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Induced Melanism in Lepidoptera.

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(Communicated by Sir Daniel Hall, F.R.S.—Received December 10, 1931.)

Introduction.

In two previous papers (Harrison and Garrett, 1926 ; and Harrison, 1928) the experimental production of melanic varieties of the moth *Selenia bilunaria*, Esper, was reported, following upon the feeding of the larvæ upon hawthorn leaves which had absorbed small quantities of lead and manganese salts respectively. These induced melanic forms were inherited in subsequent generations as recessives on ordinary mendelian lines. The production of these melanics was associated by Harrison with the occurrence of melanics in various species of Lepidoptera in industrial areas both in England and Germany where it might be expected that the food plants would become contaminated with metallic fumes.

The production of a variation so striking and heritable in what had hitherto appeared to be a fairly stable species seemed to call for further experimental investigation, especially as a natural melanic in the species had been reported (Mansbridge, 1927–28). While it is known that certain violent disturbances of the organism, as by treatment by X-rays, may induce " mutations " which are heritable, it is always difficult to be sure that the species in nature does not contain an occasional recessive gene which escapes observation because of the extreme rarity of the matings which would result in the emergence of the recessive phenotype. A fresh series of experiments was therefore initiated at the John Innes Horticultural Institution in order to ascertain if melanic variations could be induced with any regularity by the method indicated by Harrison. In any case it was difficult to understand that manganese should be a casual factor, in view of the invariable presence of manganese in plant tissues and the absence of evidence that the proportion is significantly greater in leaves in industrial areas.

Seven females of the Geometrid moth *Selenia bilunaria*, Esper, were caught in the spring of 1928 to start the experiment. These females were collected from different areas in Southern and Eastern England, as far as possible away from industrial areas. Three females were caught in the New Forest, two at Horseheath in Cambridgeshire, and one at Balsham in Cambridgeshire, and one near Warboys in Huntingdonshire. Each family was given a letter,

A–C being from the New Forest, D and F from Horseheath, E from Balsham and G from Huntingdonshire. In the course of breeding the strongest family, as judged by general behaviour, from each of the three areas was selected for the experiment and the remainder were discarded. The wild moths were in all respects typical spring brood females of *S. bilunaria*, and subsequent generations have all been normal, no signs of melanism having appeared at any time. The families have been inbred throughout, and the size of the broods has decreased very considerably as will be seen by comparing broods in the tables. Family D died out after the fifth inbred generation and the treated broods in family C after the fourth inbred generation. The experiment in this family, however, was restarted from a large control brood and two further generations on lead have now been reared. Family G has been inbred for six generations, four of which have received lead and manganese treatment. In each family no treatment took place until the third inbred generation, in order to make sure that there was no latent melanism in the stock.

Technique and Method of Treatment.

The experiments were carried out in a specially built insectary with glass roof and sides shaded with inside and outside blinds. On emergence females and males were selected at random as they synchronised, and were placed in a muslin sleeve to pair. Usually a large number of pairings were made, more especially in the later generations, as it was found that many of the pairings failed owing to infertility. Eggs were laid on the muslin, which was cut up into convenient pieces to fit into a 3-inch glass-topped tin box in the bottom of which a filter paper had been placed. The eggs were all counted as the sleeve was cut up. When the resulting larvæ hatched they were transferred to a further 3-inch glass-topped tin box containing the food plant *Cratgœus oxyacantha* (hawthorn), as it was found that placing the food plant in boxes in which the eggs were hatching was apt to produce mildew on the muslin and to decrease the hatch. If the weather was very hot a little water was dropped on to the filter paper at the bottom of the box containing the eggs. When the larvæ had developed to a sufficient size they were transferred to glass cylinders with a partially perforated zinc top and bottom, and the food plant was placed in glass tubes inside. The insects were allowed to pupate in the cylinders, after which they were transferred to shallow wooden boxes containing earth covered with a layer of silver sand. Each pupa was then covered with an ordinary glass tumbler and a small stick was placed inside each glass upon which the emerging insect might expand its wings.

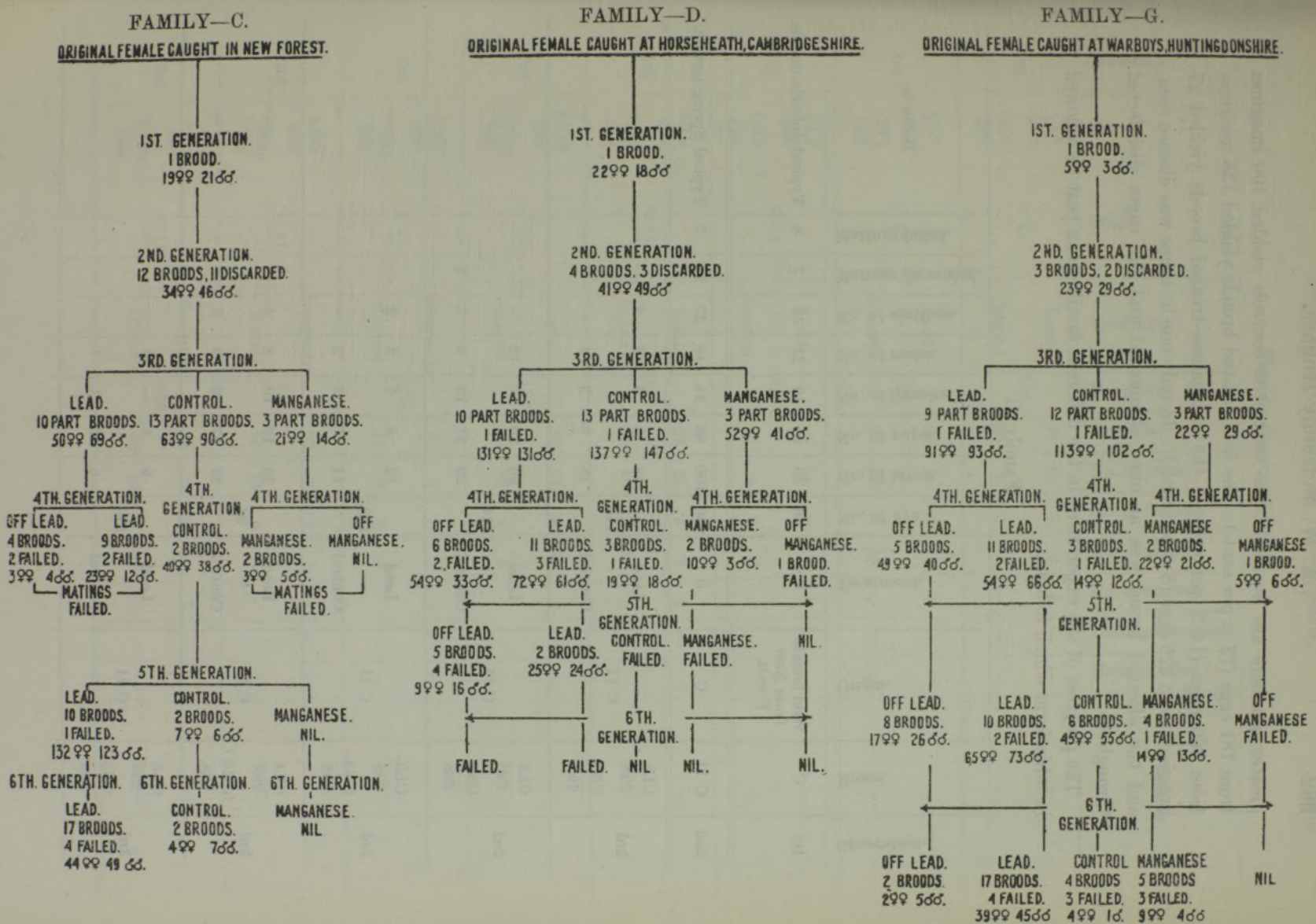
Treatment of Food Plant.

The same metallic salts as those used by Professor Harrison and Dr. Garrett were chosen for this experiment, lead nitrate and manganous sulphate. Cut twigs of hawthorn were placed with their ends in a solution of these salts in a concentration of 1 gramme per litre for at least 24 hours before use. Analyses were made from time to time to ascertain the metallic content of the foliage. In the first two treated generations a departure was made from Harrison and Garrett's method as the twigs, having been treated for not less than 24 hours, were placed in ordinary water in the breeding cylinders. In the last two treated generations the solution was also placed in the cylinders.

The Experiments.

The families retained were, C from the New Forest, D from Horseheath, Cambridgeshire, and G from Warboys, Huntingdonshire. After the first two inbred generations in which only pure untreated hawthorn was used a third generation was raised and it was decided to divide each brood into treated and control portions and to limit the controls to 50 larvæ. It was further thought that 10 lead-treated and 3 manganese-treated broods per family with their controls would be sufficient to serve as a basis for a larger fourth generation. Each brood was therefore divided and the family letter preceded the treatment letter, L meaning lead, M meaning manganese, and N meaning "nothing" or control, over the index of the year and whether Spring or July broods, S or J. Thus the first lead-treated broods were called $\frac{\text{CL1}}{29\text{S}}$, $\frac{\text{DL1}}{29\text{S}}$, $\frac{\text{GL1}}{29\text{S}}$, their controls were called $\frac{\text{CN1}}{29\text{S}}$, and so on respectively. The first manganese broods were called $\frac{\text{CM1}}{29\text{S}}$, $\frac{\text{DM1}}{29\text{S}}$, $\frac{\text{GM1}}{29\text{S}}$, their controls were differentiated from the lead controls by having alpha, beta, and gamma after them, thus $\frac{\text{CN1}\alpha}{29\text{S}}$, $\frac{\text{DN1}\beta}{29\text{S}}$, $\frac{\text{GN1}\gamma}{29\text{S}}$. In succeeding generations it was always hoped to keep at least two control broods for each family, but this was not always possible.

It will be noticed that as the families were progressively inbred their viability decreased. In the first generation from wild-collected females 107 eggs yielded 88 imagines; in the sixth generation, the one family that had been preserved in unbroken descent yielded only 109 imagines from 2497 eggs (sum of the treated and control broods). There is no evidence that the treatment had given rise to any difference of viability. In the fifth generation, for which


FAMILY—C.
ORIGINAL FEMALE CAUGHT IN NEW FOREST.
1ST. GENERATION.
1 BROOD.
19♀♀ 21♂♂.
2ND. GENERATION.
12 BROODS, 11 DISCARDED.
34♀♀ 46♂♂.
3RD. GENERATION.
LEAD.
10 PART BROODS.
50♀♀ 69♂♂.
CONTROL.
13 PART BROODS.
63♀♀ 90♂♂.
MANGANESE.
3 PART BROODS.
21♀♀ 14♂♂.
4TH. GENERATION.
OFF LEAD.
4 BROODS.
2 FAILED.
3♀♀ 4♂♂.
LEAD.
9 BROODS.
2 FAILED.
23♀♀ 12♂♂.
MATINGS FAILED.
4TH. GENERATION.
CONTROL.
2 BROODS.
40♀♀ 38♂♂.
4TH. GENERATION.
MANGANESE.
2 BROODS.
3♀♀ 5♂♂.
OFF MANGANESE.
NIL.
MATINGS FAILED.
5TH. GENERATION.
LEAD.
10 BROODS.
1 FAILED.
132♀♀ 123♂♂.
CONTROL.
2 BROODS.
7♀♀ 6♂♂.
MANGANESE.
NIL.
6TH. GENERATION.
LEAD.
17 BROODS.
4 FAILED.
44♀♀ 49♂♂.
6TH. GENERATION.
CONTROL.
2 BROODS.
4♀♀ 7♂♂.
6TH. GENERATION.
MANGANESE.
NIL
FAMILY—D.
ORIGINAL FEMALE CAUGHT AT HORSEHEATH, CAMBRIDGESHIRE.
1ST. GENERATION.
1 BROOD.
22♀♀ 18♂♂.
2ND. GENERATION.
4 BROODS. 3 DISCARDED.
41♀♀ 49♂♂.
3RD. GENERATION.
LEAD.
10 PART BROODS.
1 FAILED.
131♀♀ 131♂♂.
CONTROL.
13 PART BROODS.
1 FAILED.
137♀♀ 147♂♂.
MANGANESE.
3 PART BROODS.
52♀♀ 41♂♂.
4TH. GENERATION.
OFF LEAD.
6 BROODS.
2 FAILED.
54♀♀ 33♂♂.
LEAD.
11 BROODS.
3 FAILED.
72♀♀ 61♂♂.
4TH. GENERATION.
CONTROL.
3 BROODS.
1 FAILED.
19♀♀ 18♂♂.
4TH. GENERATION.
MANGANESE.
2 BROODS.
10♀♀ 3♂♂.
OFF MANGANESE.
1 BROOD.
FAILED.
5TH. GENERATION.
OFF LEAD.
5 BROODS.
4 FAILED.
9♀♀ 16♂♂.
LEAD.
2 BROODS.
25♀♀ 24♂♂.
CONTROL.
FAILED.
MANGANESE.
FAILED.
NIL.
6TH. GENERATION.
FAILED.
FAILED.
NIL.
NIL.
NIL.
FAMILY—G.
ORIGINAL FEMALE CAUGHT AT WARBOYS, HUNTINGDONSHIRE.
1ST. GENERATION.
1 BROOD.
5♀♀ 3♂♂.
2ND. GENERATION.
3 BROODS, 2 DISCARDED.
23♀♀ 29♂♂.
3RD. GENERATION.
LEAD.
9 PART BROODS.
1 FAILED.
91♀♀ 93♂♂.
CONTROL.
12 PART BROODS.
1 FAILED.
113♀♀ 102♂♂.
MANGANESE.
3 PART BROODS.
22♀♀ 29♂♂.
4TH. GENERATION.
OFF LEAD.
5 BROODS.
4♀♀ 40♂♂.
LEAD.
11 BROODS.
2 FAILED.
54♀♀ 66♂♂.
4TH. GENERATION.
CONTROL.
3 BROODS.
1 FAILED.
14♀♀ 12♂♂.
4TH. GENERATION.
MANGANESE
2 BROODS.
22♀♀ 21♂♂.
OFF MANGANESE
1 BROOD.
5♀♀ 6♂♂.
5TH. GENERATION.
OFF LEAD.
8 BROODS.
17♀♀ 26♂♂.
LEAD.
10 BROODS.
2 FAILED.
65♀♀ 73♂♂.
CONTROL.
6 BROODS.
45♀♀ 55♂♂.
MANGANESE.
4 BROODS.
1 FAILED.
14♀♀ 13♂♂.
OFF MANGANESE
FAILED.
6TH. GENERATION.
OFF LEAD.
2 BROODS.
2♀♀ 5♂♂.
LEAD.
17 BROODS.
4 FAILED.
39♀♀ 45♂♂
CONTROL
4 BROODS
3 FAILED.
4♀♀ 1♂.
MANGANESE
5 BROODS
3 FAILED.
9♀♀ 4♂♂
NIL

greater numbers are available, the untreated controls yielded 100 imagines from 781 eggs (12·8 per cent.); the lead-treated broods yielded 138 imagines from 960 eggs (14·3 per cent.); the manganese-treated broods yielded 27 imagines from 325 eggs (8·3 per cent.). In only one instance was disease seen, and the affected brood was at once eliminated and the cages disinfected throughout.

The progress of the experiment in the different families can best be followed in table form which follows.

Family C.

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
1st	C	Wild female from New Forest	0	46	45	40	19	21	12	7	4	Typical Juliaria insects.
2nd	C 11	C	0	200	100	85	34	46	27	11	3	Typical Spring insects.
3rd	CL1/29S	C 11	Lead	145	18	10	3	6	2	—	—	—
	CN1/29S		Control		18	8	3	4	—	—	—	—
3rd	CL2/29S	C 11	Lead	114	30	20	7	12	—	—	—	—
	CN2/29S		Control		29	21	12	9	2	2	—	
3rd	CL3/29S	C 11	Lead	123	12	8	5	2	2	—	—	—
	CN3/29S		Control		11	7	3	2	—	—	—	—
3rd	CL4/29S	C 11	Lead	94	25	19	10	8	4	—	—	—
	CN4/29S		Control		22	17	6	9	2	—	—	—
3rd	CL5/29S	C 11	Lead	136	6	4	—	3	—	—	—	—
	CN5/29S		Control		5	2	1	1	—	—	—	—

Family C—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
3rd	CL6/29S	C 11	Lead	125	24	12	3	9	2	—	—	—
	CN6/29S		Control		23	9	5	3	—	—	—	—
3rd	CL7/29S	C 11	Lead	98	23	10	3	7	2	—	—	—
	CN7/29S		Control		23	19	3	14	—	—	—	—
3rd	CL8/29S	C 11	Lead	116	12	8	6	2	1	—	—	—
	CN8/29S		Control		11	9	5	4	—	—	—	—
3rd	CL9/29S	C 11	Lead	155	29	13	5	8	2	—	—	—
	CN9/29S		Control		28	16	5	11	—	—	—	—
3rd	CL10/29S	C 11	Lead	149	30	21	8	12	2	—	—	—
	CN10/29S		Control		30	12	4	6	—	—	—	—
3rd	CM1/29S	C 11	Manganese	133	17	7	6	1	1	—	—	—
	CN1a/29S		Control		17	8	3	4	—	—	—	—
3rd	CM2/29S	C 11	Manganese	138	21	7	5	1	1	—	—	—
	CN2a/29S		Control		20	13	6	7	—	—	—	—
3rd	CM3/29S	C 11	Manganese	135	41	24	10	12	5	—	—	—
	CN3a/29S		Control		40	22	7	15	—	—	—	—

Family C—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
4th	CL1 / 29J	CL3 / 29S	0	70	15	6	1	3	—	—	—	1 female malformed, discarded.
4th	CL2 / 29J	CL3 / 29S	0	100	12	3	2	1	1	—	—	—
4th	CL3 / 29J	CL4 / 29S	Lead	99	9	2	1	1	—	—	—	—
4th	CL4 / 29J	CL8 / 29S	Lead	85	14	13	7	5	4	—	—	2 females malformed.
4th	CL5 / 29J	CL4 / 29S	Lead	149	28	11	4	5	2	—	—	1 male malformed, discarded.
4th	CL6 / 29J	CL6 / 29S	Lead	108	29	6	5	1	1	—	—	1 female malformed, discarded.
4th	CL7 / 29J	CL1 / 29S	0	75	9	—	—	—	—	—	—	All died.
4th	CL8 / 29J	CL6 / 29S	0	55	1	—	—	—	—	—	—	Died.
4th	CL9 / 29J	CL2 / 29S	Lead	65	2	—	—	—	—	—	—	Died.
4th	CL10 / 29J	CL4 / 29S	Lead	87	4	1	1	—	—	—	—	—
4th	CL11 / 29J	CL9 / 29S	Lead	112	24	1	1	—	—	—	—	—
4th	CL12 / 29J	CL10 / 29S	Lead	75	12	7	4	—	—	—	—	—
4th	CL13 / 29J	CL9 / 29S	Lead	124	6	—	—	—	—	—	—	All died.
4th	CN1 / 29J	CN4 / 29S	Control	136	117	89	39	36	13	—	—	—

Family C—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
4th	CN2 / 29J	CN2 / 29S	Control	83	5	3	1	2	—	—	—	1 female malformed, discarded.
4th	CM1 / 29J	CM1 / 29S	Manganese	42	5	4	1	1	—	—	—	—
4th	CM2 / 29J	CM2 / 29S	Manganese	109	21	7	2	4	2	—	2	—
5th	CL1 / 30S	CN1 / 29J	Lead	114	32	—	—	—	—	—	—	All died.
5th	CL2 / 30S	CN1 / 29J	Lead	92	23	15	9	6	4	1	3	—
5th	CL3 / 30S	CN1 / 29J	Lead	162	108	89	45	38	9	—	3	—
5th	CL4 / 30S	CN1 / 29J	Lead	107	57	36	17	19	5	—	2	—
5th	CL5 / 30S	CN1 / 29J	Lead	109	53	44	22	22	10	—	5	—
5th	CL6 / 30S	CN1 / 29J	Lead	127	63	26	10	14	4	2	2	—
5th	CL7 / 30S	CN1 / 29J	Lead	62	25	1	1	—	—	—	—	—
5th	CL8 / 30S	CN1 / 29J	Lead	60	18	18	6	12	—	—	—	—
5th	CL9 / 30S	CN1 / 29J	Lead	87	36	11	4	6	1	—	1	—
5th	CL10 / 30S	CN1 / 29J	Lead	139	103	40	18	6	3	—	3	—
5th	CN1 / 30S	CN1 / 29J	Control	105	44	11	5	6	4	2	—	—
5th	CN2 / 30S	CN1 / 29J	Control	89	20	2	2	—	—	—	—	—

Family C—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
6th	CL1 / 30J	CL5 / 30S	Lead	137	121	11	6	5	1	—	—	Very variable temperature during larval period.
6th	CL2 / 30J	CL5 / 30S	Lead	146	69	3	1	2	—	—	—	Very variable temperature during larval period.
6th	CL3 / 30J	CL3 / 30S	Lead	101	61	7	4	2	1	—	—	Very variable temperature during larval period.
6th	CL4 / 30J	CL3 / 30S	Lead	119	76	10	6	4	1	—	1	Very variable temperature during larval period.
6th	CL5 / 30J	CL5 / 30S	Lead	156	41	16	7	7	1	—	1	Very variable temperature during larval period.
6th	CL6 / 30J	CL5 / 30S	Lead	97	49	5	1	3	—	—	—	Very variable temperature during larval period.
6th	CL7 / 30J	CL3 / 30S	Lead	122	57	1	—	1	—	—	—	Very variable temperature during larval period.
6th	CL8 / 30J	CL3 / 30S	Lead	106	61	8	2	6	1	—	1	Very variable temperature during larval period.
6th	CL9 / 30J	CL5 / 30S	Lead	71	21	2	2	—	—	—	—	Very variable temperature during larval period.
6th	CL10 / 30J	CL4 / 30S	Lead	137	48	11	3	8	—	—	—	Very variable temperature during larval period.
6th	CL11 / 30J	CL4 / 30S	Lead	114	53	1	—	—	—	—	—	Died, very variable temperature during larval period.
6th	CL12 / 30J	CL3 / 30S	Lead	118	106	17	9	7	1	—	1	—
6th	CL13 / 30J	CL4 / 30S	Lead	142	62	4	1	3	—	—	—	—
6th	CL14 / 30J	CL6 / 30S	Lead	98	34	—	—	—	—	—	—	All died.

Family C—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
6th	CL15 / 30J	CL3 / 30S	Lead	96	31	4	2	1	1	—	1	—
6th	CL16 / 30J	CL6 / 30S	Lead	111	81	—	—	—	—	—	—	All died.
6th	CL17 / 30J	CL2 / 30S	Lead	116	51	—	—	—	—	—	—	All died.
6th	CN1 / 30J	CN1 / 30S	Control	91	25	9	3	5	2	—	2	—
6th	CN2 / 30J	CN1 / 30S	Control	103	51	3	1	2	1	—	1	—

Family D.

1st	D	Wild female from Horseheath	0	51	51	44	22	18	7	6	—	Typical Juliaria insects.
2nd	D4	D	0	189	120	97	41	49	26	13	—	Typical Spring insects.
3rd	DL1 / 29S	D 4	Lead	181	96	67	26	32	5	—	1	—
	DN1 / 29S		Control		50	39	17	21	—	—	—	—
3rd	DL2 / 29S	D 4	Lead	191	41	38	22	16	6	—	2	—
	DN2 / 29S		Control		41	28	13	15	1	—	—	—
3rd	DL3 / 29S	D 4	Lead	201	59	25	10	10	4	—	2	—
	DN3 / 29S		Control		50	24	11	12	—	—	—	—

Family D—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
3rd	DL4/29S	D 4	Lead	202	71	29	12	14	3	—	1	—
	DN4/29S		Control		50	20	11	8	1	—	—	—
3rd	DL5/29S	D 4	Lead	178	37	26	14	12	3	—	—	—
	DN5/29S		Control		37	28	15	13	—	—	—	—
3rd	DL6/29S	D 4	Lead	199	93	32	16	15	1	—	—	—
	DN6/29S		Control		50	22	11	11	—	—	—	—
3rd	DL7/29S	D 4	Lead	146	36	—	—	—	—	—	—	Given to chemist to estimate lead content.
	DN7/29S		Control		35	—	—	—	—	—	—	
3rd	DL8/29S	D 4	Lead	189	80	42	19	22	1	—	1	—
	DN8/29S		Control		50	31	15	14	—	—	—	—
3rd	DL9/29S	D 4	Lead	178	37	21	11	9	1	—	1	—
	DN9/29S		Control		37	27	14	12	—	—	—	—
3rd	DL10/29S	D 4	Lead	77	3	3	1	1	—	—	—	—
	DN10/29S		Control		2	2	—	2	—	—	—	—
3rd	DM1/29S	D 4	Manganese	195	21	14	8	4	2	—	1	—
	DN1β/29S		Control		27	8	3	5	—	—	—	—

Family D—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
3rd	DM2/29S	D 4	Manganese	127	43	29	19	10	6	4	—	—
	DN2β/29S		Control		43	25	16	10	1	—	—	—
3rd	DM3/29S	D 4	Manganese	235	98	59	25	27	1	—	1	—
	DM3β/29S		Control		50	40	11	24	—	—	—	—
4th	DL1/29J	DL4/29S	0	77	12	5	3	1	—	—	—	—
4th	DL2/29J	DL2/29S	0	113	—	—	—	—	—	—	—	Failed.
4th	DL3/29J	DL3/29S	Lead	94	4	1	1	—	—	—	—	—
4th	DL4/29J	DL3/29S	Lead	153	32	10	5	5	1	—	1	—
4th	DL5/29J	DL5/29S	Lead	78	—	—	—	—	—	—	—	Failed.
4th	DL6/29J	DL2/29S	Lead	150	29	13	4	11	2	—	2	—
4th	DL7/29J	DL1/29S	0	175	106	50	28	14	13	—	11	—
4th	DL8/29J	DL1/29S	0	172	106	—	—	—	—	—	—	Mixed with DL9/29J, discarded.
4th	DL9/29J	DL2/29S	Lead	130	60	—	—	—	—	—	—	Mixed with DL8/29J, discarded.
4th	DL10/29J	DL5/29S	Lead	121	54	26	12	14	9	—	9	—

Family D—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
4th	DL11 / 29J	DL1 / 29S	Lead	149	78	46	23	17	6	—	5	—
4th	DL12 / 29J	DL5 / 29S	Lead	121	43	8	5	2	2	—	2	—
4th	DL13 / 29J	DL1 / 29S	0	152	63	31	16	15	12	—	9	—
4th	DL14 / 29J	DL4 / 29S	Lead	79	14	—	—	—	—	—	—	All died.
4th	DL15 / 29J	DL2 / 29S	Lead	125	37	21	8	8	5	—	5	—
4th	DL16 / 29J	DL9 / 29S	0	156	48	11	7	3	2	—	2	—
4th	DL17 / 29J	DL6 / 29S	Lead	115	79	22	14	4	4	—	3	—
4th	DN1 / 29J	DN2 / 29S	Control	134	65	43	14	17	5	—	5	—
4th	DN2 / 29J	DN4 / 29S	Control	94	4	—	—	—	—	—	—	Discarded, diseased.
4th	DN3 / 29J	DN2 / 29S	Control	105	40	10	5	1	—	—,	—	—
4th	DM1 / 29J	DM2 / 29S	Manganese	157	22	8	5	2	1	—	1	—
4th	DM2 / 29J	DM2 / 29S	Manganese	145	10	7	5	1	1	1	—	—
4th	DM3 / 29J	DM1 / 29S	0	80	16	—	—	—	—	—	—	All died.
5th	DL1 / 30S	DL7 / 29J	0	95	46	29	9	16	4	—	4	—

Family D—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
5th	DL2 / 30S	DL13 / 29J	0	92	—	—	—	—	—	—	—	Failed.
5th	DL3 / 30S	DL13 / 29J	0	127	33	—	—	—	—	—	—	All died.
5th	DL4 / 30S	DL7 / 29J	0	34	—	—	—	—	—	—	—	Failed.
5th	DL5 / 30S	DL17 / 29J	Lead	90	39	35	17	18	9	—	7	—
5th	DL6 / 30S	DL13 / 29J	0	128	15	—	—	—	—	—	—	All died.
5th	DL7 / 30S	DL11 / 29J	Lead	46	27	15	8	6	3	—	3	—
6th	DL1 / 30J	DL5 / 30S	Lead	39	2	—	—	—	—	—	—	Died.
6th	DL2 / 30J	DL5 / 30S	Lead	53	3	—	—	—	—	—	—	Died.

Family G.

1st	G	Wild female from Warboys	0	10	10	8	5	3	3	2	—	Typical Juliaria insects.
2nd	G 3	G	0	199	113	71	23	29	17	2	3	Typical Spring insects.
3rd	GL1 / 29S	G 3	Lead	128	27	13	7	5	—	—	—	—
	GN1 / 29S		Control		26	15	8	7	—	—	—	—

Family G—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
3rd	GL2/29S	G 3	Lead	153	47	21	11	8	4	—	1	—
	GN2/29S		Control		46	27	11	15	2	1	—	—
3rd	GL3/29S	G 3	Lead	143	43	31	16	14	4	—	—	—
	GN3/29S		Control		43	31	15	14	1	—	—	—
3rd	GL4/29S	G 3	Lead	174	92	46	19	21	4	—	—	—
	GN4/29S		Control		50	25	11	13	—	—	—	—
3rd	GL5/29S	G 3	Lead	156	28	21	10	11	3	—	1	—
	GN5/29S		Control		27	21	7	12	—	—	—	—
3rd	GL6/29S	G 3	Lead	157	18	—	—	—	—	—	—	Given to chemist to estimate lead content.
	GN6/29S		Control		18	—	—	—	—	—	—	
3rd	GL7/29S	G 3	Lead	184	31	19	8	11	1	—	—	—
	GN7/29S		Control		30	18	12	5	—	—	—	—
3rd	GL8/29S	G 3	Lead	104	13	11	4	6	1	—	—	—
	GN8/29S		Control		13	8	5	3	—	—	—	—
3rd	GL9/29S	G 3	Lead	171	40	34	16	17	1	—	—	—
	GN9/29S		Control		40	26	14	11	1	—	—	—

Family G—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
3rd	GM1 / 29S	G 3	Manganese	113	32	17	6	11	2	—	—	—
	GN1γ / 29S		Control		32	19	11	7	—	—	—	—
3rd	GM2 / 29S	G 3	Manganese	156	63	25	10	13	1	—	—	—
	GN2γ / 29S		Control		50	23	13	9	—	—	—	—
3rd	GM3 / 29S	G 3	Manganese	195	17	11	6	5	—	—	—	—
	GN3γ / 29S		Control		17	13	6	6	—	—	—	—
4th	GL1 / 29J	GL2 / 29S	0	139	39	8	5	3	3	—	3	—
4th	GL2 / 29J	GL3 / 29S	Lead	119	43	1	—	—	—	—	—	This family died very rapidly, and the one pupa failed to emerge.
4th	GL3 / 29J	GL2 / 29S	Lead	138	68	8	5	3	3	—	2	Died rapidly in larval stage.
4th	GL4 / 29J	GL3 / 29S	0	146	36	9	6	2	2	—	—	—
4th	GL5 / 29J	GL3 / 29S	Lead	136	42	—	—	—	—	—	—	Failed, all died.
4th	GL6 / 29J	GL4 / 29S	Lead	158	72	9	3	5	2	—	—	—
4th	GL7 / 29J	GL4 / 29S	0	173	92	32	16	15	9	—	5	—
4th	GL8 / 29J	GL3 / 29S	Lead	146	37	10	2	7	1	—	1	—
4th	GL9 / 29J	GL2 / 29S	Lead	118	30	4	4	—	—	—	—	—

Family G—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
4th	GL10 / 29J	GL4 / 29S	0	181	129	44	22	18	9	—	7	—
4th	GL11 / 29J	GL4 / 29S	Lead	138	55	28	12	14	7	—	4	—
4th	GL12 / 29J	GL5 / 29S	Lead	117	23	14	4	10	3	—	3	—
4th	GL13 / 29J	GL5 / 29S	0	145	42	2	—	2	—	—	—	—
4th	GL14 / 29J	GL7 / 29S	Lead	181	72	24	10	11	6	—	5	—
4th	GL15 / 29J	GL9 / 29S	Lead	140	78	19	8	9	4	—	3	—
4th	GL16 / 29J	GL8 / 29S	Lead	144	74	15	6	7	3	—	1	—
4th	GM1 / 29J	GM1 / 29S	Manganese	164	117	42	19	19	6	—	2	—
4th	GM2 / 29J	GM1 / 29S	Manganese	121	53	7	3	2	1	—	1	—
4th	GM3 / 29J	GM2 / 29S	0	139	91	16	5	6	4	—	4	—
4th	GN1 / 29J	GN2 / 29S	Control	172	—	—	—	—	—	—	—	Infertile failed.
4th	GN2 / 29J	GN3 / 29S	Control	125	95	2	1	—	—	—	—	Very hot weather July 22–30, many died in larval stage.
4th	GN3 / 29J	GN9 / 29S	Control	150	88	30	13	12	10	—	4	—
5th	GL1 / 30S	GL6 / 29J	Lead	77	24	—	—	—	—	—	—	All died.

Family G—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
5th	GL2 / 30S	GL3 / 29J	Lead	96	35	22	8	14	7	—	3	—
5th	GL3 / 30S	GL4 / 29J	0	52	3	2	—	1	—	—	—	—
5th	GL4 / 30S	GL4 / 29J	0	128	26	6	3	3	2	—	—	—
5th	GL5 / 30S	GL6 / 29J	Lead	97	32	2	—	1	—	—	—	—
5th	GL6 / 30S	GL11 / 29J	Lead	111	62	23	7	11	5	—	2	—
5th	GL7 / 30S	GL11 / 29J	Lead	55	44	32	15	14	10	—	8	—
5th	GL8 / 30S	GL7 / 29J	0	122	30	1	1	—	—	—	—	—
5th	GL9 / 30S	GL7 / 29J	0	115	50	3	—	2	—	—	—	—
5th	GL10 / 30S	GL7 / 29J	0	50	15	6	4	2	1	—	1	—
5th	GL11 / 30S	GL15 / 29J	Lead	149	49	31	16	14	8	—	6	—
5th	GL12 / 30S	GL11 / 29J	Lead	151	39	21	11	8	7	—	5	—
5th	GL13 / 30S	GL10 / 29J	0	85	34	7	1	5	—	—	—	—
5th	GL14 / 30S	GL10 / 29J	0	165	46	15	6	6	4	—	4	—
5th	GL15 / 30S	GL7 / 29J	0	180	59	10	2	7	—	—	—	—

Family G—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
5th	GL16 / 30S	GL14 / 29J	Lead	107	26	6	—	4	—	—	—	—
5th	GL17 / 30S	GL16 / 29J	Lead	97	35	16	8	7	6	—	3	—
5th	GL18 / 30S	GL16 / 29J	Lead	20	6	—	—	—	—	—	—	All died.
5th	GN1 / 30S	GN3 / 29J	Control	133	28	16	6	9	5	—	5	—
5th	GN2 / 30S	GN3 / 29J	Control	122	23	15	5	9	1	—	1	—
5th	GN3 / 30S	GN3 / 29J	Control	149	30	20	10	10	2	—	—	—
5th	GN4 / 30S	GN3 / 29J	Control	130	31	23	8	15	7	—	6	—
5th	GN5 / 30S	GN3 / 29J	Control	91	19	7	4	3	1	—	1	—
5th	GN6 / 30S	GN3 / 29J	Control	156	26	21	12	9	4	—	3	—
5th	GM1 / 30S	GM1 / 29J	Manganese	63	38	—	—	—	—	—	—	All died.
5th	GM2 / 30S	GM1 / 29J	Manganese	111	32	12	7	4	2	—	1	—
5th	GM3 / 30S	GM1 / 29J	Manganese	53	18	11	4	7	3	—	—	—
5th	GM4 / 30S	GM1 / 29J	Manganese	98	16	5	3	2	1	—	—	—
6th	GL1 / 30J	GL2 / 30S	Lead	136	32	1	1	—	—	—	—	—

Family G—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
6th	GL2 / 30J	GL7 / 30S	Lead	80	33	—	—	—	—	—	—	All died.
6th	GL3 / 30J	GL4 / 30S	0	143	95	2	1	1	—	—	—	—
6th	GL4 / 30J	GL2 / 30S	Lead	74	15	1	—	1	—	—	—	—
6th	GL5 / 30J	GL2 / 30S	Lead	87	51	3	1	—	—	—	—	—
6th	GL6 / 30J	GL6 / 30S	Lead	65	14	—	—	—	—	—	—	All died.
6th	GL7 / 30J	GL2 / 30S	Lead	22	—	—	—	—	—	—	—	Failed.
6th	GL8 / 30J	GL7 / 30S	Lead	85	33	13	4	6	1	—	1	—
6th	GL9 / 30J	GL6 / 30S	Lead	88	61	9	5	2	1	—	1	—
6th	GL10 / 30J	GL4 / 30S	0	169	102	6	1	4	1	—	1	—
6th	GL11 / 30J	GL6 / 30S	Lead	99	37	14	8	4	1	—	1	—
6th	GL12 / 30J	GL11 / 30S	Lead	94	47	5	1	4	—	—	—	—
6th	GL13 / 30J	GL12 / 30S	Lead	99	78	3	2	1	1	—	—	—
6th	GL14 / 30J	GL17 / 30S	Lead	113	46	7	3	4	2	—	2	—
6th	GL15 / 30J	GL5 / 30S	Lead	126	116	34	13	20	4	—	2	—

Family G—(continued).

Generation.	Brood.	Origin.	Treatment.	No. of ova.	No. of larvæ.	No. of pupæ.	No. of females.	No. of males.	No. of matings.	Matings discarded.	Matings failed.	Remarks.
6th	GL16 / 30J	GL11 / 30S	Lead	29	20	2	—	2	—	—	—	—
6th	GL17 / 30J	GL17 / 30S	Lead	67	41	1	—	1	—	—	—	—
6th	GL18 / 30J.	GL17 / 30S	Lead	114	40	4	1	—	—	—	—	—
6th	GL19 / 30J	GL12 / 30S	Lead	39	20	—	—	—	—	—	—	All died.
6th	GN1 / 30J	GN3 / 30S	Control	98	38	6	4	1	1	—	1	—
6th	GN2 / 30J	GN4 / 30S	Control	109	44	3	—	—	—	—	—	Died.
6th	GN3 / 30J	GN6 / 30S	Control	44	33	—	—	—	—	—	—	All died.
6th	GN4 / 30J	GN3 / 30S	Control	41	29	—	—	—	—	—	—	All died.
6th	GM1 / 30J	GM3 / 30S	Manganese	98	38	—	—	—	—	—	—	All died
6th	GM2 / 30J	GM2 / 30S	Manganese	89	70	—	—	—	—	—	—	All died.
6th	GM3 / 30J	GM3 / 30S	Manganese	124	66	13	7	4	2	—	2	—
6th	GM4 / 30J	GM3 / 30S	Manganese	111	74	2	2	—	—	—	—	—
6th	GM5 / 30J	GM4 / 30S	Manganese	54	18	—	—	—	—	—	—	All died.

Analyses.

In the first year (1929) of feeding on treated foliage, analyses were made in order to ascertain that the lead and manganese did reach the leaves and the larvæ. In the untreated foliage the amount of lead was never measurable, but manganese was always present in amounts varying from 0·0003 per cent. early in the season to 0·002 per cent. in August. In the treated foliage the lead was always present and varied between 0·002 and 0·006 per cent. The manganese content was also increased, on occasion, to 0·004 and 0·005 per cent. These percentages are calculated on the green material.

Because of the difficulty of accurate estimation of these very minute quantities of lead and manganese, at the instance of the Ministry of Agriculture, Sir Robert Robertson was good enough to undertake some further analyses by the refined methods employed in the Government Laboratory. Leaves had been preserved at three dates, May, July, and August, 1930; the untreated material yielded respectively 0·009, 0·008 and 0·011 mgm. of lead per gram of dry material. The corresponding treated material contained 0·24, 0·20, and 0·092 mgm. of lead per gram. Of the manganese the untreated material yielded 0·05, 0·05, and a trace; the treated material gave 1·1, 0·5, and 4·4 mgm. per gram of dry leaf. The imagines reared in the third and fourth generations and also subsequently those of the sixth generation were analysed, but the amounts of lead and manganese found were too minute to be able to say conclusively that there was more lead or manganese in the treated moths than in those which were untreated.

Conclusion.

Whereas Heslop Harrison obtained melanic moths in the second treated generation, no melanism has appeared in the present experiment even after four treated generations. The final imagines obtained are the sixth inbred generation since the start of the experiment in 1928. In this experiment therefore the attempt to induce melanism in *Selenia bilunaria*, Esper, by treatment with lead nitrate and manganous sulphate has failed.

Summary.

(1) An attempt has been made to induce melanism in *Selenia bilunaria*, Esper, by feeding the larvæ on hawthorn treated with lead and manganese salts.

(2) Six generations totalling 3265 moths have been reared.

(3) No melanic individuals have appeared either in the treated or the control broods.

Acknowledgments.

Thanks are due to Sir Daniel Hall for his help and interest throughout the experiment, to Professor J. B. S. Haldane for his supervision of the work and his interpretation of results, to Mr. J. C. F. Fryer for his help with the entomological aspects of the work, to Dr. C. D. Darlington for advice in the initial stages, to Dr. E. Phillis for chemical analyses in 1929, to Sir Robert Robertson for determinations of lead and manganese content in 1930 and 1931, and to Mr. C. W. Wilkins, who assisted with the work throughout the latter part of the experiment.

Thanks are also due to Professor J. W. Heslop Harrison for much advice as to the conduct of the work, and for his interest throughout the experiment.

Professor J. B. S. Haldane has kindly contributed the following discussion.

The system of matings adopted was designed to reveal any recessive factors for melanism latent in either control or experimental stocks. Had melanism appeared in the latter and not the controls, the latter were sufficiently numerous to make it almost certain that it was due to mutation. If we call the recessive factor for melanism t, the corresponding dominant T (Harrison's terminology), the mating of $Tt \times Tt$ gives about a quarter melanics. Thus if a family of n is bred, the chance of obtaining at least one melanic is $1 - (\frac{3}{4})^n$. For example, if $n = 10$ this chance is $0 \cdot 9437$, *i.e.*, only $5 \cdot 6$ per cent. of such families should fail to reveal the recessive factor.

The 82 broods, some of whose ancestors had been treated with lead, contained 910 individuals, none of which were melanic. Harrison and Garrett record five similar broods (1923 AL, BL, LL and TL, and 1924 LL) which contained 136 types and 6 melanics. We can arrange these figures with those of Hughes in a fourfold table, and find a measure of divergence $\chi = 6 \cdot 17$. The probability of so great a divergence being due to chance is less than 10^{-9}. The use of the χ^2 formula is not, of course, quite legitimate when one of the four numbers is zero, but the order of magnitude of the probability is unaffected. Had these two experiments given parallel results, $38 \cdot 1$ melanics would have been expected among the 910 moths reared.

The comparison can be made even more strongly by comparing only broods whose parents and themselves were reared on lead-treated food. Here 652

types and no melanics were obtained, while Harrison and Garrett obtained 71 types and 6 melanics. The expectation on this basis in the Merton experiments was 50·8 melanics, and the probability that their non-appearance was due to chance was very much less than in the former case.

We can compare the results in still another way. In the 82 broods from lead-treated ancestry the average number per brood was 11·09, and 35 contained 10 or more individuals. We may take it then that somewhat over 40 parents of these broods were not both heterozygous for the factor *t*. If now the appearance of melanism was due to the heterozygosity of the parents (an hypothesis which Harrison rejects), in his case three pairs out of five were both heterozygous. Here $\chi = 5{\cdot}07$, and the probability that the divergence is due to chance is about $3{\cdot}8 \times 10^{-7}$.

The manganese results may be treated in the same way, though, being fewer, they are less conclusive. Here 12 broods whose ancestors had been reared on manganese gave 115 types and no melanics. In similar families Harrison and Garrett obtained 12 types and 8 melanics in one family (1923, DM) while Harrison obtained 310 types, 29 melanics, and 4 mosaics in five families (T1, T2, T3, T4, T5). Here $\chi = 3{\cdot}12$, and the probability that the divergence is due to chance is 0·0018.

It is thus perfectly clear that had Hughes' insects behaved in the same way as Harrison's, some melanics would have appeared. The chances of their appearing in this case were almost a billion to one.

Considering Harrison and Garrett's results, if his original female or her mate were T*t*, the other parent being TT, half the offspring would be T*t*. In such a case the chances are even that the pair chosen to breed from were also TT and T*t*, giving the all-type brood of summer 1922. Again, there is a chance of one in four that the two pairs of this family chosen for breeding were TT and T*t*. Controls were bred for the lead experiment, and presumably in this case two TT parents were chosen, and their offspring bred together for two or more generations. The chance of this event occurring in a half heterozygous family would be one in four. Thus the odds against a mendelian explanation of Harrison and Garrett's results are, so far, fifteen to one. However, as Harrison and Garrett point out, the small proportion of melanics segregating in families AL, BL and LL of 1923 is very difficult to account for on such a basis. It would readily be explicable, however, if *t* were linked with a lethal gene. Such a possibility cannot be denied, and would account for the small numbers found. It would also perhaps account for the absence of melanics in control families which might otherwise have revealed heterozygosis. Harrison's

later results with manganese were more adequately controlled, and the odds against a mendelian explanation are now 100 to 1, unless we assume linkage with a lethal. In this case the recessives should, of course, as they did, give normal mendelian results on crossing with typical insects.

It would seem, then, that although Harrison's explanation of his results is very plausible, it is not conclusively demonstrated. For this reason very full controls were bred by Mr. Hughes, so that, had a melanic appeared, the hypothesis of its origin from a recessive factor latent in the wild moths would have been most improbable.

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Investigations on Mediterranean Kala Azar. VI.—*Canine Visceral Leishmaniasis.*

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(Kala Azar Commission of the Royal Society.)

(Communicated by Sir Henry Dale, Sec. R.S.—Received February 3, 1932.)

[Plates 19 and 20.]

Following the discovery of canine visceral Leishmaniasis in Tunis by Nicolle and Comte (1908), an extensive literature has developed dealing particularly with the possible relationship between human and canine Leishmaniasis. Most authors, following Nicolle, consider *Leishmania infantum* to be the causative organism of both human and canine Kala Azar.

In the case of cutaneous Leishmaniasis, *L. tropica* has been conclusively proved by direct experiment to be the causative organism in both man and dog (1930), but in this case there is no evidence that the dog serves as a reservoir of the human disease. It is more likely that where human and canine cutaneous Leishmaniasis co-exist, human beings serve as a reservoir from which dogs are infected (1929). It is, however, difficult to carry out similar direct experi-