Heterozygotes in a Wild Population of *Gammarus chevreuxi* Sexton.

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CONTENTS.

DURING the progress of the work on *Gammarus chevreuxi* it became increasingly evident that heterozygosity must exist in the wild population.

In the hope of solving the problem, a large collection was made in 1931, the "M. Expt." (**4,** p. 381), but an outbreak of bacterial infection in the bowls rendered the experiment a failure.

In 1933 it was decided to take another dredging and to work from as large a collection as possible in order to settle the question once for all.

This is the experiment described below.

THE 1933 D. EXPERIMENT.

The animals were divided into two lots, half being kept in as near natural conditions as possible, half in the incubator.

The air temperature in the incubator was kept at 21° C., and the water in the bowls registered a half a degree lower.

For the "natural conditions" a large outside shed was prepared, facing east, but shaded so that no direct sunlight entered. Daily readings were taken with Negretti and Zambra's standardised maximum and minimum thermometers of the temperature of the air and of the water

in the bowls. The temperature ranged from 25·8° C. to —1·5° below freezing-point.

Summaries given in the following Table show the highest and lowest of the daily readings for air and water respectively and the average for the month of the maximum and minimum temperatures. The readings were taken at approximately the same time daily (10 a.m.) from February 20, 1933, to January 31, 1934.

TABLE I.

Date of Record.	Maximum temperature °C.		Date of record.	Minimum temperature °C.		Month and no. of readings.	Monthly average temperature °C.	
1933.	Water.	Air.		Water.	Air.		Water.	Air.
Feb. 22	6·5		Feb. 20	1·5	1·5	Feb. (4)	4·87	5·62
26, 28		7·0						
March 29, 30	12·0		March 1	6·0		March (30)	8·3	8·74
30		13·75	1, 21		6·0			
April 12	14·25		April 20, 22, 23	7·75		April (30)	10·25	10·35
14		15·75	20		6·25			
May 25	18·3		May 9, 10	10·75		May (28)	13·09	13·06
20		20·0	1		9·5			
June 8	22·5		June 22, 23	14·0		June (30)	16·45	16·6
9		24·5	22		12·75			
July 5	22·25		July 3	15·5		July (29)	17·98	18·26
27		24·5	1		14·1			
Aug. 7	23·8	25·8	Aug. 22, 23, 24	16·0		Aug. (25)	18·42	18·47
			22, 24		15·0			
Sept. 8	21·9		Sept. 28	9·0	7·5	Sept. (26)	16·6	16·57
4		23·0						
Oct. 1, 3	17·6		Oct. 30	7·25	6·0	Oct. (24)	12·8	12·74
3		19·0						
Nov. 3	11·4		Nov. 28	5·0		Nov. (29)	7·86	7·69
2		11·1	26, 27		4·0			
Dec. 2	8·0	8·0	Dec. 14	0·0	—1·5	Dec. (24)	3·16	3·01
1934.			1934.			1934.		
Jan. 19	9·6	10·25	Jan. 1	3·7	2·75	Jan. (24)	6·04	6·19

The time of the experiment coincided with a period of depression in the numbers of the population in the ditches. Owing to this unexpected difficulty sufficiently large numbers could not be obtained at one collection, and the dredgings had therefore to be carried on from February 20 to March 13, 1933.

Each dredging, as it was brought in, was examined, the mated pairs picked out, and half of them set out in the Incubator, and half in the "natural conditions." The preponderance of males in the catches, as will be seen in the following notes, may probably be accounted for by the floods in the Meadow preventing the nets working on the bottom of the ditches where the females and young usually live.

Feb. 20 : About 50 brought in ; 15 pairs taken out for the experiment ; the remainder being males and a few immature :

Feb. 24 : The ditches were frozen over, and the ice had to be broken

to allow of dredging; 79 animals captured, 24 pairs taken out; the rest consisting of 30 males and 1 immature:

March 3: The ditches were full of water, and only the Gammarus swimming could be caught with the nets; 191 adults were taken—168 males and 23 females; 22 pairs were set out:

March 7: The Meadow and ditches were flooded like a pond, and even the bridge was knee-deep under water. The whole catch for a day's work numbered only 40, 37 males and 3 females; 3 pairs set out:

March 10: The water had subsided sufficiently far to permit of dredging in the ditches, but the Gammarus were found higher up the Meadow than usual, in the shallower water. 117 were caught, 67 males, 47 females, and 3 immature; 28 pairs were set out:

March 13: 130 were brought in, 69 males and 61 females; 60 pairs were set out:

The experiment now numbered 76 pairs in each division.

Two later dredgings were made, one on April 20, when the total catch was 18, 5 males and 13 females; and one on May 9, of 34 animals, 19 males, 11 females, 1 immature and 3 young just extruded. Ten pairs from the latter haul were later included in the "natural conditions" division.

The numbers of the young hatched in the F_1 and F_2 generations, and the variations are tabulated below, followed by details of the more important mutating stocks in the "Natural Conditions" part of the experiment.

Natural Conditions.

It will be seen from the following Table that, in the Natural Conditions part of the experiment, the mutations or variations fall into definite groups, and that, apart from those changes in the coloured and the white pigments which have occurred in all our cultures, new groups have appeared, such as "Melanin-lag," or delay in the deposition of the black pigment; "Dwarfing," or delay in the body development; and "Gradual Normal" or increase in the white pigment. The two first of these can be directly traced to the effect of low temperature on the organism.

I. *Delay in black pigment deposition*—Melanin-lag. In Stocks *D. VII*; *VIII*; *IX*; *XIII*; *XVI*; *XX*; *XXIV*; *XXV*; *XXIX*; *XXXII*; *XXXIII*; *XXXVI*; *XXXVIII*; and *XLIX A*; and also in *XLIV* (*2*) and *LXX*.

From time to time in our work we have noted specimens showing a reduction in the amount of the melanic pigment deposited in the eye before birth. Sometimes we have been able to correlate this with a

FROM OUTSIDE MATING OF ♀.

Stock No.	F_1 Black.	F_1 Spotted.		F_2 Black.	
D. II A.	6	1	All reddish→B.	—	
D. III A.	19	3		417	40 with very thin retic.
D. IV A.	No eggs			—	
D. V A.	No eggs, ♀ very small.			—	
D. VI A.	No eggs			—	
D. VII A.	All eggs thrown off except 1			—	
D. VIII A.	8	6	2 spd. very reddish (1 irreg.) →B.	—	
D. IX A.	10	5	1 irreg.	—	
D. X A.	36	8 and 1 HN. spd. Dwarfing		439	1 HN. spd., 2 No-antenna regenerated to normal
D. XI A.	28	11		256 B., 29 BN., 1 HN.	**Nowhite recessive** F_2, 1 very irreg.
D. XII A.	Eggs thrown off			—	
D. XIII A.	18			27	F_3, 25 B.
D. XIV A.	No eggs ; ♀ very small			—	
D. XV A.	,,			—	
D. XVI A.	,,			—	
D. XVII A.	,,			—	
D. XVIII A.	,,			—	
D. XIX A.	,,	♀ very small		—	
D. XX A.	3 (♀ very small) 1 survivor, ♀×♂ from *XX* gave			26 B., 3 BN., 1 HN.	*Increase* of white pigment.
D. XXI A.	Eggs thrown off			—	
D. XXII A.	Eggs thrown off			—	
D. XXIII A.	23	4	1 irreg.	286	100 with deficiency of white pigment.
D. XXIV A.	11	2	Parent ♀ very small	—	
D. XXV A.	No eggs ; ♀ very small			—	
D. XXVI A.	,,	,,		—	
D. XXVII A.	,,	♀ Clotted-eyes		—	
D. XXVIII A.	,,	♀ very small		—	
D. XXIX A.	,,	,,		—	
D. XXX A.	,,	,,	another generation	—	
D. XXXI A.	15	1	1 reddish→B., gave B. offspring	385 B.,	**26 Red. Red-eye recessive** (r_2). Melanin-lag and *Increase* in white.
D. XXXII A.	30	5		202	1 reddish in winter brood.
D. XXXIII A.	30	16	Survivors 3 ♂, 11 ♀	263	(54 spotted).
D. XXXIV A.	19	3	Some very reddish	16	
D. XXXV A.	20		Ten survivors, all ♀	—	
D. XXXVI A.	25	1		36	1 pair gave young alm. BN. (1 spd.)
D. XXXVII A.	1 spd.			—	
D. XXXVIII A.	27	4		279 B., 1 BN., 1 HN.	*Gradual Normals.*

FROM INSIDE MATING OF ♀.

Stock No.	F_1 Black.		F_2 Black.
D. II	22		108 B., 1 HN.
(1) *D. III* ♂ & ♀	35		26
(2) *D. III* & *XXV* ♂	41		24
(3) *D. III* & *XII* ♂	40	2 ♀ white gonads→normal	232 B., 1 BN.
D. IV	70		22
D. V	27	2 Dwarf	151 B., 3 BN., 1 HN. *Nowhite*, not proved.
D. VI	10	♀ VI put with ♂ *LIII*	—
D. VII	59		168 B., 2 HN. Melanin-lag in 2 winter broods.
(1) *D VIII* ♂ & ♀	58		361 B. **18 Red. Red-eye recessive** in F_2, new. Melanin-lag in 1 brood.
(2) *D. VIII* & *IV* ♂	42		56
D. IX	65	2 Dwarf	354 B., 8 BN., 7 HN. and 10 alm. N. *Nowhite* not proved, Pigment-lag.
D. X	117		189 B., 2 **DR.**, 4 Reddish **Lilac N. Red-eye Recessive** new. Allied to Lilac. *Nowhite* variation.
D. XI	51		32
D. XII	57		234 B., 1 BN., 1 HN. (and 2 irreg. and malformed).
D. XIII	89		410 B., 17 BN., 1 HN. **Genotypic No-white** in F_2.
D. XIV	84	3 Dwarf	161
D. XV	71		209 1 irreg.
D. XVI	81	B., 3 BN., 4 alm. N. *Phenotypic Nowhite*	617 B., 13 BN., 2 HN. *Phenotypic No-white*. Melanin-lag.
D. XVII	60		87 *Increase* of white pigment.
D. XVIII	79		229 B., 2 BN., **7 Red. Red-eye recessive**, not proved. *No-white*.
D. XIX	92		242 B., 1 HN.
D. XX	53 and 2 irreg. and malformed →	almost normal	354 B., 2 BN. (and 1 alm. BN.). *No-white*, not proved. Melanin-lag.
D. XXI	40		44
D. XXII	36	In 1 brood, 3♀ out of 8, white gonads	117 17 alm. BN.→normal. *Increase* of white.
D. XXIII	99		232 22 with deficiency of white.
D. XXIV	35		421 B. and 3 irreg. Melanin-lag in 21.
D. XXV	50		107 B., 1 BN. Melanin-lag.
D. XXVI	45	*Gradual Nowhite*	102 B., 35 BN. and 2 alm. BN., **Genotypic Nowhite.**
D. XXVII	54	18 survivors, all females	—
D. XXVIII	83		253
D. XXIX	72 and 1 malformed, 2 normals→	malformed	549 B. and **7 Red. Red-eye** not proved. Irregularity and Melanin-lag in 1 winter brood.
D. XXX	60		263 *Increase* in white pigment.
D. XXXI	69 and 9 Irreg.	Irregularity	1054 B., 5 BN., 6 HN. spd. **Red-eye recessive** (r_2).
D. XXXII	65		211 Melanin-lag and *increase* of white
D. XXXIII	96	17 of these→BN. and 1 alm. N.	589 Excess of ♀ 39 to 13 ♂. *Gradual Nowhite*; Melanin-lag.
D. XXXIV	91	About 30, reduction of white pigment	84 (3 almost BN.).
D. XXXV	91		34
D. XXXVI	58		216 Melanin-lag in 2 broods.
D. XXXVII	49		159 and 1 HN.
♂ (1) *D. XXXVIII* ♀	21	Surv. 1 ♂, 8 ♀. Excess of ♀. Crossed with *XXXVIII A.* gave	8 5 B., 6 BN., 1 HN. and 1 alm. N. *Gradual Normal.*

OUTSIDE MATING.

Stock No.	F_1 Black.	F_1 Spotted.		F_2 Black.	
D. XXXIX A.	15		Survivors 2 ♀×♂ from *XXXIX* gave	75	
D. XL A.	Eggs disintegrated in pouch			—	
D. XLI A.	No eggs			—	
D. XLII A.	No eggs ; ♀ very small			—	
D. XLIII A.	,,			—	
D. XLIV A.	Eggs thrown off			—	
D. XLV A.	Infertile ♀, not Intersex, 7 matings with 3 males ; no eggs.				
D. XLVI A.	13	3	Three survivors, all ♀.	—	
D. XLVII A.	17	7	Two survivors, ♂	—	
D. XLVIII A.	22	6		95	
D. XLIX A.	22	3		203	B., 4 BN. and 10 alm. N.→ B. normal. *Increase* in white. Melanin-lag.
D. L A.	2		One survivor, ♂	—	
D. LI A.	24	3	One survivor ♂×♀ from *LIV A.*	27	B., 2 BN. *Nowhite*, not proved.
D. LII A.	8	3		19	
D. LIII A.	9 B. (2 spd.), **2 Red** (1 spd.) **Red-eye recessive** (r_2)			28	B. **5 Red.** 2 Dwarfs.
D. LIV A.	37			67	
D. LV	13			16	
D. LVI	9			3	
D. LVII A.	2	1	Survivors 2 ♂	—	
D. LVIII A.	20	1		13	
D. LIX A.	10	1		2	
D. LX A.	21			14	
D. LXI A.	1 spd.			—	
D. LXII A.	15	3		—	
D. LXIII A.	10	1		17	
D. LXIV A.	29	2		145	B., **3 Red. Red-eye recessive** (r_2).
D. LXV A.	6	2		—	
D. LXVI A.	15	1		1	
D. LXVII A.	23			6	
D. LXVIII A.	10	3		4	
D. LXIX A.	2	1	Survivors, 2 ♀	—	
D. LXX A.	27	1	♂×♀ of *LXX* stock gave	30	
D. LXXI A.	7			—	
D. LXXII A.	No eggs			—	
D. LXXIII A.	,,			—	
D. LXXIV A.	,,			—	
D. LXXV A.	3		Survivors, males		
D. LXXVIII A.	4	1	Survivor 1 ♀	—	
D. LXXIX A.	15	2		24	
D. LXXX A.	19	1		13	
D. LXXXI A.	24	2		2	
D. LXXXII A.	25			7	
D. LXXXIV A.	18			31	
D. LXXXV A.	33			7	
D. LXXXVII	21	1		34	B. and 2 HN. *Nowhite* not proved. In F_3 1 HN., white gonads.

INSIDE MATING.

Stock No.	F_1 Black.		F_2 Black.	
♂ (2) *D. XXXVIII* ♀	39	Surv. 2♂, 13♀. Crossed with *XXXVIII A*.	105	B., 1 HN. spd. and 4 alm. BN.
D. XXXIX	71	"White body" variation	62	B., 1 BN., 2 HN. "White body." *Nowhite*, not proved.
D. XL	100		48	
D. XLI	46		257	
D. XLII	65	Surv. 12 ♂, 7 ♀. 1 ♀ white gonads	45	2 ♂ and 1 ♀ "white body," 1 ♀ pale eggs.
D. XLIII	55	(19 spd.) excess of ♀, 41♀ to 8 ♂	286	(46 spd.) and 2 Irregulars.
D. XLIV	98		172	3 premature DR.→B.
(♂ 1) *D. XLVI* ♀	29		175	B. and **18 Red. Red-eye recessive** (r_2). Reduction of white.
(♂ 2) *D. XLVI* ♀	19			
D. XLVII	49		262	(6 almost BN.)
D. XLVIII × *LXX* ♂	10		41	(1 alm. BN.).
D. XLIX	50		258	B., 1 BN. spd., 2 HN spd.
D. L	70		186	B., 1 HN.
D. LI	55		123	B., 4 BN., 2 HN. *Nowhite*, not proved.
D. LII	29		14	
D. LIII	62		253	B., 1 BN., **23 Red, 5 RN. Red-eye recessive** (r_2) and *Nowhite*.
(2) *D. LIII* × ♂ *LXX*	37		22	B., **2 Red.**
(3) *D. LIII* × ♂ *LXI*	34		13	B., 1 HN., **3 Red** (3 B. very thin ret.)
D. LIV	71		105	
D. LV	48		131	B., 3 BN. *Phenotypic Nowhite* in F_2.
D. LVI	58		55	B. and 1 BN. malformed and much spotted.
D. LVII	16		190	
D. LVIII	47		170	
D. LIX	74		209	
D. LX	93		189	
D. LXI	62		64	B., 8 BN., 5 HN. *Nowhite*, not proved.
D. LXII	60		19	
D. LXIII	49	*Gradual Nowhite ?*	74	B., 3 BN. (and 2 alm. BN.).
D. LXIV	89	1→HN.	323	B., **20 Red. Red-eye recessive** (r_2).
D. LXV	53		114	B., 6 BN. and 65 almost BN.→B. normal. *Increase* of white.
D. LXVI	51	Excess of females, 10 to 1 ♂	11	
D. LXVII	56		41	1 sl. irreg. and much spotted.
D. LXVIII	30	B., 5 BN. Survivors, 6♂, 14 ♀	199	B., 13 BN., 17 alm. BN. *Phenotypic Nowhite*.
D. LXIX	63	Survivors, 9 ♂, 16 ♀	40	
D. LXX	58		60	Premature extrusion, 1 DR.
D. LXXI	40		92	
D. LXXII	87		28	
D. LXXIII	66	Excess of males, 15 to 6 ♀	25	
D. LXXIV	61	Only 5 survivors, 4 ♂, 1 ♀	—	
D. LXXV	74	Excess of females, 10 to 3 ♂	26	
D. LXXVIII	19	Survivors, 1 ♂, 6 ♀	8	
D. LXXIX	10		19	
D. LXXX	40		12	
D. LXXXI	35		7	
D. LXXXII	43		3	
D. LXXXIV	102	Excess of males, 17 to 4 ♀	34	
D. LXXXV	81		86	
D. LXXXVII	17	Survivors, 1 ♂, 5 ♀	5	

sudden rise in the temperature accelerating the last stages of embryonic development, by which hatching may be so hastened that the young animal is born with the eyes Reddish Black, or even Dark Red, instead of the normal jet-Black, and a few days may elapse before they darken to the right intensity.

For many years the effect of high temperatures on the growing organism has been studied, particularly in relation to the eye-pigments, but until this experiment was started nothing had ever been observed as to the effect of extreme cold. It was of course known that by lowering the temperature, the embryonic development could be retarded to a great extent but it was now found that continued extreme cold during the incubatory period could and did in some cases so delay the deposition of the black pigment as to inhibit its action completely until after the young had left the brood pouch, the eyes at birth being a bright clear red colour with no trace of the dark pigment.

D. VII.

In this stock the first brood was hatched in mid-winter, on December 2, 1933, and extruded from the pouch on December 4, 5 very small young =1 Black, 1 Reddish Black, and 3 bright Red. On the following day, two had commenced to darken, and by December 29, before the first moult, all five were jet-black, and remained so. Eggs were laid on December 4, but did not hatch ; two later broods were all Black at birth.

In another brood of 7, from the same Stock, 5 hatched before the others, viz., 2 very Reddish Blacks, and 3 bright Reds. Four days later, all had become jet-Black and the 2 remaining in the pouch were extruded, both black-eyed.

D. VIII.

A brood of 19, extruded on February 12, 1934, from eggs laid during a period of intense cold, at the end of December, consisted of 8 Black, 8 Reddish Black, and 3 Dark Red. The dark pigment took several weeks to develop, but all eventually became quite Black, and gave normal Black-eyed offspring.

D. IX.

The delay in the deposition of the pigments, retinular as well as interommatidial, was very noticeable in this stock. In one brood of 22 (8 spotted and 1 HN.) (*D. 2185*) all were hatched with more red than black showing in their eyes ; many of them were a clear deep Red colour. All became Black before maturity ; and gave Black offspring. In another brood of 9 almost Nowhite, 4 were hatched Dark Red→Black normal-eyed and gave Black offspring.

A third brood of 15 consisted of 4 Black, 5 BN., and 6 Dark Red; all died immature.

In a fourth brood, 2 were hatched, one a very Reddish Black, and one dark clear Red. Both darkened, and became quite Black in a few days.

In another brood of 18 (extruded on the same day as the first brood of 22), 1 had the right eye normal Black, and the left, bright Red and irregular, with a large spot on the same side (Cf. *D. XXXIII*, p. 328; and p. 356). It died before reaching maturity.

D. XIII.

One midwinter brood, hatched January 10, consisted of 3 very small Black, 1 spotted and 2 very reddish→Black.

D. XVI.

In one brood hatched on December 12, 1933, with the temperature below zero, 12 young were noted, as very small and humped up, with bright Red eyes. By December 29, and before the first moult had taken place, the eyes were all jet-Black (2 animals almost BN., with the white pigment failing).

D. XX.

One brood of 3, consisting of 2 Black and 1 dark clear Red. The Red darkened rapidly and in two days became quite Black.

D. XXIV.

One midwinter brood, hatched on November 25–26, consisted of 20 "Red": examined again on November 27 the eyes had darkened and became Black.

D. XXV.

In one midwinter brood of 12, 3 were born with bright Red eyes. Before maturity was reached the eyes had become jet-Black. The one survivor, a ♀, was mated with 2 Black males of the same brood, with no results.

D. XXIX.

In one midwinter brood, December 2, 1933, 3 out of a brood of 6 were Dark Red on hatching, but were quite Black ten days later.

D. XXXII.

One winter brood of 3 contained 1 Black normal and 2 Dark Red on hatching. These became Black in ten days, but did not survive to mate.

D. XXXIII.

Out of 26 pairs, one pair in 2 winter broods gave some bright Red-eyed young with the Black. The first, extruded with the temperature below zero, consisted of 24 Black (2 spd.) and 10 Red (1 spd.). The Reds were a bright clear colour which darkened gradually to RB., and then to Black.

In the second brood, examined within a day or two of extrusion, 9 were Black, 2 Dark Red, and 1 had a bright Red eye on the right side, and a normal Black left eye (cf. *D. IX*, p. 327). The two DR darkened; the Half-Red died.

D. XXXVI.

In 2 midwinter broods 1 very reddish was extruded on January 11, and 1 very reddish in a brood of 4 B. on January 17. Within a week the eyes had darkened to Black.

D. XXXVIII. A.

One brood of 7 just extruded were all dark Red, but darkened within a day or two to Black.

D. XLIX. A.

One brood hatched in midwinter, December 12, 1933, consisted of 6 young, 3 Dark Red (1 spd.), and 3 bright Red; others were in the pouch, but died. For 2 days the eye-colour remained unchanged; then it gradually darkened until, 17 days later, all six were definitely Black. By January 11, 1934, all were dead.

Two other examples, *D. XLIV* (*2*) and *D. LXX*, in which some of the young were Dark Red on extrusion, are referable to a different cause—not so much to a delay in pigment deposition, as to a premature hatching before the development in the egg was completed. In both cases the females were being examined under the microscope. In *XLIV* (*2*) three out of nine eggs were seen to break and the young emerge. Two had no white pigment, and one hardly any. They were examined 4 days afterwards, with the six others which had hatched later, all were found to be jet-Black, and to have the full amount of white pigment reticulation.

In *D. LXX*, when examining the female, 1 young was hatched and extruded on the slide. It was a rusty Dark Red, which became jet-Black within a day or two.

II. *Dwarfing.*

Many instances of greatly retarded development or "dwarfing" occurred throughout the stock, both in the F_1 and F_2.

A few typical instances are quoted below:—

D. V.

In an F_1 brood of 5, hatched on May 18, there were 3 survivors on September 15, 1 ♂ fully mature, normal size, and 2 Dwarfs. These two did not reach maturity till October 11; they mated on October 22, but their first brood was not hatched till December 11. They were still exceedingly small, but the young produced though few in number were normal size. Three broods were given in six months, 14 B., and 3 BN.—of which four, 3 B. and 1 BN. survived, all normal size, but died without offspring.

D. VIII.

Out of an F_1 brood of 28 Black, 19 survived, 2 "dwarfs exceedingly small," were seen four months later when most of the others were mature. At 9 months of age, 1 had become a very small female, the other was marked as "not yet mature." Two months later, it died "size and condition unaltered."

D. IX.

An F_1 brood, eggs laid April 4, 1933, hatched April 26; 27 Black young. On September 3, there were 14 survivors, 5 of which were mature, 7 others normal size, nearly mature, and 2 exceedingly small, which were put in a separate pot for observation. Though of over four months age, they were no larger than young animals three weeks old. By the end of September one had started to grow, and had attained normal size by October 23, a ♂; the ♀ was also sexually mature, but still exceedingly small, with 2 eggs in her pouch. These were thrown off; other broods were laid, of only 1 or 2 eggs, the female's pouch being too small to hold more. None hatched until February 15, 1934, when 1 normal sized spotted Black young was born (died immature). The female laid another batch of eggs, and was eaten by her mate. In the F_2 brood, *D. 2168*, in which melanin-lag occurred, there were 5 B. and 4 Dark Red; 1 of the 5 Black was a dwarf, and looked, when the others were mature, as if it had only just reached the first growth stage.

D. X. A.

The Outside brood was hatched on March 16, 1933, and consisted of 37 Black (9 spd. 1 HN.), of which 29 survived. Six months later, September 14, 3 of these were still so small as to look like the next generation. On October 16 they were still not mature: on November 1 they looked like young ♂ and ♀, but died without breeding.

D. XIV.

In one F_1 brood of 17 Black, hatched on May 26, 1933, and divided into two lots, seven in one bowl were mature and breeding by the beginning

of October, i.e. mature at 4 months, whilst in the other at the same date 3 only were left, exceedingly small. One of these survived but at 8 months had made no appreciable growth, and died without attaining sexual maturity.

D. LIII. A.

In one F_2 brood of 11 Black, *D. 595*, August 17, 1933, 5 were found on November 18, 3 just mature, and 2 very small and now Half Nowhite. Three months later they were still marked as exceptionally small, with no appreciable growth. One—a very small female—survived to maturity, May 24, 1934, but died without mating.

III. *Gradual Normals.*

Gradual Nowhites, with the characteristic feature of steady and gradual reduction of the interommatidial white pigment, have been recorded in different stocks from 1917 onwards.

The effect of the reduction-factor was the gradual change from normality to nowhite; but another variation, the Gradual Normal, has been found in this experiment in which the reverse action obtains, a gradual change from nowhite to normality.

Some factor or factors modifying the rate of the formation and deposition of the white pigment are evidently present in this type. In some instances the extreme cold would seem to be the excitant cause of this appearance, the low temperature retarding the formation of the white in the later embryonic stages. The result is an eye nowhite at birth, the absence of the pigment being made good later by a rapid increase in the rate, bringing the amount deposited up to normal.

It is an interesting fact that these *reduction* and *increase* factors can exist in the same stock with the Nowhite factor inhibiting the appearance of any white pigment—see e.g. *D. XIII*, with genotypic Nowhite, and Gradual Normals in the same brood. Instances of the gradual increase of white making good the deficiency caused by the extreme cold, are *D. XXXII*, and *D. XLIX*.

D. XXXII. Increase in white pigment.

Black Nowhites were given by two pairs, the young hatching on the same day, March 12, 1934. One brood numbered 5 Black normal, 4 BN.; and the other 3 B., and 5 BN. In ten days, white pigment had been deposited to the usual amount, and two animals had even developed spots. Two reached maturity and gave Black young perfectly normal-eyed.

D. XLIX. A. Increase in white pigment.

Out of the 4 F_1 pairs of this stock, one pair only gave Nowhite in its offspring. The brood was a mid-winter one, 14 in number, consisting of 10 Black almost BN. (1 spd.), with only 1 or 2 flecks of white in the eyes, and 4 BN. (1 spd.). All developed the white reticulation and became perfectly normal-eyed, and gave 6 normal-eyed young. The other broods of the pair, 1 B.; 2 B.; 4 B. (2 spd.); and 22 B. (5 spd.) were all normal-eyed.

Instances which cannot be attributed to cold and of which the cause is not evident, will be found in *D. XXX.* (see also *D. IX.*), and *D. XXXVIII.*

D. XXX. Black Nowhites appeared in the F_2 from 1 pair only, out of 15 effective pairs. The brood hatched in midwinter consisted of 20 Black (4 spd.), all with very thin reticulation, and 3 BN. All became perfectly normal-eyed, the BN. showing the commencement of a very faint white reticulation 9 days after birth, and developing the full quantity of white pigment before maturity. Two of the BN.→ normal mated and gave broods as follows: 7 Black norm.; 2 B. norm.; 19 B. norm. (2 spd.), and 1 thin retic.; and then a brood of 10 Black, 4 almost nowhite, and 3 BN. (1 spd.); and 2 B. norm. and 1 almost Nowhite.

All the members of the fourth brood→quite normal and developed the full amount of white pigment. The delay in the formation of the white pigment in this case could not be attributed to the effect of low temperature as the brood was hatched in August.

D. XXXVIII.

The ♀ *XXXVIII* produced a brood of 27 Black normals (4 spd.) in her mating with an Outside male, *XXXVIII A.* Sixteen survived, 6♂ and 10♀ and an F_2 of 281 B. was hatched.

The only appearance of anything out of the normal occurred in the third of 4 broods from Pair 2; the broods numbered 4 B.; 9 B.; 2 B. spd., 1 BN., 1 HN. spotted; and 29 Black (1 spd.) All died immature —so that the nowhite could not be tested, but they are probably the same as the Nowhites from the female's other matings.

The ♀'s brood from her "Inside" mating, *D. XXXVIII (1)*, numbered 21 Black normals, 9 reaching maturity, 1 ♂ and 8 females.

One F_2 brood of 8 B. normal was hatched in the brood-bowl, the male and female died, and to the remaining seven females, a male from the ♀'s previous brood was added (*Pair×2*). This male unfortunately proved a cannibal, and after two more F_2 broods had been hatched (*D. 2271* and *D. 2426*), all the females and some of the young were eaten.

Brood D. 2271 consisted of 8 young, none of them with normal reticulation, and all but one spotted, viz., 2 BN. spotted both sides; 2 BN. spd. right side; 2 BN. spd. left side; 1 HN. right side, only a fleck or two on left side and spotted both sides; and 1 almost BN. unspotted. This brood had an interesting development; hatched on March 30, 1934, it was re-examined on April 19—7 left, none BN.; the 1 unspotted→normal; the HN. spd. still HN. on right side, but normal on the other; of the other 5, "BN. spd.," 4 had become normal one side, and 1 on both sides, while in some the spots had changed shape and position. Re-examined later, six were found, all had become perfectly normal, with white reticulation (except one still half nowhite left side), and the spots had again altered. One male and 4 females had become mature, and had given 23 Black young (1 spd.) all normal-eyed.

Brood 2426 numbered 5 Black normal, none survived.

The ♀ then mated with a third male, *D. XXXVIII (2)*, and had 3 broods, 39 Black. Fifteen survived, with again a great preponderance of females, 13 to 2 males. Intermatings of the 2 ♂♂ and 6 females produced 105 Black (4 spd.) and 1 HN. spotted→normal later. The 6 remaining females were put with a male from *XXXVIII A.* (the female's previous brood) (*Pair*×*3*) and gave an F_2 95 young, viz., 67 Black normal, 20 BN. (1 spd.), 1 HN., and 7 almost BN. (1 spd.). The first brood of these numbered 27, none normal (*D. 2272*), 20 BN. (1 spd.), 1 HN., 6 almost BN., hatched on March 30, 1934; examined three weeks later all had become perfectly normal-eyed, and in July when they were found breeding, 47 young were taken out, all with normal eyes (and 2 spotted).

It may be worth noting that these 3 stocks (with the same female parent) gave B. normals when mated *inter se*, 8 B. and 106 B. (1 HN.), but when crossed with the other stocks, a large number were born with greatly reduced or with no reticulation, e.g.:

XXXVIII (1)×*A.* gave 5 normals to 8 greatly reduced.

XXXVIII (2)×*A.* gave 67 normals to 28 greatly reduced.

D. LXV. Increase of white pigment.

Five out of seven F_1 pairs gave young showing great deficiency of white pigment at birth up to nowhite. 118 were normal-eyed; 45 had very little white reticulation; 17 were practically nowhite, with only 1 or 2 flecks of white; and 6 were completely Nowhite. All without exception developed the normal amount of white, one of the "BN." even becoming spotted. There was no F_3 generation.

This is the stock referred to in the last paragraph (**3**, p. 693), but by a clerical error the total was put at 254 instead of 186. It should read "an F_2 of 186 Black young was hatched, consisting of 118 normal-eyed. . . ."

IV. *Variations in the interommatidial pigment.*

The two definite types of Nowhite eye were represented in this experiment, Genotype and Phenotype; in addition also Gradual Nowhites and Gradual Normals occurred and, as will be seen in Tables II and III, numerous cases of BN. and HN. were recorded in many stocks appearing sporadically. Few of these could be tested owing to the high mortality amongst them.

Several stocks are described in detail below :—

D. IX.

This stock would appear to be one of those in which several modifying factors are present. Although only a few of the variant specimens survived to maturity yet they are sufficient for evidence of the *increase* of the white pigment after birth, and of the *Phenotypic* Nowhite variation, in which the animal affected, though it never develops the white itself, is yet able to transmit it to its offspring.

The F_2 consisted of 354 Black normal eyes, 8 BN., 7 HN. and 10 almost Nowhite.

Of the 8 BN. (7 from winter broods, 1 from a June brood) 6 died before they could be proved; one, spotted, developed white pigment on the left side, none on the right, i.e. Half Nowhite. It was mated with another HN., right side, and gave Black normal young. The eighth BN. reached maturity unchanged, a male, and was proved Phenotypic by a cross with a genotypic BN., from *Stock D. XIII.* The offspring consisted of 19 Black, all with the normal white reticulation, showing that the white was brought in by the phenotype, the genotypic nowhite possessing a factor inhibiting its appearance.

Of the 7 HN., 5 were nowhite on the right side, 2 on the left. All died without offspring except one, a female; she was mated with a phenotypic BN. from Stock *D. XVI*, and gave 22 Black normals (9 with thin reticulation).

Nine of the 10 almost Nowhite survived to maturity. In 8 of these the white pigment increased to the normal quantity in both eyes; in the ninth it developed in one eye only, the left.

D. XI A., probably *Genotypic Nowhite.*

A brood of 28 Black with marked instability of the white pigment. Eleven were spotted at birth, with rather unusual uniformity in the position of the spots, 6 being situated on the dorsum, and 5 on the right side of the head.

Twenty-one reached maturity, 14 males and 7 females, but of these only 15 were effective. Seven pairs were set out, and one female after

giving 7 Black young with one male, was mated with a second male, and had 10 Black.

One pair only, Pair 2, unspotted, gave Nowhites. The offspring consisted of 65 Black (4 spotted), 28 Black Nowhites and 1 Half Nowhite (spotted on the same side as the Nowhite eye). The same female had one brood in the brood-bowl (probably with the same male) of 3 Black and 1 BN.

Another pair gave 1 with a very irregular left eye and spots on both sides, and 1 almost Nowhite out of 53 Black young, whilst a third pair had 4 almost Nowhite (2 of them with large spots both sides) amongst their 25 Black offspring.

All the other pairs hatched normals.

Four only of the BN. reached maturity, 3 males and 1 female, and of these 3 died without offspring. The remaining BN. male mated with a B. normal female of its own brood, and had 1 brood of 2 B. normal and 1 BN.

This, though not proved, is probably the *Genotypic Nowhite.*

D. XIII.

Genotypic Nowhites appeared in this stock in the F_2. They were proved by crossing with other known genotypic nowhites, with which all BN. offspring were given—and also with seven different phenotypic stocks, with which only Black normals were produced.

Seventeen F_1 pairs were effective, and out of these, three gave some Nowhites in their broods, as follows :—

Pair 8, 12 B., 1 BN. ; *Pair 11*, 9 B., 2 BN. ; *Pair 15*, 33 B., 11 BN. and 7 almost BN. at birth (→normal later) ; and 2 females, mating in the brood-bowls gave 6 B., 1 BN. and 4 B., 2 BN., respectively. All the other matings gave normal-eyed young.

A good example of the *Gradual Normals* appearing in the same family with the genotypic nowhites will be found in the *Pair 15* offspring, the 7 hatched "almost nowhite" had become fully normal a month later. In another family (*Pair 13*) 1 hatched almost Nowhite →HN.→ perfectly normal ; a second one, almost N.→HN. on the right side, perfectly normal on the left, gave all normal-eyed young.

D. XVI. Phenotypic Nowhites.

The Nowhites appeared in the F_1. Four broods were hatched, the first with all the young, 17, normal-eyed. The second numbered 33, one almost BN. ; one of the normals →BN. later and one →HN. in the right eye, left eye part nowhite. The third consisted of 19 B., 1 BN. and 3 partly BN., all with the reticulation thin and broken. One normal →BN. and one of the part-Nowhites →BN. female.

The latter female, and one of the normal eyed B. females had rows of "*white-eggs*" in the gonads (cf. Lilac, **4**, p. 394). Like the Lilac "white-egg" females the normal B. female gave no offspring, but the BN. female, though her gonads looked the same, laid eggs tinged with pale green colour, and evidently fertile, two small broods of 4 B. normals hatching out.

The dilution or absence of colour in the gonads and eggs appears now and again through this stock. For example, a sister female of the two just mentioned gave a brood of 3, one a BN. which had gonads and eggs of a bright emerald-green shade instead of the usual dark green verging on black.

The fourth F_1 brood consisted of 12 Black, one almost Nowhite and 2 BN.

A number of test matings were made, crossing the phenotypic nowhites of this stock with the genotypic Nowhites of *Stocks I*, *D. XIII* and *D. XXVI*; with phenotypic Nowhites of other stocks; and finally mating them together in the stock. In all the cases, only normal-eyed Black young were produced, all with the usual amount of white pigment, but it is a curious fact and not without significance that all the survivors without exception reached maturity with very little or none of the white pigment left, as if the deposition of the pigment must have been reduced or ceased soon after hatching.

One pair of the F_1 survivors of the *D. XVI* BN. matings gave a brood of 3 young, 1 Black and 2 BN.

D. XVIII. Phenotypic Nowhite.

The Nowhite occurred in one brood only, of 8 Black (1 spd.), 2 BN., from a back cross mating of the parent ♂ *XVIII* with an F_1 B. ♀.

The 2 BN. reached maturity, male and female, and mated. The female was eaten, and the male was then tested with two different genotypic Nowhite stocks, *D. XIII* and *D. XXVI*, and gave Black normal-eyed with both. The survivors, as recorded for similar matings in the previous Stock *XVI*, all suffered reduction and loss of the white pigment.

D. XX A. and *XX.*

Six pairs and 13 mixed matings were made amongst the F_1 survivors of the Inside mating of ♀ *XX*. Only one pair gave anything out of the ordinary, *Pair 1*, producing 2 BN. in a brood with 25 Black. The male was then mated with the only survivor of the Outside *XX* brood, a Black female—and had offspring, 25 Black normal, 3 BN. and 1 almost BN→ perfectly normal (increase of white pigment) and 1 HN.

D. XXVI. Genotypic Nowhite.

The Nowhite of this stock differs from *D. XIII*, the other genotypic Nowhite which arose in the D Experiment, in that a *gradual* reduction of the white pigment ending in Nowhite (in appearance) took place in the F_1 generation, and the pure heritable Nowhites segregated out in the F_2. The gradual reduction is not exactly comparable with our "Gradual Nowhite" variation (**3**, p. 693).

Eleven F_1 survived, but only 3 pairs gave offspring—and one Back Cross of ♂ of *Pair 3* with the F_2 BN. ♀ from *Pair 1*. The results were as follows :—

Pair 1. Both parents had become practically BN. ; they gave 1 brood of 8 Black (1 spd.) and 1 BN. (→♀ and mated with ♂ *3*).

Pair 2. Parents with thin reticulation, had 55 Black (10 spd.), 2 almost Nowhite (1 spd.).

Pair 3. The male had become completely Nowhite, the female almost. Six broods were hatched, 41 Black (14 spd.) and 34 BN. (1 spd.).

The figures for the different broods are, 2 B. (spd.), 6 BN. ; 3 B. (2 spd.) ; 14 B. (4 spd.), 11 BN. (1 spd.) ; 3 B. (2 spd.), 10 BN. ; 16 B. (3 spd.), 6 BN. ; and 3 B. (1 spd.), 1 BN.

After six weeks 15 survivors of the B. normals were examined, 11 had remained normal, but 3 were now BN. and 1 almost BN. There were 15 survivors of the BN, which, mated later *inter se*, gave all BN. offspring.

The Back Cross referred to above of the BN. male *Pair 3* with the F_2 BN. female from *Pair 1*, was a mating between two types of Nowhite (phenotypic and genotypic). One brood was produced, 1 Black and 5 BN. One only survived, a BN. ♀, which was mated with the Genotypic Nowhite of Stock I and gave 20 BN. young. Many other crosses were made with the Nowhites of other types, giving as to be expected, Black normals with the phenotypic and Black Nowhites with the genotypic.

D. XXXIII is a typical example of the *Gradual Nowhite.*

Five broods were hatched in the F_1, all perfectly normal-eyed at birth, but all the survivors suffered the gradual reduction of the white pigment characteristic of this variation, ending in 17 cases as completely Nowhite and in 1 other case almost nowhite. Fifty-two animals reached maturity ; females were greatly in excess, 13 males to 39 females (cf. also *XXXI A*. 3 ♂, 11 ♀ survivors).

The offspring from their matings, 589 in number, were all born with normal-eyes, but these, as with their parents, steadily lost the white pigment. Out of the 33 survivors, only 16 remained fairly normal, 1 had very thin thread-like reticulation, 6 were almost BN. with only 1 or 2 flecks of white and 4 were completely BN.

An F_3 of 66 was given ; they were examined as they were approaching maturity, when 54 were found to be practically normal, 1 RB., 3 with very thin reticulation, 4 almost BN. and 4 quite BN. Two matings were made with the Black Nowhites crossed with the genotypic BN. of *D. XIII*, and one with the phenotypic Nowhite of *D. XVI*. All the offspring were normal-eyed Black.

D. LI.

Nowhite not proved. In the F_2 4 BN. and 2 HN. were given, all from the intermatings of one F_1 brood. 2 BN. and 2 HN. came from mixed matings in the brood-bowl ; the other 2 BN. from a pair out of the same bowl. (They had young as follows : 3 B. ; 7 B., 1 BN. ; and 8 B., 1 BN.) All six were unchanged at maturity, but none survived to breed.

D. LV. Phenotypic Nowhite.

Out of an F_1 of 48, only 3 males and 7 females survived to breed. The offspring from the different matings were all normal-eyed Black with the exception of 4 broods from *Pair 2*, viz. 6 Black (1 spd.) all with thin reticulation ; next 8 B. (1 spd.), 1 BN. ; 2 B. (1 spd.) ; and 4 B., 2 BN., a total of 20 Black and 3 BN.

Only 4 of the Black normals reached maturity, 2 ♂♂ (one→HN. left eye) and 2 ♀♀ (one→HN. right eye→BN.).

The HN. ♂ and ♀ mated and gave 1 brood of 3 B. normals (1 spd.) (died). The normal-eyed mated, giving 40 Black (2 spd. and 1 thin retic.) ; and mixed matings had 14 B. (2 spd.) and 1 HN. left side (the HN.→BN. ♀ and died).

The 3 *Black Nowhite*→♂♀♀. The male mated with one female and had 2 broods, 9 B. normal-eyed (1 spd.) *D. 3090* ; with the second female, 1 brood of 3 Black (1 almost nowhite) and 3 BN. (*D. 3138*, all died). This female was then tried with one of the normal males and gave 1 brood of 1 B., 1 BN. (→BN. ♀).

She was tested with a genotypic Nowhite from *D. XIII* stock, and gave 3 broods, 8, 10 (1 spd.) and 10 (2 spd.), all Black normal-eyed.

In the F_3 there were 4 survivors of the *D. 3090* brood, 2 ♂♂ and 2 ♀♀, all with greatly reduced pigment ; one pair giving 1 B., 1 HN. (*D 3216*), and the other a brood of 2 BN. (D. 3217).

These 2 BN.→♂ and ♀ mated (the third BN. mating in the stock) and had 1 brood of 3 Black (2 spd.) normal-eyed but with the reticulation rather thin.

D. LXI.

Nowhite not proved. 62 F_1 were hatched in 3 broods. One brood gave offspring, 26 B., 3 BN., 2 HN. ; the second, 64 B., 5 BN. and 3 HN.

The third brood had only 1 survivor, a male evidently cannibal. The Nowhites and Half Nowhites remained unchanged, but none reached maturity. The normal-eyed matings gave an F_3 of only 6 Black normals.

D. LXIII. Gradual Nowhite ?

Six of the F_1 at maturity showed a great reduction of pigment, 2, ♂ and ♀, reaching the completely nowhite stage.

These two, *Pair 1*, gave 1 brood of 6 Black normal-eyed, but in a mating with another male (normal→thin reticulation) the female hatched a brood of 9 B. (3 spd.), 3 BN. and 2 almost BN. All the F_2 died immature.

D. LXVIII. Phenotypic Nowhite.

The F_1 numbered 30 B. and 5 BN.—but it must be noted that 10 of the Black and the 5 BN. were not examined until they were two months old, and though they may have hatched BN., yet the possibility of their being normals→BN. cannot be overlooked—one of the 10 normals became BN. at maturity.

Seven males, 6 B., 1 BN., and 14 females, 11 B., 3 BN., reached maturity. The BN. male and 3 females mated in the brood-bowl and gave 7 B. normal and 4 BN. young. The male died and the females were tried with normals of their own brood. One mating B. ♂×BN. ♀ had 66 B. (3 spd.), 8 BN. and 2 almost BN.; another of the same type produced 17 B. (3 spd.); one normal B. pair gave 18 B. (1 spd.), 1 BN. and 3 almost BN.; the other normal matings had normal offspring only.

The Nowhite was tested by crossing with the genotypic nowhite of *D. XIII*, Black normal-eyed young resulting from the mating.

V. *Changes in retinal pigment.*

There were several instances in the experiment of the changes in the retinal pigment, 6 Red-eyes and 1 new variant type, near to the Lilac-eye.

In *D. VIII*, *XVIII* and *XXIX* it was not possible to prove the constitution of the recessives owing to their scarcity and delicacy, except that in *D. VIII* the r_1 or r_2 genes were shown to be absent.

The r_2 gene reappeared in four other stocks, in *D. XXXI A.* and *XXXI*, carried by the female; in *D. XLVI* probably by the male; in *LIII A.*, where it appeared in the F_1, by both male and female; in *LIII*, *LIII* (2), *LIII* (3) by the female; and in *LXIV A.* and *LXIV* by the female.

D. VIII. Red-eye recessive, not r_1 nor r_2 gene.

Out of 58 F_1 33 survived to maturity, 10 males, 1 male (?) and 22 females. 7 males only were effective, and were mated first with females from their own brood-bowls, and six of them later with other untried

females. One pair out of them all gave Red-eyed young amongst the Black, 59 B. to 18 R. The male of the pair was mated with another female, and 16 B. young were produced, but all died immature.

There were only 9 survivors of the Reds, all males. They were tested with the Red Nowhite of Stock I and with the Red of Stock II, and proved distinct from both.

The Black intermated, but only a very small F_2 was given, 36 all Black.

The Red factor was probably introduced by ♂ *VIII*. The female gave only Black in her "Outside" mating, and in another Inside mating, with ♂ *IV*, an F_1 of 42 B., and F_2 56 B.

D. XVIII.

Red-eye recessive, not proved. Two out of eleven families gave some red-eyed in their offspring, but, counting the individuals proved for the red factor, eight were heterozygous, and one of these, ♀ 11, gave Nowhite as well.

The numbers were as follows in the broods: 12 B., 2 Red; 2 B., 1 Red; 7 B., 3 Red; and 4 B., 1 Red.

The colour was a clear bright red, more concentrated in the lower part of the eyes, with no tinge of darker pigment. It remained unchanged, but unfortunately the gene involved could not be tested as the only two males which came to maturity, and were mated, died without giving any offspring.

D. XXIX.

Red-eye recessive, not proved, and *Irregulars*. Five F_1 broods were hatched.

In the first brood of 16 Black, one animal had its head malformed on the right side, the right eye very irregular, and the white reticulation much broken→♂. Later, two others in the brood developed the same malformation→♀♀. The male and one of these females were put together as *Pair 1*, and had 21 normal-eyed B. young; the second female mated with a normal B. male, and hatched 28 normal-eyed Black. The survivors were all normals.

Twenty-five F_1 pairs were set out, 19 proving effective and 9 mixed matings. Only 2 of the pairs gave Red-eyed offspring, *Pair 8* and *Pair 14*.

A peculiarity in this strain was an irregularity in eye-structure, especially in the left eye—7 Black (6 Left, 1 Right) and 4 Reds (2 Left, 2 Right) which occurred in several broods. It varied in degree from a slight change in shape to the extreme cases in which the ommateum was broken up, with patches of ommatidia lying outside and separate from it.

The details of the appearance of the Red and the Irregularity are as follows:—

From *Pair 5*, 7 broods ; the fifth consisted of 8 Black, with thin broken reticulation, 1 almost nowhite with a large very irregular Left eye, in two separate nowhite portions, divided transversely by a bar of white pigment.

From *Pair 7*, 3 broods ; the last, of 1 Black with a very irregular Left eye, with separate patches of ommatidia.

From *Pair 8*, 7 broods ; the second (*679*) was composed of 6 Black and 1 Red ; the third (*693*) of 11 Black (4 spd.) and 5 Red ; the fourth (*1098*) of 7 Black, 4 of them very reddish and irregular ; the other broods were normal-eyed Blacks.

Brood 679. The Red-eyed specimen had an irregular, much broken-up Right eye, part nowhite. Died immature.

Brood 693 just extruded. All with more or less irregularity, and varying quantity of white pigment, and only one or two of the Blacks fairly normal. Many of the Blacks were very reddish, and 1 had an irregular Left eye drawn out behind. The Reds were the faded red colour, characteristic of this appearance, 3 of them with irregular eyes, 2 Left and 1 Right.

Brood 1098. The 4 irregular-eyed Black had patches of ommatidia almost separate from the ommateum, 3 Left eye and 1 Right. All died.

From *Pair 14*, 2 broods, 1 of 5 Black and 1 of 1 Red-eye.

There were 7 Reds altogether, but unfortunately their constitution could not be proved as none lived to maturity.

The colour was a distinct clear red without any admixture of the dark pigment, rather more dilute than the shade we call "normal red," and in consequence less brilliant and more faded looking. It did not change with growth.

The Black matings failed, only 4 F_3 being produced before the stock died out.

D. XXXI A. and XXXI. Reappearance of the r_2 gene.

The brood from the Outside mating of ♀ *XXXI* numbered 15 (1 spotted and reddish). All survived to maturity, and 6 pairs were set out in addition to mixed matings in the brood-bowl. Two of the pairs, *1* and *2*, gave red-eyed in their offspring, and ♀ *4* also proved heterozygous. Two other males, *4* and *6*, were found to be homozygous, but the rest died before their constitution could be tested.

The Red was proved to carry the r_2 gene, and to belong to the "unstable" class of eye-colour, but with no great range of colour, from bright Red to cream or Flushed white.

The results given by the heterozygous pairs are interesting for comparison.

Pair 1, 5 broods, 46 Black (3 BN. at birth and 5 almost BN. all→quite

normal later) and 17 Red. The Reds were a pale clear bright red on hatching, and remained so through life.

Pair 2, 4 broods, 16 Black and 4 Red. All 4 were the palest cream colour at birth, and developed only a faint pink in 2 or 3 central ommatidia, though their young, some produced by mating in the brood-bowl, and others with the mates of the same type of colour from another r_2 stock (*D. LXIV A.*), all had bright red eyes.

The heterozygous male *2* was mated with ♀*4* (which had given 33 Black with ♂ *4*) and gave 12 young, 5 B.; and 5 Black and 2 Reds, the same coloration as the *Pair 2* offspring.

The Red-eyes from the Inside mating of ♀ *XXXI* appeared from matings in the very irregular *D. 151* brood—a pure red, pale and clear.

Pair 20 had 7 broods: 12 Black, 1 Red; 3 Black, 1 Red; 3 B.; 5 B.; 4 B.; 14 B.; and then again 9 Black and 2 Red. Descendants of these are still living.

The male of *Pair 20* was mated with the F_1 female (of his first brood) and gave 4 B.; 4 B. (1 spd.) and 2 Red; 2 B.; 7 B.; and 2 B.

Another pair had 1 brood, 6 B., 4 BN. and 1 Red—no survivors.

D. XLVI. Reappearance of the r_2 gene.

The ♀ had three matings: (1) With an Outside ♂, 1 brood of 13 Black (3 spd.); survivors, 3 females. (2) With a male of the same dredging, Inside mating, offspring 29 Black (2 spd.); survivors, 4 males, 5 females. (3) With ♂ *D. XX*, giving 19 Black (1 spd.); survivors, 3 males and 9 females.

The two Inside matings are treated together here. The factor for Red-eye was evidently carried by the ♀ *XLVI*; both matings gave red in the F_2 when mated *inter se* or when crossed together. An F_2 of 175 Black and 18 Red was produced.

The Reds were proved and found to carry the r_2 gene, and to belong to the "unstable eye-colour" class. The colour at birth varied from bright clear red to almost white, in the same family, but the changes and fluctuations with growth were remarkable. Nine were born with bright red-eyes, 4 survivors, pale pink, almost white; 9 were pale primrose, or a faint pinkish shade, 3 survivors, full clear red at maturity.

The proportions of black to red in the broods giving the recessives were as follows:—

Pair 1. ♂♀♀, 8 B., 2 Red pale; 3 Black.

Pair 4. ♂ and the 2 ♀♀ of *Pair 1*; 12 B. (1 spd.), 3 Red pale.

Pair 4. ♂ and ♀, 2 B., 3 Red (primrose); 12 B. (1 spd.), 3 Red (1 spd.); 6 B. (1 almost BN.).

Pair 4. ♂ and 2 ♀♀ (*226*), 39 Black, 4 Red bright; probably one of the females gave Blacks only.

The 2 ♀♀ (*226*) with another ♂ of their own brood ; 1 brood, 3 B., 3 Red (2 bright, 1 pale).

All died without offspring, except the male which was crossed with a ♀ from *dd. LXXIII* (r_2 gene) and gave 3 broods of bright Red-eyed, 11, 18 and 2.

D. LIII A. Reappearance of the r_2 gene. (**2**, p. 27.)

The brood of the heterozygous ♀ *LIII* in her Outside mating consisted of 9 Black (2 spd.) and 2 Red (1 spd.), showing that the male with which she had mated in the wild was heterozygous also.

This was the first time we had had colour changes in the F_1 from the wild, and the first *direct* proof of heterozygosity existent in the wild population (see p. 355). The Red colour in these and in the F_2 reds was pale and clear, but altered later, some gaining in intensity, others losing.

LIII.

The parent female in her Inside mating gave 62 Black (3 spd.) in 2 broods.

LIII (2).

She was then mated with ♂ *D. LXX*, and gave 1 brood of 37 Black. *LIII* (3) then with ♂ *D. LXI* and had 34 Black young in 2 broods. All 3 matings gave Red-eyes in the F_2 generation.

The colour in the recessives covers a greater range than in the Main Stock II, or in any of the other reappearances of the r_2 gene, from colourless, which developed faint lemon, pink or red tints, through all shades of red and lilac to deep ruby and purple. The interommatidial pigment also varies extensively from normal reticulation, thin reticulation, to subwhite and nowhite.

The experimental work on this stock is not yet finished, and the results therefore cannot be included here, but will be discussed in a later paper.

D. LXIV A. and *LXIV.*

Reappearance of the r_2 gene " unstable eye-colour division " proved by crossing with Stock II Red (*D. XXXI A.*).

The Red factor was evidently carried by the female parent ; it appeared in the F_2 of both her Outside and Inside matings.

LXIV A.

The mixed matings in one of the brood-bowls gave 3 broods ; 12 B. (3 spd.), 1 Primrose ; 6 B. (1 spd.) ; and 13 Black (1 spd.), 2 Primrose.

Although all three had eyes so pale at birth as to be almost white, the red pigment developed later, denser in the lower part of the ommateum.

Two survived, ♂ and ♀, and gave 1 brood of 3 Red-eyes like their parents with the pigment dense in the lower part.

In *LXIV*, the Inside mating, the F_2 numbered 323 Black (20 spd.) and 20 Red (5 spd.). Out of 9 effective pairs and about 15 mixed matings Red-eyes appeared in three instances; in one mixed mating 3 B. to 1 Red; in the offspring of *Pair 4*, 32 B. (1 spd.) to 17 Red (5 spd.); and in the mating of the male of *Pair 4* with the female of *Pair 5* (gave all B.), 12 B. (1 spd.) and 2 Red.

As is usual in the " unstable " division of Stock II Red-eyes the colour varied, from a pale pink to a bright full red.

D. X. New variant type.

This is a quite distinct stock, bearing a great resemblance to the Lilac Stock (**4**, p. 395) in the colour of the recessives, the two Lilac types, Creamy and Reddish, being represented; in the behaviour of the white pigment varying from heavy reticulation to Nowhite; and in the pale colour of the gonads and eggs. Whether or not it is genotypically the same, it is not yet possible to say, owing to the scarcity and low viability of the recessives.

The F_1 numbered 117 Black (3 spd.).

Mixed matings in the brood-bowls gave an F_2 of 131 (10 spd.), all of which were normal Black, with one exception, viz. 1 almost Nowhite, with very reddish black eyes. It died immature.

In addition to the matings in the bowls, 26 pairs were set out, but only 25 of the animals proved effective, giving the small number of 64 offspring, 58 Black, 6 Red-eyed.

The Reds came from two pairs, *Pair 10* and *Pair 15*, as follows:

Pair 10 had 2 broods, neither examined at birth, one of 4 Black (1 spd.); and the other, *D. 2727*, of 1 Black and 1 Dark Reddish Lilac Nowhite.

Pair 15 had 3 broods: one, *D. 1198*, examined when just extruded, of 2 Black (1 spd.) and 2 Dark Red.

One, *D. 2206*, examined within two days of hatching, of 3 Black (2 spd.) and 3 Dark Reddish Lilac Nowhite.

And one, *D. 2474*, of 2 Black, examined when about a month old. Taking the broods in order of time :—

D. 1198. The 2 Dark Red died within a short time of extrusion.

D. 2206. The red of the 3 Nowhites was a deep clear colour when examined on February 26, 1934, a day or two after hatching. By May 14, one only was left. It had a patch in each eye of 6 clear creamy yellow ommatidia thinly outlined in dark pigment, surrounded by a broad band of deeply pigmented reddish lilac ommatidia. Re-examined on June 14, the reddish colour had intensified to purple, and the creamy patches had clouded over but were still plainly visible.

On July 10 it was marked as "Nowhite ♂, eyes all over dark reddish purple, almost black; the clear spaces are now covered."

It was mated with a Black female from *D. 2727* which had pale eggs, and white mixed with the green of the gonads. Later, the eggs darkened to the normal dark green and the white in the gonads disappeared. A brood of 5 Black was produced, *D. 3108*.

Only one of the *D. 3108* young survived to maturity, a female, which was mated with an F_4 male from *D. X A.* (the Outside mating of ♀ *X*), as no male of her own stock was available. Two broods of 16 Black (2 dorsally spotted) were given, *D. 3192*, all with "heavy reticulation" (i.e. an excess of the white pigment), and from their intermatings the heavy reticulation in the Blacks, as well as the Dark Reddish Lilac type normals and nowhites, have appeared again, but so far none of the Creamy Lilac type.

D. 2727. 1 Black and 1 Dark Reddish Lilac Nowhite; about 3 to 4 weeks old when examined. In the latter there was a patch in the centre of both eyes of light yellowish ommatidia (like the Creamy Lilac) surrounded by a deeply pigmented Dark Red band along the front margin. The specimen died immature; examined after death, the dark surrounding band had intensified to black and the colour in the centre patches had also deepened, to dark yellow. The 1 Black became a ♀ and was mated with a *B.* ♂ of *X A.*, giving a brood of 5 Black (1 spd.), all with heavy reticulation, and 2 Reddish Lilac Nowhites. Examined when nearly mature, the colour showed as a clear bright red in the centres, surrounded by pale purple; as they grew older the colours deepened in tone.

The Blacks are now breeding and have given offspring, all so far with heavy reticulation, and several spotted.

VI. *Irregularity.*

(*a*) *Irregularity of eye-shape.*

Irregularity was of frequent occurrence throughout the stock, but usually appearing sporadically not often in successive generations.

The stocks are as follows:—

D. VIII A. F_1: 1 very irregular, spotted and reddish at birth, →Black.
D. VIII F_2: 1 irregular and almost Nowhite.
D. IX A. F_1: 1 very irregular, Right eye, only 2 ommatidia.
D. XI A. F_2: 1 very irregular, Left eye.
D. XII F_2: 2 irregular and malformed heads (1 R.S. and one both sides).
D. XV F_2: 1 very irregular, Left eye.
D. XX F_1: 2 irregular malformed →practically normal-eyed and gave normal-eyed young.

D. XXIII A. F_1: 1 irregular, Right eye.

D. XXIV F_2: From 1 pair, which gave 48 B. offspring; in one brood of 8 all almost BN., 2 very irregular in both eyes; in another brood of 7 B., 1 irregular in Left eye and spotted.

D. XXIX (see p. 339) F_1: 1 with malformed head, 2 normals→malformed; great irregularity in the stock.

D. XXXI.

This stock is peculiar for the appearance of an extraordinary variety of eye-irregularity in one brood of the F_1. The brood (*D. 151*) consisted of 21 normal-eyed Black (3 spd.) and 9 irregulars. No two of these were alike, some had the eyes divided in separate pieces, others looked as if different pieces had been joined together, some had one eye much larger than the other, some were flattened, others constricted in the middle, whilst many had the ommateum outline broken and black streaks or spots outside the margin. All 9 came to maturity and were mated, 8 in 4 pairs and the ninth, a female, with a brother male of the same brood. All the matings produced young with perfectly formed normal Black eyes, and curiously enough, none of the parents' irregular types reappeared in this, or in the following generation, from their intermatings.

D. XLIII.

Two F_1 females gave irregulars. One produced only 1 Black young with an irregular Right eye, and normal Left eye at birth. The Right eye was very wide with two portions carrying ommatidia separate from the ommateum. By the time maturity was reached the two pieces had joined together vertically and coalesced with the eye, giving it an extraordinarily broad appearance; the Left eye had also become irregular with small pieces of one or two ommatidia each, separate. The animal was a female, and was mated to a normal-eyed male. One brood was produced of 6 normal-eyed Black (3 much spotted), one with a deep sub-white dorsal patch.

In the second instance, the female had 24 Black young, 12 of them with many spots and 1 with a normal Right eye and a very irregular much larger Left eye with a large piece "broken off" on the top margin.

D. LVI F_2: 1 BN. malformed and much spotted.

(*b*) *Irregularity in body and gonad colour.*

Though the genotypic White-body type did not appear in this experiment, a certain amount of variation and lack of colour in body and gonads occurred scattered through the stock. "White-eggs" had been noted before in the Lilac Stock, but in the cases recorded (**1**, p. 202) the body and gonads were the normal green shade, i.e. the eggs formed in the ovaries

showed as dark green, though when they were extruded from the oviducts into the pouch they were pearly white, and always infertile.

In the " White-eggs " females of this experiment the eggs in the ovaries were translucent white, in startling contrast to the green body colour, but by the time they had reached the pouch they had become tinted a pale nile-green shade, and were fertile. Some instances are given below :—

D. III (3).

Two F_1 females in one brood-bowl had " white-eggs " in the gonads. Microscopically examined later, one was found with the contents of the ovaries part white and part green, and the other with all green. Both extruded normal-coloured fertile eggs.

D. XVI.

In one F_1 brood-bowl, 7 females matured, 2 with " white-eggs " and 5 with dark green. When examined, 1, a Black Nowhite, had just extruded a batch of eggs into the pouch, a very pale green tint, not the normal dark colour. The second female gave no offspring.

D. XXII.

Out of 8 females in one F_1 brood, 3 had " white gonads " and 5 dark green. Two died without mating, the third gave the pale green eggs, fertile.

D. XXXIX.

The F_1 took an exceptionally long time to mature, and only 13 survived out of 71 hatched. They varied greatly in size, some looking as if they came of another generation. Five, 2 males and 3 females, had the typical *White-body* appearance, with no tinge of colour in body or gonads at maturity. It was found to be quite definitely *not* the same as the genotypic White-body (described in **4**, page 374), for while the body remained white through life, a certain amount of colour developed in the gonads and eggs. It proved impossible to trace the inheritance of this variation owing to the dwarfing, the high mortality and the difficulty of finding " white-body " animals suitable in size to mate together (only 3 of such matings were obtained) or with normal-body. There seemed to be some sort of hereditary factor running through the generations, as will be seen from the instance given below from the first " white-gonad " female to mature.

This female reached maturity at the age of six months, and was put with a male from the same brood, January 10, 1934. Both were pearly white, body and gonads. The ovaries showed as rows of round translucent white eggs. On February 4 it was noted that a pale shade of nile-green could be seen in the ovaries under the microscope. May 2, a brood of 2

Black young, both white-bodied (*D. 2411*), was hatched and new eggs laid, green in colour, but not the blackish green of the normal eggs. On June 26, again examined microscopically after another brood had hatched 3 Black (*D. 2850*), it was seen that the ovaries contained green eggs, mixed with the white ones. The ♀ was examined at intervals after broods were extruded, and it was found that more and more of the eggs formed in the ovary were coloured, and that the eggs laid were darker, though the colour concentration of the normal was never reached, and that as they ripened in the pouch they were yellow, much paler than the usual orange tone.

This pair gave 39 Black young, but only 2 survived, a normal-body male from *D. 2850* and a white-body female (*D. 2411*). The latter was mated with a white-body male and gave 2 white-body young (*D. 2924*), one of which became a female and was put with the normal body ♂ *2850*.

At the time of maturity 16 " white-eggs " were counted in the ovaries. The eggs when laid were examined microscopically and were seen to be lying in 4 rows of two in a row in the pouch, the first 2 very small, the next 2 three times the size, the next a little smaller and the last 2 smaller again. Four young were hatched (1 spd.), " pale body-colour but not white-body " (*D. 3060*); they did not survive to maturity, but the colour was unchanged at 4 months age.

D. XLII.

One F_1 female out of 19 survivors (12 ♂ and 7 ♀) had the appearance of " White-body." There was no tinge of colour in the body, but the eggs laid were the dark green normal tint. In the F_2, 3 animals out of a brood of 17 were very white, 2 males and 1 female, but were not White-body, the female having pale-coloured gonads. Died without offspring.

Incubator Conditions (Table III).

I. *Temperature and Development.*

The *temperature* in the Incubator was approximately 21° C., only rising slightly at midday, and in the coldest weather dropping a little in the early hours of the morning. The average of the daily readings (recorded on the thermograph) at 10.30 a.m. ranged from 20·13° to 20·8° February to April, 1933, 21° to 21·75° May to September, and 20·75° to 20·13° October to December.

Development.

F_1. The time taken by the parents from the wild for the eggs laid in the Incubator conditions, counting from oviposition to extrusion of the broods, was practically constant, 9 days being the usual period for all (very occasionally 10 days).

TABLE

INCUBATOR CONDITIONS.

OUTSIDE MATING.

Stock No.	F_1 Black.	F_1 Spotted.		F_2 Black.	
DD. I	1			—	
DD. II	No eggs			—	
DD. III	,,			—	
DD. IV	2			—	
DD. V	No eggs			—	
DD. VI	Eggs thrown off			—	
DD. VII	No eggs			—	
DD. VIII	,,			—	
DD. IX	15			118	B. and 1 RB. very reddish and sl. irreg.
DD. X	Eggs thrown off			—	
DD. XI	No eggs			—	
DD. XII	,,			—	
DD. XIII	,,			—	
DD. XIV	,,			—	
DD. XV	13	12	survivors 2 ♂, 8 ♀	12	
DD. XVI	20	2	1 ♂ 2 ♀→thin retic.	201	(43 spd.)
DD. XVII	23	2		151	B., 3 BN. spd., 3 HN. spd. (BN. and 1 HN. irreg.). *Nowhite*, not proved.
DD. XVIII	3 heavy retic. and 2 spotted			—	
DD. XIX	24	5	1 ♂→sl. irreg.	25	
DD. XX	Eggs thrown off			—	
DD. XXI	No eggs			—	
DD. XXII	,,			—	
DD. XXIII	,,			—	
DD. XXIV	,,			—	
DD. XXV	,,			—	
DD. XXVI	,,			—	
DD. XXVII	Eggs thrown off			—	
DD. XXVIII	,, ,,			—	
DD. XXIX	1			—	
DD. XXX	No eggs			—	
DD. XXXI	Eggs thrown off			—	
DD. XXXII	,, ,,			—	
DD. XXXIII	,, ,,			—	
DD. XXXIV	No eggs			—	
DD. XXXV	,,			—	
DD. XXXVI	,,			—	
DD. XXXVII	,,			—	
DD. XXXVIII	,,			—	
DD. XXXIX	,,			—	
DD. XL	,,			—	
DD. XLI	,,			—	
DD. XLII	32	12	Survivors, 12 ♂, 8 ♀	111	
DD. XLIII	17	11		2	
DD. XLIV	23	3		—	

III.

D EXPERIMENT.

INSIDE MATING.

Stock No.	F_1 Black.	F_2 Black.
dd. I	201 B. and 8 B. irreg. (5 ♀ irreg. out of 9 in 1 brood)	227
dd. II	85 6→thin retic.	23
dd. III	70 *Gradual Nowhite*	35 B., *1 HN. spd.
dd. IV	50	—
dd. V	110 2 ♀→RB.	26
dd. VI	127 (27 spd.)	141 B., 1 HN. spd.
dd. VII	62	45
dd. VIII	123 B., *1 HN., 5→almost BN.	49 B., 2 BN., 2 HN. *Nowhite*, not proved.
dd. IX	118 B., *2 BN. spd., *7 HN. spd. (1 ♂→ BN.) *Spotted Nowhite*	10 B., *1 HN. spd. In F_3 17 B. and *1 HN.
dd. X	140	23 B., 1 HN. spd.
dd. XI	117	184
dd. XII	94 1 ♀→BN., 4→alm. N.	144 B., 1 BN., 1 HN. irreg.
dd. XIII	91	15
dd. XIV	104	—
dd. XV	142	—
dd. XVI	110	—
dd. XVII	68	123 B., 1 BN. spd., 1 HN. spd.
dd. XVIII	84 (21 spd.) 1 ♂→alm. BN.	—
dd. XIX	126	—
dd. XX	81	147 B., 16 BN., 1 HN. *Nowhite.*
dd. XXI	44	—
dd. XXII	152	—
dd. XXIII	147	60
dd. XXIV	55	23
dd. XXV	54	10
dd. XXVI	124 Parents irreg. eyes and ♀ "clotted"	50
dd XXVII	16	—
dd. XXVIII	172 B., *1 BN. spd., *9 HN. spd. *Spotted Nowhite*	84 B., *1 BN., *2 HN.
dd. XXIX	46	—
dd. XXX	62	—
dd. XXXI	49	—
dd. XXXII	96 1 ♂→alm. BN.	3 B., *1 HN.
dd XXXIII	37 B., 1 HN.	—
dd. XXXIV	19 1 ♂→alm. N.	—
dd. XXXV	30	—
dd. XXXVI	8	—
dd. XXXVII	90	—
dd. XXXVIII	57	—
dd. XXXIX	42	2 B., *3 BN., *1 HN. **Red-eye recessive** (7 : 6, 18 : 4), prob. F_3.
dd. XL	23	—
dd. XLI	39 B., *1 BN. spd., *1 HN. spd.	120 1 heavy retic., 1 thin retic.
dd. XLII	38	9
dd. XLIII	94	180 B., 1 RB., **18 Red, Red-eye recessive** (r_2) 1 irreg.
dd. XLIV	35	—

* Not examined at birth.

TABLE III

OUTSIDE MATING.

Stock No.	F_1 Black.	F_1 Spotted.		F_2 Black.	
DD. XLV	14	6		14	
DD. XLVI	15	1		—	
DD. XLVII	No eggs			—	
DD. XLVIII	9	2	*Gradual Nowhite*	6	
DD. XLIX	Eggs thrown off			—	
DD. L	19	2		—	
DD. LI	No eggs			—	
DD. LII	20		3→alm. BN.	8	
DD. LIII	11	3		—	
DD. LIV	10			—	
DD. LV	18	9	*Gradual Nowhite*	—	
DD. LVI	10	2		—	
DD. LVII	22 B., 1 HN., 4 spd.			—	
DD. LVIII	No eggs			—	
DD. LIX	1			—	
DD. LX	No eggs			—	
DD. LXI	19	7		—	
DD. LXII	22	6	*Gradual Nowhite*	14	
DD. LXIII	16	9		—	
DD. LXIV	No eggs				
DD. LXV	13	8 spd. (7 dorsally)		36	(9 spd., 6 dorsally).
DD. LXVI	Eggs thrown off			—	
DD. LXVIII	44	4	1 ♀ × ♂ from *dd. LXVIII* gave	28	
DD. LXIX	14	2		8	
DD. LXX	19			—	
DD. LXXI	22	8	1 ♂ 4 ♀ survivors	42	(16 spd.).
DD. LXXII	36	1		—	
DD. LXXIII	27			31	
DD. LXXIV	12	1		7	
DD. LXXV	20	2	2 ♂♂→alm. HN.	33	
DD. LXXVI	28			5 B., *3 BN., *3 HN.	*Nowhite* not proved.

* Not examined at birth.

—*continued.*

INSIDE MATING.

Stock No.	F_1 Black.		F_2 Black.	
dd. XLV	66		—	
dd. XLVI	102		—	
dd. XLVII	22		—	
dd. XLVIII	60		—	
dd. XLIX	36		40	
dd. L	51		—	
dd. LI	5		—	
dd. LII	52		—	
dd. LIII	73		—	
dd. LIV	19		—	
dd. LV	66	♂ parent heavy retic.	9	BN. spd., probably F_3.
dd. LVI	37		—	
dd. LVII	48		—	
dd. LVIII	58		—	
dd. LIX	52		4	
dd. LX	47	B., *4 BN. spd., *3 HN spd. (1 BN. and 2 HN. irreg.). *Spotted Nowhite*	177	
dd. LXI	52		—	
dd. LXII	88		—	
dd. LXIII	21		1	
dd. LXIV	90		34	(8 irreg.-eyes).
dd. LXV	79	(13 survivors of 1 brood were all ♀)	—	
dd. LXVI	87		8	RB., 1 alm. Dark Red.
dd. LXVIII	38		*8	RB. (3 alm. DR. Gave B. (2 irreg.) in F_3.
dd. LXIX	27		21	2 irreg.
dd. LXX	17		13	B., 2 RB., **6 Red. Red-eye recessive** (r_2).
dd. LXXI	39		47	1 irreg., 2 "clotted."
dd. LXXII	25		—	
dd. LXXIII	42	1 *Half-Red*	538	B., 2 HN., **71 Red. Red-eye recessive** (r_2). Reduction of white pigment.
dd. LXXIV	35		12	B., *1 BN.
dd. LXXV	2	Parent ♀ heavy reticulation	—	
dd. LXXVI	76	Reduction of white, 1 ♂ "clotted"	107	B., *2 HN.

* Not examined at birth.

The age at which the F_1 became mature and started breeding varied greatly, the average for the first 3 months was from 75 to 80 days, but there was no uniformity. The shortest time was in 42 days, the longest recorded was 131, but other broods hatched at the same time as this took 56, 62, 70, 80, 84, 86, 88 ; one brood matured at 3 months but did not begin breeding till 5 months old ; another brood was not yet mature at 5 months.

It is remarkable that the development of the F_1 incubator young should in many instances take the same length of time as the F_1 in the "Natural Conditions" part of the experiment. Some of those in the heat hatched in 9 days in March and became mature at 5 months ; those in the cool laid during the same period took 34 to 36 days to hatch and 5 months to develop. In April, broods hatched at the same time after 9 days' incubation in the heat, and 23 to 25 in the cool, reached maturity in the first case at 52 days, 4 months, and $4\frac{1}{2}$ months, and in the second case at 69 days, 4 months, 4 months and 5 months.

F_2. The F_2 broods took on the average 9 to 10 days to hatch ; a few in July and August at 8 days ; only one record of 7 days, in May.

Owing to the great mortality, a large number did not reach maturity, so that there are not many records for the age of F_2 parents at the time of their first oviposition. To take one stock only, *dd. LXXIII*, the age in days varied from 37 to 74, the first mentioned being from a brood extruded on July 5, and the last from a brood extruded July 24. An August brood was 68 days old when mature and was not breeding even then.

II. *Variations in the Interommatidial Pigment.*

An interesting point in the Incubator experiment is the number of stocks in which variations of the interommatidial pigment occurred, all tending to reduction of the amount of white normally deposited in the embryonic eye.

Where such cases occurred in the "Natural Conditions," the tendency was for the amount to be made good by a rapid increase in the rate of deposition bringing the white up to the normal in the early growth-stages, but no such increase took place in these Incubator stocks.

Nowhites and Half Nowhites were frequent, but unfortunately their constitution could not be tested owing to the high mortality.

Spottedness was another feature, i.e. excess of the white pigment, as shown in spots and patches apart from the eye.

Twenty-five cases of slight Irregularity in eye-shape were also noted, in the BN., HN. and normals, and 3 Clotted-eyes were found.

The details are as follows :—

dd. III. Gradual Reduction, probably a Case of the Gradual Nowhite.

In one F_1 brood 26 Black were hatched normal-eyed on extrusion. 11 survived, but developed very slowly; microscopically examined at the age of 4 months, all were still small, only 3 males and 4 females were mature; all showed reduction of the white pigment. Seven weeks later the only survivor, a female, had become completely Nowhite. The young from the intermatings in this bowl numbered 11 B. normal-eyed and 1 HN. spotted. None survived.

dd. IX. Half Nowhites.

One F_1 brood was remarkable for the number of Half Nowhites in it; out of 30 hatched, 2 were BN. and 7 HN., 3 with the left eye nowhite and 4 with the right eye, and all the 9 were spotted (3 dorsally). None survived.

DD. XVI. Spottedness.

The F_1 " Outside " brood numbered 20 Black (2 spd.), of which 5 males and 8 females survived, 3 with a slight reduction of pigment. An F_2 of 201 was produced with 43 spotted, but 25 of these came from 1 female, mated with 2 different males. With the first she gave 13 spotted out of 17, and with the second 12 spotted out of 23.

DD. XVII. Spotted Nowhites.

Six appeared in the F_3, 3 BN. and 3 HN. all spotted, and 3 of the BN. and 1 HN. had irregular-shape eyes also.

The Nowhite was probably brought in by the female, as in a mating with another male 1 BN. and 1 HN., both spotted, appeared in the F_2. None reached maturity.

dd. XVIII. Spottedness.

In the F_1 21 were spotted out of 84 Black. No F_2.

dd. XX. Nowhite, not proved.

The three F_1 broods totalled 81 B. normals (5 spd.). Nowhites appeared in the F_2 in the offspring from two of the broods; in *Brood 23* 1 BN. from the mixed matings in the bowl and 8 BN. from one pair; in *Brood 72* 4 BN. and 1 HN. from mixed matings, and 3 BN. from one pair. None survived to mate.

dd. XXVIII. Nowhite, not proved.

The curious point about the F_1 from this pair was the appearance of a large number of Half Nowhites in the sixth and last brood. The numbers

were: 24 B.; 38 B. (1 spd.); 24 B.; 42 B. (1 spd.); and 20 Black normals (13 of them spotted), 1 BN. spotted and 9 HN.

The only F_2 from the BN. and HN. matings were 2, 1 BN. and 1 HN., both spotted. The offspring from the normals and the other broods numbered 84 normals (3 rather thin reticulation) and 1 HN.

DD. XLVIII. Gradual Reduction, probably the Gradual Nowhite Variation.

The Outside brood consisted of 9 Black (2 spd.), of which 4 survived—1 ♂→almost Nowhite, 2 ♂♂ almost Half Nowhite and 1 ♀→ thin reticulation. Mixed matings gave 6 B. normal-eyed young. None survived.

DD. LV. Gradual Reduction.

The Outside brood numbered 18 B. (9 spd.). There were 8 survivors—1 ♂ and 1 ♀→BN., 2 ♂♂, ♀ and 3 nearly mature became almost Nowhite. No young were produced.

dd. LX. Nowhite, not proved.

This stock is like *dd. XXVIII* both in the appearance of Nowhites and Half Nowhites in one brood out of six, and in the large proportion of spotted in the same brood. The first brood numbered 8 B. (3 spd.), the second 15 Black normal-eyed (of which 13 were spotted), 4 BN. spotted, and 3 HN. spotted. The Nowhite did not reappear in the next generation.

DD. LXII. Gradual Reduction, probably the Gradual Nowhite Variation.

This Outside brood took 4 months to reach maturity. There were then 3 males and a female with thin reticulation, one male practically BN. They gave 14 B. normal-eyed young.

DD. LXV. Spottedness.

A remarkable number of spotted must be noted here as coming from a mating in the wild, 8 out of a brood of 13 being spotted (of which 7 were in the dorsal position). In the F_2 there were 9 spotted in a total of 36, 6 being dorsal.

dd. LXXVI Gradual Reduction.

Out of an F_1 of 76 Black, 1 ♂→ almost BN.; 1 ♂ and 2 ♀♀ → thin reticulation, and 1 ♂ had rather "clotted" eyes.

In the F_2, 2 HN. appeared out of 109 animals (1 thin reticulation). The F_1 from the Outside mating of the same ♀ numbered 28 Black normal, and gave an F_2 of 11, viz. 2 ♂♂ and 2 ♀♀ normal; 1 ♀ almost nowhite; 1 ♂ with only a trace of white in the left eye, right eye nowhite; 1 ♂ and 1 ♀ with right eye Nowhite; and 2 ♂♂, 1 ♀ completely Nowhite.

III. *Changes in retinal pigment.*

Four of the stocks gave Red-eye recessives in the F_2 generation. These were tested, and all were found to carry the r_2 gene, and to belong to the "stable eye-colour" category.

dd. XXXIX Red-eye recessive (r_2).

F_1 consisted of 42 Black normal-eyed. From mixed matings an F_2 of 2 B., 3 BN. and 1 HN. was produced, but as these were not examined until maturity, it is not known whether this is a case of nowhite at birth or of gradual reduction.

Some small young were found in the same bowl and judged to be F_3, 7 Black and 6 Red. Another brood from the same BN. ♀ in a bowl with 1 B. ♂ and 2 BN. ♂♂ numbered 18 Black and 4 Red.

Eight Reds survived, 7 males and 1 female, eaten later. One male became almost nowhite. One was tried with a Black female of the same brood and had 4 B. young. A second was crossed with a Red ♀ from *dd. LXXIII* stock and gave all Red young as would be expected, both carrying the r_2 gene.

dd. XLIII Red-eye recessive (r_2).

The F_1 numbered 94 Black. Three pairs survived of 1 brood and *Pair 3* gave 15 B. to 16 R. In another brood mixed mating gave 7 B., 2 R. A heterozygous B. ♂ of the F_2 was mated with a Red ♀ from *LXXIII* Stock and the Red was found to be the same constitution.

dd. LXX Red-eye recessive (r_2).

There were two small broods in the F_1, 12 B. and 5 B. respectively. From mixed matings in the first brood were obtained 10 B., 5 R. and from the second brood 3 B. ♂♂♂, 2 RB. (♂ and ♀) almost Dark R. and 1 R. ♀.

The Reds were tried with Stock *dd. LXXIII* and gave Red-eyed offspring. They were also crossed with Stock I Red Nowhite and gave Black.

dd. LXXIII. Appearance of a One-sided Red (r_2) *in the* F_1.

This is the second instance in the D. experiment of the appearance of a recessive in the F_1 from the wild (see p. 342, *D. LIII A.*).

The F_1 consisted of 42 Black normal-eyes, and 1 specimen a Half-Red, with the left eye black and the right eye bright red. It became a male and proved to behave genetically as a heterozygous Black. Mated with 2 heterozygous females, it gave 77 B. and 22 Red with one, 25 B. and 12 R. with the other. It was then mated with 3 F_2 Red females giving 11 B., 10 Red with one, 10 B. and 11 R. with the second, but with the third (from its own mating with one of the B. females) the proportions were 2 B. to 20 Red.

The occurrence of a " one-sided " animal, i.e. an animal with the two eyes different from each other, is not infrequent in *Gammarus chevreuxi*, although such a difference in the retinal coloured pigment as shown in this instance, is very rarely found.

Two other specimens in the experiment were noted as definitely One-sided Red or Half-Red at birth, viz. one in *D. IX* (p. 327) and one in *D. XXXIII* (p. 328), but these would appear to be in another category. Neither survived, but from the fact that a slight deposit of melanin was found in the Red eyes after preservation it seems probable that the red in these eyes was due to a slowing down or a temporary cessation of melanin deposition on one side, and that if the animals had lived, the melanin deficiency would have been made good later. In the *dd. LXXIII* specimen under discussion, the red of the eye remained unchanged through life.

In the white interommatidial pigment, on the other hand, there are many records of One-sided Whites, or Half-Nowhites (HN.), i.e. animals in which at birth one eye has the normal white reticulation, and the other none. Cases also of this peculiarity in relation to eye-structure have occurred in which one eye is regular in structure, and the other irregular (see p. 344 for examples).

REFERENCES.

1. 1933. Sexton, E. W., and Clark, A. R. Further mutations in the Amphipod *Gammarus chevreuxi* Sexton. Nature, Vol. 131, pp. 201–202.

2. 1934. Sexton, E. W., and Clark, A. R. New developments in *Gammarus chevreuxi* Sexton. Nature, Vol. 133, p. 27.

3. 1936. Sexton, E. W., and Clark, A. R. Variations in the White Pigment of the Eye in *Gammarus chevreuxi* Sexton, with a description of a New Genetic Type, the " Clotted Eye." Journ. Mar. Biol. Assoc., N.S., Vol. XX, No. 3, pp. 691–699.

4. 1936. Sexton, E. W., and Clark, A. R. A Summary of the work on the Amphipod *Gammarus chevreuxi* Sexton carried out at Plymouth Laboratory (1912–1936). Journ. Mar. Biol. Assoc., N.S., Vol. XXI, No. 1, pp. 357–414.