On the Evidence Against the Chemical Induction of Melanism in Lepidoptera.

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1. Introductory.

McKenny Hughes (1932) has reported an experiment, carried out at Merton, in which the moth *Selenia bilunaria* Esper was fed on leaves treated with lead nitrate and manganous sulphate. In the generations following these treatments no instance of a moth showing the melanic recessive mutation, reported by Harrison and Garrett (1926), was recorded.

In the discussion contributed by Haldane (McKenny Hughes (1932), p. 400) it is argued that the results are significantly in conflict with the findings of Harrison and Garrett in that these authors recorded 6 melanics out of 142 moths of the generations following treatment, while McKenny Hughes found no melanics among 910 moths. This difference, as Haldane claims, would be highly significant, if the several individuals counted had an independent chance of being melanics. In both lots, however, the moths were in reality closely related, and the chances cannot on any theory be considered independent. The other calculations in Haldane's discussion are open to the same criticism.

The value of a negative finding, unlike that of a positive result, such as that reported by Harrison and Garrett, rests exclusively on the extent of the evidence. The different broods reported by McKenny Hughes are not only of different sizes, but of several different kinds, having very different weight as negative evidence. Thus, to take the lead series only, in family C there are 21 broods, comprising 132 moths, all in the first generation following treatment with lead. In family D there are (*a*) 12 broods comprising 220 moths in the first generation following treatment, (*b*) two broods of 49 moths after two generations of treatment, and (*c*) one brood of 25 moths the parents of which had been off lead for one generation. Finally, in family G there are (*a*) 14 broods comprising 209 moths in the first generation following treatment, (*b*) 8 broods comprising 138 moths after two generations of treatment, (*c*) 8 broods comprising 43 moths off lead for one generation, (*d*) 12 broods comprising 83 moths after three generations of treatment, and (*e*) 2 broods comprising 7 moths off lead for two generations.

Table I shows the combined totals for the three families.

Table I.—Number of broods and moths bred.

	One generation treatment.	Two generations treatment.	Three generations treatment.	Off lead one generation.	Off lead two generations.	Total.
Broods	47	10	12	9	2	80
Moths	561	187	83	68	7	906

The small discrepancies from Haldane's values are presumably to be ascribed to errors of transcription. It will, however, make no appreciable difference whether the error is in the tables printed with McKenny Hughes' paper, or in the totals given by Haldane.

The value of the material as negative evidence may be measured by the rates of mutation which, in the light of the observations, can be shown to be highly improbable. An infinitude of observations would be required to show that lead treatment had actually zero effect on the mutation rate. Any body of data showing no mutations may be accepted as proving the non-existence of mutation rates above a certain critical level; the more extensive the observations, and the more relevant they are to the point at issue, the smaller will this critical level be made. In particular we may ask, for the body of material presented, for what mutation rate the probability of observing no melanic moths will be reduced to $0 \cdot 05$ or $0 \cdot 01$. The experiment might then be interpreted as proving, with degrees of certainty measured by these two levels of significance, that the mutation rate did in fact not exceed the corresponding value found. These critical mutation rates will be evaluated in the following sections.

2. *Definition of Mutation Rate and Form of Calculation.*

The probability of the absence of visible mutants in a given series of affiliated progenies will be determined not only by the mutation rate, but by the stage at which the mutations are supposed to occur. By hypothesis A we shall denote the view that a mutation rate μ per generation implies that a non-mutant individual will after treatment produce a fraction μ of mutant gametes, and a fraction $(1 - \mu)$ of non-mutant gametes. A heterozygous individual, on the other hand, will produce $\frac{1}{2}(1 + \mu)$ mutant gametes, and $\frac{1}{2}(1 - \mu)$ non-mutant gametes. There will, therefore, on this hypothesis, be only three types of mating to be considered, as shown in Table II.

Table II.—Frequency of the three kinds of offspring from the different types of mating.

Mating.	Offspring.		
	MM.	Mm.	mm.
MM × MM	$(1 - \mu)^2$	$2\mu(1 - \mu)$	μ^2
MM × Mm	$\frac{1}{2}(1 - \mu)^2$	$\frac{1}{2}(1 - \mu)(1 + 2\mu)$	$\frac{1}{2}\mu(1 + \mu)$
Mm × Mm	$\frac{1}{4}(1 - \mu)^2$	$\frac{1}{2}(1 - \mu^2)$	$\frac{1}{4}(1 + \mu)^2$

Thus if μ is 3 per cent., the proportionate frequencies of melanic moths in the three types of mating will be 0·0009, 0·01545, 0·265225, and if the progeny yields s moths, the probabilities that all shall be non-melanic will be $(0·9991)^s$, $(0·98455)^s$ and $(0·734775)^s$. Columns (c), (d) and (e) of Table III give, in reverse order, these probabilities for the sixth generation (the third after treatment), using $\mu = 3$ per cent. for the offspring of treated moths, and $\mu = 0$ for the offspring of the untreated. It will be observed that families over ten have only a small probability of being the offspring of two heterozygotes, but that even the large family of 33 might well have been the offspring of a heterozygote and a homozygote.

The probabilities of the three possible matings producing the sixth generation may be used to calculate the probabilities of these same alternatives in the fifth generation. Thus from a mating of homozygotes the probability that both the offspring chosen for mating shall be homozygotes will be $(1 - \mu)^4$, the probability that one shall be a homozygote and one a heterozygote will be $4\mu(1 - \mu)^3$, and the probability that both shall be heterozygotes will be $4\mu^2(1 - \mu)^2$. If, therefore, the mating, which produced the fifth generation progeny, was one between non-mutant moths, the probability that a pair of its members mated *inter se* will produce s offspring non-mutant in appearance, will be

$$4\mu^2(1 - \mu)^2 \{1 - \tfrac{1}{4}(1 + \mu)^2\}^s + 4\mu(1 - \mu)^3 \{1 - \tfrac{1}{2}\mu(1 + \mu)\}^s$$
$$+ (1 - \mu)^4 \{1 - \mu^2\}^s,$$

or, with a 3 per cent. mutation rate, the sum of the values in columns (c), (d) and (e) of Table III, multiplied in order as they stand by 0·003387, 0·109521, 0·885293, respectively. The values in column (h) in Table III have been obtained by using these factors.

Table III.—Calculation of probabilities—first stage.

Sixth generation brood.	Moths.	Fifth generation mating.			Fourth generation mating.			Brood of parents.
		Mm × Mm.	MM × Mm.	MM × MM.	Mm × Mm.	MM × Mm.	MM × MM.	
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
GL18/30J	1	0·73478	0·98455	0·99910	0·47003	0·89157	0·99481	}GL17/30S
GL17/30J	1	0·73478	0·98455	0·99910	0·47003	0·89157	0·99481	
GL14/30J	7	0·11563	0·89674	0·99371	0·29458	0·68426	0·97833	
GL13/30J	3	0·39670	0·95436	0·99730	0·37847	0·78721	0·98877	GL12/30S
GL16/30J	2	0·53989	0·96934	0·99820	0·41777	0·83250	0·99169	}GL11/30S
GL12/30J	5	0·21418	0·92510	0·99551	0·32594	0·72442	0·98336	
GL15/30J	33	0·00004	0·59820	0·97072	0·19431	0·21484	0·92489	}GL7/30S
GL8/30J	10	0·04587	0·85581	0·99104	0·26741	0·64543	0·97125	
GL11/30J	12	0·02476	0·82957	0·98925	0·25587	0·62676	0·96672	}GL6/30S
GL9/30J	7	0·11563	0·89674	0·99371	0·29458	0·68426	0·97833	
GL5/30J	1	0·73478	0·98455	0·99910	0·47003	0·89157	0·99481	}GL2/30S
GL4/30J	1	0·73478	0·98455	0·99910	0·47003	0·89157	0·99481	
GL10/30J	5	0·23730	1	1	0·37182	0·80932	1	}GL4/30S
GL3/30J	2	0·56250	1	1	0·45312	0·89062	1	

Similarly column (g), representing the supposition that the fifth generation brood was derived from the cross of a homozygous non-mutant with a heterozygote, is obtained by multiplying the same quantities by the factors

$$\tfrac{1}{4}(1-\mu)^2(1+2\mu)^2, \qquad \tfrac{1}{2}(1-\mu)^3(1+2\mu), \qquad \tfrac{1}{4}(1-\mu)^4,$$

or numerically by 0·264299, 0·483717, 0·221323 for a 3 per cent. mutation rate, or by 0·25, 0·50, 0·25 for zero mutation rate.

Finally, the factors for the supposition that the fifth generation brood was derived from the mating of two heterozygotes are

$$\tfrac{1}{4}(1-\mu^2)^2, \qquad \tfrac{1}{4}(1-\mu^2)(1-\mu)^2, \qquad \tfrac{1}{16}(1-\mu)^4,$$

or, numerically, 0·249550, 0·235013, 0·055331 and 0·25, 0·25, 0·0625 for $\mu = 0·03$, and zero respectively, These factors are used to obtain the values in column (f).

We are now in a position, for any progeny of the fifth generation, to express the probability of each of the three possible types of mating from which it might have been derived, taking into account not only the composition of the fifth generation, but also of that of the broods derived from it. Thus brood

GL17/30S consists of 15 moths of which three pairs have been used in the production of the sixth generation broods GL18/30J, GL17/30J and GL14/30J. Apart from the sixth generation the probability that this fifth generation brood had been derived from a mating of two heterozygotes would have been $(0 \cdot 734775)^{15}$; taking the sixth generation into account it is now seen to be $(0 \cdot 734775)^9$, $(0 \cdot 47003)^2$, $(0 \cdot 29458)^1$, or $0 \cdot 00406$ as set down in Table IV. The index of the first power is 9 and not 15, since the chance that the 6 moths used as parents should be non-mutants has already been taken into account in the three corresponding factors. Columns (c), (d) and (e) of Table IV, derived thus from columns (f), (g) and (h) of Table III, show that many of the fifth generation progenies were sufficiently large to exclude, as improbable, the idea that they were from the mating of two heterozygous mutants; while for only one of the progenies, GL7/30S, a large brood from which two satisfactory broods had been derived, is there shown a low probability of its having one mutant parent.

Table IV.—Calculation of probabilities—second stage.

Fifth generation brood. (a)	Moths not mated. (b)	Fourth generation mating.			Third generation mating. (h)	Brood of parents. (i)
		(c)	(d)	(e)		
GL17/30S	15–6	0·00406	0·47280	0·96038	0·90200	GL16/29J
GL11/30S	30–4	0·00004	0·40231	0·95262	0·88741	GL15/29J
GL16/30S	4	0·29148	0·93962	0·99640	0·98600	GL14/29J
GL12/30S	19–2	0·00201	0·60414	0·97375	0·92823	⎫
GL7/30S	29–4	0·00002	0·09395	0·87830	0·78784	⎬GL11/29J
GL6/30S	18–4	0·00101	0·34487	0·93391	0·86456	⎭
GL5/30S	1	0·73478	0·98455	0·99910	0·99481	GL6/29J
GL2/30S	22–4	0·00086	0·60061	0·97373	0·92782	GL3/29J
DL7/30S	14	0·01337	0·80414	0·98746	0·96231	DL11/29J
DL5/30S	35	0·00002	0·64665	0·96894	0·92862	DL17/29J
GL15/30S	9	0·07508	1	1	0·99507	⎫
GL10/30S	6	0·17798	1	1	0·99542	⎬GL7/29J
GL9/30S	2	0·56250	1	1	0·99672	⎪
GL8/30S	1	0·75000	1	1	0·99735	⎭
GL14/30S	12	0·03168	1	1	0·99492	⎫GL10/29J
GL13/30S	6	0·17798	1	1	0·99542	⎭
GL4/30S	6–4	0·09477	0·72081	1	0·96456	⎫GL4/29J
GL3/30S	1	0·75000	1	1	0·99735	⎭
DL1/30S	25	0·00075	1	1	0·99482	DL7/29J

For an experiment extending over many generations the cycle of operations set out above may be repeated indefinitely. Since in the experiment under discussion, the third generation was the first to be treated, the only hypothesis to be considered as to the origin of the fourth generation is that its parents were in each case both homozygous non-mutants. Consequently, columns (f) and (g) will not be needed in Table IV, and only column (h) will have to be calculated; the formula for treated moths is at this stage used in every case. The 19 pairs of the fourth generation, which had progeny, have accounted for 38 moths, leaving 523 others each with a probability 0·9991 of being a mutant. The product of the 19 values shown in column (h) of Table IV, multiplied by 0·9991 to the power of 523, will therefore give the probability of the observed negative result, on the hypothesis of a 3 per cent. mutation rate specified by hypothesis A.

The product of the 19 values comes to 0·43209, and the power of 0·9991 to 0·62446. The final probability of the whole series of observations is therefore 0·26982; hence the experiment is far from excluding the possibility of a mutation rate of 3 per cent.

On repeating the calculations with a 10 per cent. mutation rate, the product of the contributions of the 19 families of the fifth generation is 0·0013216, while 0·99 to the power of 523 contributes a factor 0·0052145. The probability of such a negative result as that observed, if there had been a mutation rate, as defined, of 10 per cent., is only 6·8915 millionths. The observations thus effectively exclude this possibility.

The logarithm of the probability evidently increases, in the range considered, more rapidly than the first power, though not quite so rapidly as the second power of the mutation rate. Interpolating logarithmically, the 1 per cent. point is found to be at a mutation rate of about 6·0 per cent., and the 5 per cent. point at about 4·7 per cent.

Since the logarithm of the probability increases proportionately with the bulk of the data, supposing these to be of the same kind, it may also be inferred that to exclude mutation rates exceeding 1 per cent. per generation would have required about 17 times as many observations as those reported if we were satisfied with the 5 per cent. level of probability, or about 26 times as many to reduce the probability to 1 per cent.

3. *Alternative View of the Action of the Mutations.*

The data of Harrison and Garrett are not in accordance with the possibility that some 5 per cent. of the gametes of moths treated as larvæ carry the mutant

gene, for then only about 1 in 400 of each brood of the second generation could show the mutation. On the contrary, Harrison and Garrett reported no broods without melanics, and two broods showing 3 melanics and 55 normals. Data of this kind suggest that the mutation, if not already present in the stock, occurred at an earlier stage than has been supposed, so that when a mutation is induced a large proportion of the gametes are affected. We may therefore consider the alternative supposition (B), or, rather, the other extreme of the range of possibilities ; namely, that a single mutation affects the whole germ tract, so that a treated non-mutant has a probability $(1 - \mu)^2$ of propagating as a non-mutant, a probability $2\mu (1 - \mu)$ of propagating as a heterozygote, and a probability μ^2 of propagating as a homozygous mutant. This extreme supposition is not that best suited to Harrison and Garrett's data, for it would require those broods in the second generation which contain melanics to show 25 per cent. melanics ; actually they show only about 5 per cent., which would be near to expectation if a single mutation affected about half the germ tract of the animal in which the mutation took place. We shall not, however, consider this special possibility, since its characteristics will doubtless be brought out by the more extreme and definite hypothesis to be considered.

The practical computation is, in the case of hypothesis B, a little more complicated than in hypothesis A since there are 5 instead of 3 types of mating to be considered. The two additional types arise when individuals normal in appearance are transformed as progenitors into homozygous mutants. Two types of mating give a proportion of mutant offspring ; namely, Mm × Mm giving 25 per cent. melanics, and Mm × mm giving 50 per cent. The probabilities of a mating yielding s normal individuals being of these kinds are therefore given by the values $(\frac{3}{4})^s$ and $(\frac{1}{2})^s$ respectively.

A normal individual from either the mating MM × mm or Mm × mm is bound to be heterozygous before mutation, and after treatment will be germinally heterozygous in $(1 - \mu)$ cases, and homozygous in μ cases. A pair of such individuals mated will therefore give a mating of type Mm × Mm in $(1 - \mu)^2$, of type Mm × mm in $2\mu (1 - \mu)$ and of type mm × mm in μ^2 cases. Hence the contribution of any progeny of s to the probability that the parent progeny was of either the types MM × mm or Mm × mm will be

$$\mu^2 (0)^s + 2\mu (1 - \mu) (\tfrac{1}{2})^s + (1 - \mu)^2 (\tfrac{3}{4})^s,$$

which we may write in a more general notation as

$$2\mu (1 - \mu) p_{011} + (1 - \mu)^2 p_{121},$$

where p stands for the probability, as judged by its observed composition, and that of its descendants, that a progeny is of the theoretical composition indicated by the suffix.

Similarly if the parent progeny is of type Mm $\times$ Mm, the probability that a normal offspring will propagate as a homozygous normal is $\frac{1}{3}(1-\mu)^2$, as a heterozygote $\frac{2}{3}(1-\mu^2)$, and as a homozygous mutant $\frac{1}{3}\mu(2+\mu)$. The contribution of the offspring of a pair of such normal offspring to the probability that the parental mating is of this type will therefore be

$$\tfrac{4}{9}\mu(2+\mu)(1-\mu^2)\,p_{011} + \tfrac{4}{9}(1-\mu^2)^2\,p_{121} + \tfrac{2}{9}\mu(2+\mu)(1-\mu)^2\,p_{010}$$
$$+ \tfrac{4}{9}(1-\mu^2)(1-\mu)^2\,p_{110} + \tfrac{1}{9}(1-\mu)^4\,p_{100}.$$

In like manner the contribution to the probability that the parental mating is of type MM $\times$ Mm is

$$\tfrac{1}{2}\mu(1+2\mu)(1-\mu^2)\,p_{011} + \tfrac{1}{4}(1-\mu)^2(1+2\mu)^2\,p_{121} + \tfrac{1}{2}\mu(1-\mu)(1-\mu^2)\,p_{010}$$
$$+ \tfrac{1}{2}(1+2\mu)(1-\mu)^3\,p_{110} + \tfrac{1}{4}(1-\mu)^4\,p_{100},$$

and the contribution to the probability that it is MM $\times$ MM is

$$4\mu^3(1-\mu)\,p_{011} + 4\mu^2(1-\mu)^2 p_{121} + 2\mu^2(1-\mu)^2 p_{010}$$
$$+ 4\mu(1-\mu)^3\,p_{110} + (1-\mu)^4 p_{100};$$

so that starting from the terminal progenies we may calculate, as before, the probability of the series of non-melanic progeny observed for any chosen mutation rate.

Using a mutation rate of 10 per cent. the probability of the series of normal families observed by McKenny Hughes is found to be $0 \cdot 0097551$, so that 10 per cent. per generation is just over the 1 per cent. value for hypothesis B. One obvious reason for the lower sensitivity of the experiment to hypothesis B, compared with hypothesis A, is that in the first generation following treatment the chance of showing no mutant for a brood of 1 is $0 \cdot 99$ on both hypotheses. On hypothesis A, however, any further members of the brood have an equal and independent chance, so that the probability of s non-mutants is $(0 \cdot 99)^s$; while on hypothesis B the probability of a non-mutant brood, however large, cannot fall below $0 \cdot 9639$, for this is the probability that one or other of the parents is a non-mutant. The 21 broods of Series C, for example, comprise 132 normal moths, but the probability on hypothesis B of this series of observations is $0 \cdot 57372$, equal to that of about 55 individual moths on hypothesis A, or to 55 broods of one on hypothesis B. Many other examples show emphatically how nearly impossible it is by judgment alone, and without explicit calculations, to gauge the value of negative evidence of this kind.

4. *Discussion.*

The observations reported by McKenny Hughes are sufficiently extensive to exclude as improbable mutation rates exceeding 6·0 per cent. per generation induced by lead treatment, on the hypothesis that mutations are induced independently in the gametes ; for the series of normal families observed would have a probability of occurrence of less than 1 per cent. if the mutation rate had exceeded this value. Less decisively, that is, with a 5 per cent. level of significance, are mutation rates exceeding 4·7 per cent. excluded.

Using the same sort of observations, the amplitude of the material would have to be increased 17-fold in order to exclude mutation rates over 1 per cent. on the lower standard, and about 26-fold to exclude them on the higher standard of significance.

On the alternative view that the mutations affect not individual gametes independently, but the whole germ tract of the individual affected, the observations are still less conclusive, for the probability of the series of normal families observed is only just under 1 per cent. with a mutation rate as high as 10 per cent.

Harrison and Garrett, after a single generation of treatment, mated four moths, all of which acted as partial heterozygotes. Apart from exceptional good fortune, this suggests an enormous mutation rate ; for, with a mutation rate of 8 per cent., only about 30 per cent. of the treated moths should act as semi-heterozygotes. With *Tephrosia bistortata*, on the other hand, only a single brood after four generations of treatment yielded a melanic, a result suggestive of a much lower, though still absolutely large, mutation rate.

It is not the writer's purpose to attempt to justify the very remarkable claim put forward by Harrison and Garrett, and it appears by no means impossible that the mutants observed were in reality segregates from a mutation pre-existing in the stock. If it is true that the melanics are less viable than normal moths, the paucity (5 per cent. against 25 per cent. expected) of melanics in the broods in which they first appeared would be explained. The system of experimentation adopted at Merton is, however, quite insufficient to show that chemical agencies do not induce mutations with even more than the high mutation rate of 1 per cent.

To view the matter in perspective we may note that of known physical agencies, the most effective in inducing mutations, namely, irradiation with X-rays, seldom causes a rate of more than one in several thousands at a particular locus. Thus Timoféeff-Ressovsky (1930), whose work I cite as pre-eminent in its thoroughness and extent, reports 15 back mutations in *Drosophila*

melanogaster, out of 213,567 irradiated chromosomes, or a little less on the average than 1 in 14,000. Even to establish the absence of mutation rates exceeding 1 per cent., though sufficient to require a reinterpretation of Harrison and Garrett's observations, would only show that the chemical agency (lead) is not more than 140 times as effective as X-rays are ordinarily found to be.

Had it been possible to test for mutations not by inbreeding only, but by back-crosses to the melanic form, little difficulty should have been encountered, with material not considerably greater than that used by McKenny Hughes, in excluding mutation rates over 1 per cent. For, without inbreeding, but using crosses between different treated families, and in the absence of disease, there can be little doubt that satisfactorily large broods, at least exceeding 10 moths, would have been readily obtained after three generations of treatment. A hundred such broods formed by crossing treated moths with melanics would test 200 chromosomes. With a mutation rate of 1 per cent. for three generations, about 3 per cent. of these should contain mutant genes, on either view of the incidence of mutation, and the absence of all melanics, when six affected families were expected, would be good evidence against a mutation rate of 1 per cent.

The method of attempting to reveal possible mutations by inbreeding, not only introduces difficulties by impairing the stock, but is exceedingly inefficient compared to back-crossing. Since all scientific experiments are limited by the amount of money and labour which can be expended upon them, it is highly desirable that any experiment should be designed so as to use the available resources to the best advantage.

Summary.

A method is given of assessing by calculation the value of evidence of the non-occurrence of recessive mutations under experimental conditions. It appears that the evidence, against the induction of melanic mutations in moths by feeding with lead, is insufficient to disprove the existence of mutation rates up to 5 per cent. or 8 per cent. according to the stage at which mutation is postulated.

Mutation rates of this magnitude would be far greater than those which can be certainly induced by any other agency.

The use of back-crosses instead of inbreeding would increase the value of experimental data of this kind by approximately thirty-fold.

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