30, 12 were left, all clotted in different degree, three medium, and nine of the extreme type with eyes irregular in shape and the white-pigment thick and raised ; 6 of these had only specks of black in both eyes ; 1 had two spots of black in the left eye, none in the right ; 1 had a few tiny black specks in and around the right eye, none in the left ; and 1 was white all over, with a few colourless lenses showing, and no trace of black. This is the one figured from the living animal on August 12 (Fig. 1, *b* and *c*) and again on August 14 after the white had been dissolved out.

Of the Reds, the fourth one became clotted ; three reached maturity, one male and two females, but although the male was tried with the females, and with a Black one from the same stock, no young were produced.

The Clotted eye strain has now reached the F_6 generation, and the character has remained permanent in the laboratory cultures, but in two other cases now to be described, it seems as if the character is not inherited at all. Cf. e.g., the heritable White Eye of Stock II (**3**, p. 195) with the non-heritable White Eye of Stock I (**1**, p. 287).

Clotted eyes, as has been mentioned above, have very occasionally appeared, in other stocks or in the wild, but only one or two specimens at a time.

One such, in the *D* experiment, brought in May 9, 1933, was mated with two normal-eyed females of the same dredging, and gave an F_1 of 40, an F_2 of 113 and an F_3 of 47, all perfectly normal Black eyes.

This scarcity of specimens held good till about a year ago, when numbers were found in two dredgings. On January 15, 1935, 301 were brought in, 220 normal and 81 clotted ; on February 12 the numbers were 189 normal to 35 clotted. The degrees of " clotted " ranged from medium to the extreme type, as in the laboratory cultures.

Forty-three pairs were set out, 19 with one mate normal, and one clotted ; and 24 with both of them clotted. An F_1 of 1139 Black young was hatched, 1131 quite normal with 29 inclining to heavy reticulation, 3 Half No-whites and 5 with thin reticulation, but few survived to mate. The F_2 numbered only 180, viz., 173 Black normal, 2 Black No-whites, 1 Half No-white, 3 with thin and 1 with heavy reticulation.

Apart from the few instances quoted, of reduction of the white-pigment, the eyes are hatched normal and remain normal. Experiments are still being carried on with them but so far no sign of the clotted condition has shown itself in any of the offspring from these pairs.

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