An Experiment on Breeding Wild Pairs of *Gammarus chevreuxi* at a High Temperature, with an account of Two New Recessive Types of Red Eye.

By

G. M. Spooner, M.A.,

Assistant Naturalist at the Plymouth Laboratory.

With 1 Figure in the Text.

1. Results of Rearing the F_2 from 13 Wild Pairs.

On each of two former occasions when specimens of *Gammarus chevreuxi* Sexton have been brought from the wild into an incubator and kept at 21° C. or more, red-eyed recessive types have been reared among the F_2 progeny (**4**, pp. 190 and 194). In November, 1930, an opportunity occurred for making further tests on this point. Twenty-three wild pairs, taken in Chelson Meadows on November 19, were placed on the following day in the incubator, the temperature of which at first averaged between 21° and 22° C., but was raised after a few days to an average of between 22° and 24° C. The young which were extruded from eggs laid in the wild were discarded. Those of subsequent broods were reared with a view to obtaining as many F_2 families in each stock as would give a reasonable opportunity for segregating recessive characters to appear. The F_2 young were examined for recognisable variations that might indicate the presence of a recessive character.

Of the original pairs, 8 gave no young; 2 a small F_1 but no F_2, while 13 gave a smaller or larger number of F_2 families. The results obtained from the rearing of the 13 stocks are summarised in Table I.

The eyes of the original pairs and all the F_1 were of the normal type; that is, black with white accessory pigment.

It may be noted, however, that neither the black nor the white pigment was always present in a full concentration. Among those brought in from the wild, many showed a slight deficiency of black, appearing very dark purplish or slightly reddish rather than jet-black. The deficiency shown in a tendency to reddishness was accentuated in many of the F_1 families. In most of the stocks definitely Reddish Black (**4**, Plate VIII) individuals were noted. Reddishness was most conspicuous in stocks *II*, *XIV*, *XVIII*, and *XX*. On the other hand a more typical concentration of black seemed characteristic of *IX*, *XVI*, *XXII*, and a cross between *XXI* and *XXIII*.

Some of the original wild specimens also showed a noticeably thin reticulation of white pigment. A greater or lesser deficiency in this pigment was found subsequently in all stocks, particularly in *XIV*, *XVIII*, *XXII*, and *XXIV*. The deficiency, which involved thinning of the reticulation or irregular clumping of the pigment, developed gradually during life.

Among the F_2, as the table shows, 10 stocks produced nothing but blacks of the normal type, but 3 stocks gave, in some of their families, certain conspicuous variations in eye pigmentation, which were found to behave as mendelian recessive characters. These recessive types included the following.

1. *No-White*, appearing in stock *XVI*, has the white accessory pigment of the eye completely lacking. It resembles (and indeed proved to be

TABLE I.

RESULTS OF REARING THE F_2 FROM 13 PAIRS INTRODUCED FROM THE WILD INTO INCUBATOR (NOV. 1930).

No. Stock.	1. Character of F_2.	2. OCCURRENCE OF RECESSIVES. Number of families in which recessives appeared.	Family examined in which recessive was first found.	Ratio Recessive : Normal.	3. Total number of F_2.	4. Number of adequate F_2 families, or approximate equivalent.
I	All Black Normal				108	4
II	All Black Normal				255	13
VI	All Black Normal				259	4 or 5
IX	All Black Normal				212	6 or 7
XI	All Black Normal				56	2 or 3
*XIV**	"*Flesh*" *Red-eye* appears in some families	7 out of 35	2nd	approx. 1 to 3 ratio	2,917	c. 40
	"*Beet*" *Red-eye* appears in some families	6 out of 35	4th	approx. 1 to 3 ratio		
XV	"*Flesh*" *Red-eye* appears in some families	2 out of 3	1st	apparent 1 to 3 ratio	54	3
XVI	Black "*No-white*" eye appears in some families	3 out of 7	3rd	apparent 1 ro 3 ratio	234	6 or 7
XVIII	All Black Normal				116	6
XX	All Black Normal				158	10
XXII	All Black Normal				178	9
XXIII	All Black Normal				28	1
XXIV	All Black Normal				152	6 or 7

TOTAL NUMBER OF PAIRS 13

EFFECTIVE NUMBER OF PAIRS (i.e. equivalent value of pairs giving a fully adequate F_2, calculated from probabilities given by figures in column 4) . (approx.) 10

Hence 4 recessive genes occurred in an equivalent of 10 pairs (= *circ.* 20 individuals).

N.B. Only those Stocks which gave an F_2 are included. The young were not generally examined immediately after extrusion.

genetically identical with) the already familiar No-White recessive form which occurred in the earliest of the Mutant Stocks (1).

2. *Flesh Red-eye* appeared in two separate stocks, *XIV* and *XV*. The eye is typically of a pale red colour. The production of black pigment is postponed and greatly retarded, so that even in old specimens there is no more than a central darkening. (For further description see p. 345.)

* This stock (*XIV*), containing two recessive forms, is being maintained as Mutant Stock VII.

3. *Beet Red-eye* appeared in stock *XIV* and segregated independently of Flesh Red-eye. The eye has a red colour at extrusion, but there is an appreciable amount of dark pigment present, which gives it the appearance of New, Intermediate, or Dark Red (**4**, Plate VIII). During the earlier growth stages the eye darkens rapidly to a Reddish Black, or even Black, the final state varying among individuals. Beet Red is quite unlike any other recessive form hitherto discovered (see p. 350).

There is no reason for supposing that the occurrence of these recessive forms is in any way connected with the tendency to reddishness or deficiency in white pigment noted above. Not only did the latter conditions exist in stocks which produced no recessives, but in those stocks in which the recessives occurred, pure and impure dominant were affected alike.

The manner of occurrence of the recessive forms is susceptible of the simple explanation that one of the original parents of the stock had been heterozygous for the recessive factor involved. Half the F_1 would therefore be heterozygous, and on the average 1 in 4 F_2 families would contain recessives in a 1 to 3 ratio. That this condition actually held is strongly supported by evidence from data dealt with in subsequent sections of this paper.

This implies that, among the 26 original parents, there existed 4 recessive genes in a heterozygous state, the gene for Flesh occurring twice. The question whether any of the original parents may have mutated or whether the genes were brought in from the wild, is now receiving special investigation, and cannot be discussed at this stage.

Since the probability of detecting the recessive genes carried among the animals which constituted the original pairs is dependent on the number of F_2 families obtained from an in-bred F_1, there is in practice always a greater or lesser chance that any present will escape notice. In this case, the presence of 4 genes was detected among the 26 animals ; but for an estimate of the *total* number of such genes as were present, this constitutes a minimum figure. Calculation, based on the number of F_2 families in each case, shows that among the 13 stocks there was an approximately 77% chance of recessives appearing. If 77% probability gives 4, 100% probability should give 5—hence for an estimate of the number of recessive genes carried among the animals introduced into the incubator, the experiment shows 5 in 26. This can also be expressed as 4 in approximately 20. While the figures are far too small to indicate the true proportion, whether it be of the number of genes in the wild population, or of the mutation rate, it is convenient for purposes of comparison with similar experiments to state the results in this way. The estimated 10 pairs may be described as the " effective " number of pairs, this being an estimate of the number of pairs that would, in this case, give 4 recessive characters if a full chance were allowed for all recessives to appear.

2. Occurrence of Recessive Forms.

"*No-White.*"

No-Whites occurred in three out of seven F_2 families in stock *XVI*, constituted as follows :

	F_1 *pair.*	F_2 *family.*
1	8 Black Normal	
2	45 B. Normal	
3	21 B. Normal	4 B. No-White
4	4 B. Normal	
5	44 B. Normal	
6	52 B. Normal	9 B. No-White
9	14 B. Normal	7 B. No-White

In addition, there were 18 B. Normal and 6 B. No-Whites in a brood bowl which contained, among others, ♀ and ♂ of Pair 6.

Though, on the average, only 1 in 4 adequate families would be expected to contain No-Whites, the above results are not incompatible with the supposition that No-White appears as a consequence of the heterozygosity of one of the original pair *XVI*.

It so happens that the original ♂ *XVI* was involved in two other matings. An F_2 generation was obtained from each, and in each case No-Whites appeared.

(1) Mating with ♀ *VII*.

F_1 survivors : ♂, 2 ♀♀.

F_2	25 Black Normal	5 B. No-White.

(2) Mating with ♀ *XV*.

The F_1 were divided among five bowls, several reaching maturity in each. The F_2 young were periodically removed, the total proportions in each bowl being constituted as follows :

Bowl 1	44 Normal	1 No-White
2	19 Normal	6 No-White
3	72 Normal	12 No-White
4	43 Normal	—
5	147 Normal	8 No-White
Total	325	27

Random mating would be expected to give a 15 to 1 proportion, with a wide deviation for samples of a few of each sex.

These results are sufficient to warrant the conclusion that ♂ *XVI* was heterozygous for No-White.

From matings between these No-Whites and those of Mutant Stock I (i.e. the Stock containing the original Red-eye recessives, Allen and Sexton, **1** : for No-White see p. 326), No-Whites only were obtained. It is therefore concluded that the same gene (*w*) is involved. Since No-Whites have occasionally been found in the wild (this is stated on Mrs. Sexton's

authority), this recessive gene is doubtlessly distributed among the wild population. There is no need to go further afield for an adequate explanation of the appearance of the recessive No-Whites.

"Flesh Red-eye."

Animals with pure red eyes, varying from "Normal Red" (**4**, Plate VIII) to almost colourless, occurred in several of the various F_2 families of stock *XIV*, as well as among the few of stock *XV*.

In stock *XV* the F_2 families were as follows :

Pair 1	8 Black	3 Flesh Red
♀4 ♂4	6 Black	
♀4 ♂3	13 Black	
Pairs 6 (2 ♀♀, 3 ♂♂)	20 Black	3 Flesh Red

In stock *XIV* Flesh Reds were given by the following matings :

Pair 6	59	Black	24	Flesh Red
Pair 16	39	,,	20	,,
♀18× ♂6	47	,,	15	,,
Pair 22	83	,, †	29	,,
♀28×♂21	54	,, †	23	,,
♀49b×♂14	53	,,	24	,,
♀10× ♂42	25	,, †	9	,,
♀10× ♂46	36	,, †	12	,,
Pair 33	29	,, †	6	,,
♀33×♂36	11	,, †	6	,,
♀4× ♂2	15	,,	5	,,
♀9× ♂2	27	,,	10	,,
♀12 ♂14	17	,, †	5	,,
♀12 ♂21	8	,, †	2	,,
♀30×♂36	15	,,	6	,,
♀13b ♂16	7	,, †	6	,,
♀13b ♂22	16	,,	4	,,
♀31× ♂31	25	,,	10	,,
♀31× ♂32	10	,,	4	,,
♀54×♂31	13	,,	2	,,
♀ 7*	5	,, †	2	,,
♀10*	4	,, †	3	,,
♀16*	4	,,	1	,,
♀22*	6	,,	1	,,
	608		229	

N.B. The broods were not necessarily examined at extrusion. The recessives, however, gave not the least indication of being less viable than the dominants.

* Mated in brood bowl. † Including Beet Reds (see below).

The total ratio in these families is 608 to 229, or 2·66 to 1. The divergence from the expected 628 to 209 proportion (for which the larger families are responsible) is less than twice the standard deviation and is not to be regarded as significant.

A considerable number of the F_1 of stock *XIV* were mated among each other, the matings being controlled so that the genetic potentialities of as many individuals as possible might be revealed (see section 3). Among 72 (36 ♀♀ and 36 ♂♂) which gave any F_2, 28 (16 ♀♀ and 12 ♂♂) were involved in matings giving "Flesh Reds," while 28 (11 ♀♀ and 17 ♂♂) gave adequate families containing no Flesh when mated with individuals that were known to carry it. There were, in addition, 4 pairs of unknown constitution which gave adequate families containing no Flesh.

This result is in agreement with the expected genetic interpretation that approximately half the F_1 were heterozygous, and half homozygous dominant. Stated in genetic terms, therefore, 28 were shown to be heterozygous; while the number of homozygous is 30—including 28, proved directly, and a further 2, representing the probable excess of homozygous over heterozygous in the 4 pairs mentioned above. So, if the recessive gene concerned is denoted as *f*,

30 represents the number of *FF* types
28 ,, ,, *Ff* ,, .

Ample evidence that the Flesh Reds in stock *XIV* were genetically identical with those in stock *XV* was obtained by different crosses between the two stocks.

The ♀ of original pair *XV* was also mated with the ♂ of *XVI* (see p. 340). The F_2 from this mating has already been described. Though ample opportunity was given for recessive characters to show (cf. appearance of No-White), no Flesh Reds appeared. Hence it may be concluded that ♀ *XV* did not carry Flesh Red. The onus therefore falls on the ♂.

The above results fall in line with the supposition that one member of each of the original pairs *XIV* and *XV* was heterozygous for a recessive gene *f* (in *XV* it must have been the ♂). The *ff* types have a red eye, characteristically of a pale normal shade. (For further details of the character of *ff* types see p. 345.) The expected equal proportions of homozygous and heterozygous in the F_1 is well seen in stock *XIV*.

It may be further noted that the recessive Flesh Reds gave no signs of being weaker, less productive, less vigorous, or in any way less viable than the normal dominants. This is all the more noteworthy as the whole stock was conspicuously healthy.

The 80 F_2 Flesh which reached maturity included 35 ♀♀ and 45 ♂♂.

"*Beet Red-eye.*"

Individuals with darkening red eyes occurred among the young in some of the F_2 families of stock *XIV*. It was soon found that these

showed a characteristic course of colour change, falling into a definite category to which the term " Beet Red-eye " was applied (see p. 350). While it was always possible to distinguish Beets from Flesh Reds, Beets could only be unquestionably separated from Blacks in the earliest stages.

It was also soon evident that the Beets were segregating in the manner of a recessive character, the segregation being independent of that of the Flesh Reds. Some matings gave Flesh only, others Beet only, and others gave both. The composition of the families in which Beets appeared was as follows :—

a.	♀10 × ♂42	20	Black	5	Beet Red (f)
	♀10 × ♂46	28	,,	8	,, (f)
	♀10 × *	2	,,	2	,, (f)
	♀13a × ♂36	18	,,	6	,,
	♀13b × ♂22	14	,,	2	,, (f)
	♀13b × ♂43	37	,,	9	,,
	Pair 14	0	,,	2	,,
	♀19 × ♂14	33	,,	3	,,
	♀19 × ♂20	10	,,	4	,,
	Pair 22	9	,,	3	,, (f)
	♀28 × ♂21	44	,,	10	,, (f)
	Pair 33	20	,,	9	,, (f)
	♀33 × ♂36	7	,,	4	,, (f)
	♀35 × ♂22	6	,,	2	,,
	♀43 × ♂22	1	,,	2	,,
	♀43 × ♂42	31	,,	7	,,
	♀43 × ♂43	9	,,	3	,,
	♀43 × ♂52	15	,,	1	,,
	♀49a × ♂14	27	,,	4	,,
	♀12 × ♂21	6	,,	2	,, (f)
		337		88	
b.	♀10 × ♂18	48	Black	17	Beet Red
	♀4 × ♂17	5	,,	1	,,
	♀7 *	3	,,	2	,, (f)
	♀12 *	3	,,	2	,,
	Pair 14	2	,,	2	,,
	♀15 *	2	,,	2	,,
	♀31 *	6	,,	1	,,
		69		27	

a. Broods examined within 2 days of extrusion.

b. Broods examined a little time after extrusion, but Beets still absolutely distinguishable from the Blacks.

Some other families and broods in which the Beets could not be separated with certainty from the Blacks, owing to age, are not included.

(f) These families also contained Flesh Reds.

* Mated in brood bowl.

The figures show an excess of Blacks over Beets on the expected 3 to 1 ratio, which is very clearly not due to the postponement of examination of the broods. If attention is confined to those examined early (a), the excess is twice the standard deviation and may indicate a significant divergence from the simple mendelian proportions. The excess is greater still, being nearly $2\frac{1}{2}$ times the standard deviation, if only the larger families (from both a and b) of over 15 are taken into account, the proportion then being 325 to 81. The figures are too small to justify any more definite pronouncements, but at least provide grounds for more detailed investigation.

Beets were subsequently found to breed true, thus confirming their genetic character. It may be supposed that a recessive gene *t* is involved.

The tests for the genetic constitution of the stock *XIV* F_1, as described for Flesh Red, can be applied at the same time to Beet Red (see p. 342). If *t* is taken to denote the recessive gene involved, the results give

31 *TT* (12 ♀♀, 17 ♂♂, 2 sex unknown).
30 *Tt* (16 ♀♀, 13 ♂♂, 1 ,, ,,).

This conforms with expectations. Presumably, therefore, either the ♂ or the ♀ of pair *XIV* was heterozygous for *t*.

Among the 80 F_2 Beets which survived to maturity, 42 were ♀♀ and 38 ♂♂.

3. Stock *XIV*—Distribution of Heterozygotes among the F_1 Generation.

From the results given by various matings among them, it was possible to deduce the genetic constitution of a considerable number of the F_1

TABLE II.

	♀	♂	sex unknown	Total.
FF	11	17	2*	30
Ff	16	12		28
Total	27	29	2	58
TT	12	17	1† 1*	31
Tt	16	13	1†	30
Total	28	30	3	61

FFTT			*FFTt*		
♀ 4	♂ 11	Total 15	♀ 5	♂ 4	Total 9
FfTT			*FfTt*		
♀ 6	♂ 4		♀ 9	♂ 7	
sex unknown 1		Total 11	sex unknown 1		Total 17

* To allow for certain pairs which gave no recessives; a correction which places the chances of detecting homozygotes and heterozygotes on equality.

† In this case shown by the constitution of the young.

family of *XIV*. It is assumed that two recessive genes, *f* and *t*, are involved. Reference has already been made to the results, which are summarised in Table II.

It is seen that homozygous dominants and heterozygotes of both types occurred in approximately equal numbers, and that the two recessive genes are at least largely independent of each other. The figures involved, however, are not sufficient to show minor deviations from the normal, as, for instance, partial linkage between *F* and *T*.

The distribution of the 72 F_1 adults investigated among the different broods of the F_1 family is as follows :—

Brood 1	♂ *FfTT*	♂ *FfTt*	2 ♂♂ unknown	
	♀ *FFTT*	♀ *FF–*	2 ♀♀ unknown	
,, 2	♂ *FFTT*	♂ *FFTT*	♂ *FFTT*	♂ *FFTT*
	♂ *FFTT*	♂ *Ff–*	♂ *FFTT*	♀ *FfTT*
	♀ *FfTt*	♀ *FfTt*	♀ *Ff–*	♀ unknown
,, 3	♂ *FFTt*	♂ *FF-*	♂ *FfTt*	♂ *FfTt*
	♀ *FFTt*	♀ *FfTT*	♀ *–Tt*	♀ *–TT*
,, 4	♂ *FFTT*	♂ *FFTt*	♂ *FFTt*	♂ *FfTt*
	♂ *FfTt*	♂ *–TT*	♂ *–Tt*	♂ *–Tt*
	♀ *FFTt*	♀ *FFTt*	♀ *FfTt*	♀ *FfTt*
	♀ *FfTt*	♀ *FfTT*	♀ *–TT*	♀ *FFTT*
,, 5	♂ *FFTT*	♂ *FFTT*	♂ *FfTT*	
	♀ *FfTT*	♀ *FfTt*	♀ *FfTt*	
,, 6	♂ *FFTT*	♂ *FFTT*	♂ *FfTt*	♂ *FfTT*
	♀ *FfTt*	♀ *FF–*	♀ *–Tt*	
,, 7	♂ *FF–*	♂ *FfTt*	♂ and ♀ unknown	
	♀ *FFTT*	♀ *FfTT*	♀ *FFTt*	
Broods 8 and after		♂ *FFTt*	♂ *FfTt*	♂ *FfTt*
	♂ *–TT*	♀ *FFTt*	♀ *FfTt*	♀ unknown
	♀ *FFTT*			

From the above it may be seen that the genotypes are not irregularly distributed. It may also be pointed out that heterozygotes of both kinds appeared in the first brood that the *XIV* pair produced in the laboratory.

4. Character of "Flesh" and "Beet" Recessives.

We may now consider more fully the appearance of Flesh and Beet recessives, and express more precisely the effect of the genes *f* and *t*.

This account may be compared with that of other recessive " red-eye " characters surveyed elsewhere (Sexton, Clark, and Spooner, p. 333 of this Journal, Vol. XVIII).

ff types.

The earliest examples of this class had eyes of a " Normal Red " shade (**4**, Plate VIII), but much paler than the typical " Normal " Red of Mutant Stock I. The appearance suggested a pinkish flesh colour, and the name " Flesh Red " was given to the recessive type. Among those reared in the incubator this pale red was the most usual colour, but the intensity of the red was subject to variation, and eye-colours ranging from typical Normal Red to colourless were obtained. At the same time, as the animals grow older, the central ommatidia begin to show darkening, owing to the production of melanic pigment.* In examples of more advanced age a thin deposit of melanin, concentrated in the centre, had formed over the whole eye.

When reared at laboratory temperatures (*circ.* 15° C.), the *ff* types did not show the same tendency to lose red pigment. The eyes were of a typical Normal Red or only slightly lighter. Though examples with very pale, and even colourless, eyes occurred in certain families, the contrast between the eye-colours in the laboratory and those in the incubator was most striking. A further difference lay in that in the laboratory darkening occurred either not at all or only to a very slight degree.

Though the above variations in the red pigment call for special consideration, for our present purpose of defining the character of *ff* types the red pigment has only secondary relevance. The essential difference between the *ff* recessives and the normal black-eyed form is that the eyes of the former are very greatly or entirely deficient in melanin. In the absence of melanin the existing red pigment becomes visible. This point is discussed in connection with other " red-eye " types (p. 308 of this Journal, Vol. XVIII). All these are more accurately termed " melanin-deficiency " types ; for it is only in respect to melanin formation that we are certain that the presence of the recessive gene is felt. Admittedly the production of red pigment, though involving a very different kind of chemical process, may yet be affected more or less directly ; but (a) there is no means of detecting any difference, as the red pigment is invisible in the black eyes of the dominants, and (b), even if there were a difference, it has still to be shown that it is not a secondary phenomenon dependent on the actual presence of black pigment and not on the genes affecting it. While, in the light of known instances of one gene affecting two widely different visible characteristics, it is not impossible that the black and red pigment should be affected at the same time, this should

* See footnote, p. 308.

not be assumed until there is definite reason for doing so. As yet no such reasons are forthcoming from possible sources of positive evidence. For instance, (i) no other body characters are known to be visibly influenced by the melanin deficiency genes, and there is no greater probability of the red pigment being affected than other characters. (ii) Evidence that the genes affect the red pigment would be obtained if there were differences in the state of the red pigment between two recessives, and if the difference were proved constantly to be associated with these recessive types. A notable difference exists, for example, between the r_1r_1 types and the *ff* types of their respective Main Stocks, at temperatures of 22° to 24° C.; a full concentration of red pigment being shown by the former and a marked deficiency by the latter. But, as is shown in the results of a cross-mating described below, the difference is not maintained when the two genotypes segregate in the same family.

In discussion of the gene *f*, it is seen from the above considerations that in the present state of knowledge we have to regard its sphere of action as limited to the process of melanin production.

The effect of *f* is very similar in kind to that of the original Red-eye gene, r_1. The process which normally takes place in the 2 or 3 days preceding extrusion, resulting in rapid accumulation of melanin in the eye, is very greatly, if not completely, retarded. At laboratory temperatures the younger mature specimens have usually not yet even started to produce melanin, and at most show only a very slight deposit in the central ommatidia. All older specimens have produced at least a little, but the darkening does not go far. In the incubator, the same effect is seen as in the case of the r_1r_1 types, namely, that the production of melanin is accelerated. Darkening may start in later immature stages, and most mature specimens have an appreciable central dark patch. It was, however, noticeable that the *ff* types darkened distinctly less readily than r_1r_1 types from the Oxford Stock which were being kept at the same time. This difference is borne out in the results of the cross-mating described below (see Fig. 1).

A mating was made between a Flesh Red ♂ and a ♀ of the above-mentioned Stock I r_1r_1 types (which was also a No-White). The F_1 were all Black, and an approximate 9 : 7 ratio of Blacks to Reds was obtained from matings among them.

	Obtained	Expected.
Black	89 (28 No-Whites)	86 (21 No-Whites)
Red	63 (14 No-Whites)	66 (17 No-Whites)

The F_2 " reds," among which would be expected recessives for each type (r_1r_1F and ffR_1), as well as double recessives, were reared to maturity and

TABLE III.

SUMMARY OF "REDS" IN F_2 OF CROSS BETWEEN "FLESH" RED AND "STOCK I RED."

	At Birth.	Reached Maturity.	Survived for Mating.	Proved Steriłe.	Proved Flesh (*ff*).	Proved Stock I Red (*rrF*).	No Result.
Normal White	49	33 { 21♂ 12♀	26 { 19♂ 7♀	4 { 2♂ 2♀	13 { 8♂ 5♀	8 { 8♂ –	1 { 1♂ –
No-White	14	4 { 3♂ 1♀	3 { 3♂ –	—	2 { 2♂ –	1 { 1♂ –	
Total	63	37 { 24♂ 13♀	29 { 22♂ 7♀	4 { 2♂ 2♀	**15** { **10**♂ **5**♀	**9** { **9**♂ –	1 { 1♂ –
Preserved			26 { 21♂ 5♀	2 { 2♂ –	14 { 9♂ 5♀	9 { 9♂ –	1 { 1♂ –

Genetic constitution		
	1 *ffRR*	3 *rrFF*
	2 *ffRr*	6 *rrFf*
	1 *ffrr*	–
	11 *ff* ?	9
	—	
	15	

as many as possible were tested by back-matings with the Flesh Stock, etc., for the purpose of ascertaining whether they were " Flesh " (ffR_1 or ffr_1r_1) or Stock I Red only (r_1r_1F). The results are given in Table III.

It may be noted at this point that the majority of the reds, though most

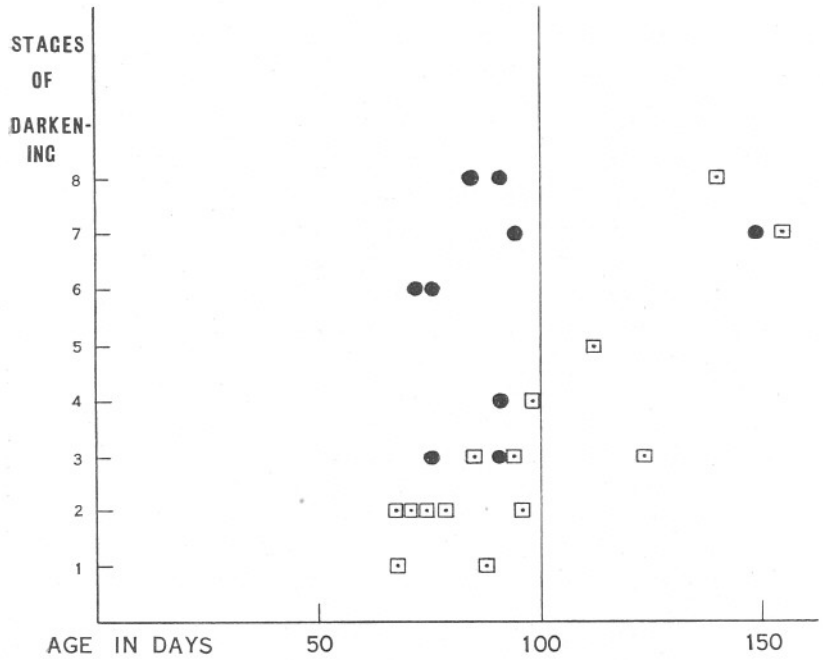

FIG. 1.—Showing that f inhibits melanin to an appreciably greater extent than r_1.
● = r_1r_1F types (maximum age value).
⊡ = ff types (minimum age value).

N.B. Since for several specimens the data of extrusion was known only within limits, the age-value cannot always be expressed exactly. However, in all cases a *maximum* has been given to r_1r_1F types, and a *minimum* to ff types. This has a considerable effect in reducing the difference between the two types, but even so the average darker state of the r_1r_1F types is unmistakable.

had plenty of red colouring when young, became deficient in red pigment as they grew older. In some cases it was almost entirely lost. There was also considerable variation in the rate of darkening. While it was always possible from later immature stages onwards to predict with some certainty that particular individuals would prove rrF and others ff, in earlier stages, and often later on, it was quite doubtful as to which type the specimen belonged.

After a sufficient family had been procured to prove genetic constitution, the F_2 " reds " were immediately preserved in spirit. When time had elapsed for the red pigment to dissolve away, examination was made of the amount of melanin that was left deposited in the eye. The 25 specimens preserved were separated into 8 classes according to the amount of melanin present. (These bear no relation to the 14 stages between red and black, used by Ford and Huxley.)

In the case of 23 it was known whether the specimen was an *ff* or an r_1r_1F type. The stage of darkening attained in these is plotted (Fig. 1) against age when preserved, the two genetic types being distinguished. The amount of pigment acquired in any given time is seen to vary considerably—no doubt partly owing to the segregation of modifying factors ; but it is quite evident that on the average the r_1r_1 types darken quicker than the *ff* types. This is particularly noticeable between the ages of 70 to 100 days. Since all other factors that might affect melanin production are, at least on the average, the same for both classes, it may be concluded that the gene *f* has a greater inhibitory influence (at these temperatures) than r_1.

With reference to the state of the red pigment among these " reds," it has been mentioned that the majority developed a marked deficiency. This variation is characteristic of the Flesh Stock, but in Stock I " reds " the pigment maintains a full concentration at incubator as well as at laboratory temperatures. Nevertheless the variation in the F_2 of the cross applied to r_1r_1F, as well as to *ff*, types. One particularly striking instance was provided by a ♂ which had a typical dark centre, but had the red colour almost completely lacking. The resulting effect was a lilac shade, very dark in the centre and fading out towards the periphery.

The question of hereditary factors underlying the variation in the red pigment requires special investigation. It has been pointed out elsewhere how in some in-bred strains a uniform concentration is maintained, while in others instability occurs through the strain, whatever recessive " melanin-deficiency " types may be contained in it.

Beet Reds.

At extrusion Beet Reds have red eyes, but, as there is always a certain amount of melanin intermixed with the red pigment, the colour is never of a pure " Normal Red." It varies, in fact, from " New Red " to " Dark Red " (**4**, Plate VIII). Subsequently, during the earlier growth stages, the eye darkens fairly rapidly, until it reaches a point at which the intensity of melanin remains more or less stable. The eye colour at this stage varies considerably among individuals. While typically approximating to a Reddish Black shade (**4**, Plate VIII), it is sometimes decidedly more reddish, and sometimes indistinguishable from Black.

Evidently the gene *t* retards the process of melanin deposition to degree comparable with a rapid-darkening r_1r_1 type (see Ford and Huxley, **2**). The resemblance, however, between the two cases cannot be carried further. In the first place, there is no striking dissimilarity between those reared in the incubator and others reared in the laboratory. No exact data are so far available, but the indication is strong that the difference in temperature has little influence on pigment deposition relative to body growth. Secondly, examination of specimens preserved in alcohol shows that at each stage during the process of darkening the melanin is uniformly distributed over the whole eye, and not concentrated in the older, central ommatidia, as in darkening r_1r_1 types. At each moult the new ommatidia show the same concentration of melanin as those in the more central part of the eye. While in the r_1r_1 types each ommatidium undergoes a similar course of pigment change, in the Beets the ommatidia are affected as a group, irrespective of their age. It appears, therefore, that the gene *t* affects some stage in the processes underlying melanin production other than that affected by r_1 and *f*.

Comparison of the Effects of Different Genes on Melanin Deposition.

An increasing number of genes are being found to influence the course of melanin deposition in the eye of *Gammarus chevreuxi*. A close resemblance is seen between the effects of some, a striking difference between others. Thus the effects of r_1 and r_2, so far as is known, are indistinguishable. Both retard very considerably the rate of melanin deposition. The gene *f*, as has been seen above, acts in essentially the same way, but has not quite such a powerful retarding influence. On the other hand, there exist between the effects of r_1 and r_5, or r_1 and *t*, differences which seem to be differences of kind rather than of degree. This suggests that different stages in the chemical processes underlying melanin production are affected, and calls for more exact comparison of the types concerned.

In their detailed investigation of r_1r_1 types, Ford and Huxley (**2** and **3**) gave prominence to the fact that the essential action of the gene r_1 is a retardation of the normally rapid process of melanin deposition in the individual ommatidia. At the same time they noted that the process was in the average case not brought to completion—a state of equilibrium was attained before the concentration of melanin was still decidedly below maximum. Except in the most rapidly darkening forms, this equilibrium phase was reached at temperatures of 20°–23° C. approximately at the time of onset of maturity. With this state of affairs the condition in r_5r_5 types may be contrasted.

An account of the influence of the gene r_5 has been given elsewhere

(see p. 328 of this Journal, Vol. XVIII). Two phases in the course of melanin production are distinguished. It is now suggested that the second phase—in which (i) no marked increase in darkening takes place,* (2) temperature differences have apparently no effect, and (3) all the ommatidia attain to an equal concentration of melanin—corresponds to the equilibrium phase among darkening r_1r_1 types. The *main* result of the presence of the gene r_5 is therefore that the stable phase is brought on very much sooner, in fact, some time during the earliest growth stages. The first phase, during which melanin is being accumulated, is of very much shorter duration in r_5r_5 types. It takes place, for the most part, before extrusion, sometimes almost as rapidly as in normal eyes. On the other hand, in r_1r_1 types, the melanin does not begin to appear until after extrusion, and at room temperature may not appear at all unless the animal lives to a considerable age.

If this comparison is justified, then (i) during the first phase in r_5r_5 types darkening should be more advanced in the centre of the eye, and should be accelerated by heat; and (2) during the second phase of r_1r_1 types, increase of temperature should have no effect, and the pigment should become uniformly concentrated over the whole eye. These points are susceptible of verification.

During the second phase, some influence is apparently at work which prevents the concentration of melanin in any part of the eye from passing a certain limit. Individual ommatidia, it seems, can darken to a certain point, but no further. Hence a uniform concentration is attained over the whole eye. A possible explanation for this phenomenon is that a precursor of the melanin cannot be formed at a sufficiently rapid rate.

While r_2, r_6, and f fall into the same category as r_1; r_4, and apparently r_3, have an effect of the kind exhibited by r_5. The r_4r_4 types, as is apparent from those born with a Dark Red eye, evidently enter on the second phase at about the period of extrusion, if not before. They differ from the r_5r_5 in showing a less variable rate of darkening in phase 1, and in a more complete inhibition of melanin production in phase 2.

The effect of the gene t, however, is different from any of the above in certain essentials. Among the tt there is uniform distribution of pigment over the whole eye during the time darkening is in progress. Since the new ommatidia at each growth-stage acquire their pigment rapidly, it follows that the rate of melanin deposition is not greatly retarded. This indicates the presence of a limiting factor of the kind seen in the second phase of, e.g., r_5r_5 types. This factor at first imposes severe restrictions, but as growth proceeds its influence becomes progressively less. The process of darkening seen during immature stages is thus the

* The relative concentration of melanin may gradually become less during this phase, especially if the phase starts early in life.

result, not of the retardation of the normal darkening process within the individual ommatidia (as in the r_1r_1), but of gradual increase of the maximum concentration attainable in the eye as a whole, owing to the gradual removal of this limiting factor.

There are, to sum up, indications that genes may influence the course of melanin production in the following ways : (1) in retarding the normal process of melanin deposition in the individual ommatidia ; (2) in imposing a limit on the concentration of melanin attainable in any part of the eye ; and (3) in shifting this limit. Following up these differences would seem to promise a fruitful line of study, which, if brought in connection with the chemistry of melanin formation, should go far towards stating the actions of the different genes in terms of reference to particular chemical processes.

My grateful acknowledgements are due to Mrs. E. W. Sexton, who has freely acquainted me with the details of previous investigations, and other useful information arising from her intimate knowledge of Gammarus. I am indebted to Miss A. R. Clark for supervising the stock during the summer of 1931 and for the help of her experience in such matters as distinguishing shades of eye-colour. I have finally to thank Dr. E. J. Allen for his kind interest and valued advice.

REFERENCES.

1. 1917. Allen, E. J., and Sexton, E. W. The Loss of Eye Pigment in *Gammarus chevreuxi*. A Mendelian Study. Journ. Mar. Biol. Assoc., N.S., XI, No. 3, 1917.
2. 1927. Ford, E. B., and Huxley, J. S. Mendelian Genes and Rates of Development in *Gammarus chevreuxi*. Brit. Journ. Exp. Zool., V, No. 2, Dec., 1927.
3. 1929. Ford, E. B., and Huxley, J. S. Genetic Rate-factors in *Gammarus*. W. Roux' Archiv f. Entwick. Mech. u. Org. Bd. 117, 67. Zweiter T., Berlin, 1929.
4. 1930. Sexton, E. W., Clark, A. R., and Spooner, G. M. Some New Eye-colour changes in *Gammarus chevreuxi*. Part I. Jour. Mar. Biol. Assoc., N.S., XVII, No. 1, 1930.