THE MATERNAL INHERITANCE OF A SEX-LIMITED LETHAL EFFECT IN *DROSOPHILA MELANOGASTER*

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SUMMARY

The present paper describes the maternal inheritance of a lethal effect in *Drosophila melanogaster* which leads to the early death of a majority of female zygotes, and which in consequence gives rise to an average sex-ratio among adults of one female to 5.5 males. The effect may be transmitted both through the male and through the female; the genetic basis involves a recessive gene in the second chromosome group. The missing females probably die in the egg stage. The maternal effect may be attributed to an influence of the chromosomal composition of the mother upon the eggs which will give rise to the offspring exhibiting the lethal ratio previous to the time when these eggs leave the mother's body. The presence in such eggs, after fertilization, of the female-determining chromosomal complex as contrasted with the presence of the male-determining chromosomal complex is responsible for the differential death rate.

[1] The data included under these two headings were obtained while the writer was National Research Fellow in Zoology at COLUMBIA UNIVERSITY.

HISTORICAL

It has long been known, as shown for example by the extensive work on reciprocal species crosses in echinoderms and teleosts, that the developing embryo may for a longer or shorter period exhibit characters typical of the race from which the egg was derived. But an intrinsic difficulty from the genetic standpoint in much of this work is that the hybrids cannot be raised to maturity and bred. It is only when succeeding generations are obtained that we have the data necessary for determining whether a given case of maternal inheritance is quite independent of the nucleus or is the result of a deferred nuclear influence. The intimate relation of the egg with the mother's body, as well as the fact that preceding reduction it contains the chromosome complex of the mother, afford a basis for the latter type of maternal inheritance.

In regard to those characters which are shown to be determined by the genic composition of the mother and to which in consequence the term "maternal inheritance" is applied, it should be noted that this term is not happily chosen. For although the characters are immediately fixed by the constitution of the mother, they are just as definitely inherited from the father as from the mother, as becomes apparent in following generations.

The work of TOYAMA (1912 and 1913), TANAKA (1924), and others has demonstrated that cases of maternal inheritance shown by egg characters (shape, color, voltinism) of the silkworm moth may be explained on the basis of segregating genes. The color characters, which have been most extensively studied, depend upon the yolk (deposited while the egg is still part of the maternal body), the shell (derived from the epithelium of the oviduct), and the serosa. UDA (1923) has recently questioned the conclusions of TOYAMA regarding the transmission of brown versus slate, dependent upon serosa pigmentation; but it is clear from UDA'S paper, as has been pointed out by TANAKA (1924) and PELLEW (1925), that his data, as well as the discrepancies in TOYAMA'S papers, are readily explainable on the basis of an influence of the maternal genetic composition combined with an influence of the composition of the embryo itself.

STURTEVANT (1923) has suggested that the data of BOYCOTT and DIVER (1923) on the heredity of the direction of coiling in the snail, *Limnaea*, might be interpreted in terms of maternal inheritance. The recent paper of DIVER (1925) indicates that in addition to the parental nuclear influence the genetic composition of the zygote itself may be a further factor; there are possibly other as yet undetected influences at work in determining this character.

The studies of GOLDSCHMIDT (1924) on the transmission of the larval pattern resulting from crosses of geographic varieties of the gypsy moth, *Lymantria dispar*, have led him to conclude that besides the hereditary differences dependent upon segregating genes there is evidence of further differences which depend upon the plasma furnished by the egg and by the spermatozoon. In this respect, as is to be expected, the egg is the more influential. It may be recalled that GOLDSCHMIDT'S work on intersexes in this form shows that the "femaleness" factors are inherited, without paternal influence, exclusively from the mother. They were previously (1916 and 1917) attributed to the cytoplasm of the egg. It is now (1922 and 1923) suggested that they are transmitted by the "Y" or "W" chromosome and that a deferred effect is exhibited. This latter explanation is based upon the appearance of individuals assumed to be non-disjunctional.

Occasional characters are reported for *Drosophila melanogaster* whose expression shows more or less influence of the unreduced genetic composition of the mother. Such a maternal effect was reported by MORGAN (1912 and 1915) for rudimentary: by LYNCH (1919) in a study of a number of mutant forms showing partial or complete sterility in certain crosses; these forms include fused, rudimentary, morula, dwarf, and reduced bristles: and by MOHR (1925) for a third-chromosome recessive minute. An interesting point established by Miss LYNCH is the partial restorative effect upon viability at some stage of the presence of the normal allelomorph (for example of fused). This restorative effect may be exercised: (1) by the normal allelomorph of fused when present in the unreduced egg, even though the mature egg may contain fused and may be fertilized by a spermatozoon carrying fused, or (2) by the introduction of the normal allelomorph of fused by the spermatozoon.

Typical egg characters in *Drosophila melanogaster* are known to be determined by the genetic composition of the mother. MOHR (1922) found that the singed gene causes the eggs of homozygous females to be short and deformed. WARREN'S study (1924) of egg size in various races indicates that this character is dependent upon genes in all four linkage groups and that it is determined by the genetic constitution of the mother exclusively. This is to be expected since the size of the egg is fixed before the entrance of the spermatozoon. An interesting character of this type has recently been found by J. C. LI (unpublished work from the Columbia laboratory) in the eggs of Minute-1 females. These eggs are shorter and rounder than normal eggs and are considerably more transparent so that developing structures are readily observed.

ACKNOWLEDGMENTS

The work, a report of which follows, was conducted under the direction of Professor S. J. HOLMES of the Department of Zoology of the UNIVERSITY of CALIFORNIA. Laboratory accommodations were extended by the Division of Genetics. I am grateful also to the following for stocks and for suggestions: Professor H. J. MULLER of the UNIVERSITY of TEXAS, Professor R. E. CLAUSEN of the UNIVERSITY of CALIFORNIA, and Dr. C. B. BRIDGES of COLUMBIA UNIVERSITY.

FIRST APPEARANCE OF THE LETHAL

The original culture exhibiting a sex-count with the low proportion of females appeared in February, 1923, among the progeny of a cross involved in an experiment designed to measure the rate of lethal mutation in the X-chromosome of *Drosophila melanogaster*. Three generations previously a female from wild type stock had been mated to a male from eosin-vermillion-forked stock. (Eosin, vermilion and forked are sex-linked recessives with the following symbols and loci: eosin, w^e, 1.5: vermilion, v, 33.0: forked, f, 56.5 BRIDGES, 1921). A phenotypically wild female from this cross, with one X-chromosome of the wild type, the other containing eosin, vermilion and forked, was mated to an eosin-vermilion-forked male. A similar selection was made among the progeny for the parents of the next generation; these were the parents of the lethal culture.

The expectation in such a cross is approximately equal numbers of males and females and approximately equal contrary classes. By the procedure of mating normal-appearing females with eosin-vermilion-forked sibs in successive generations, a new sex-linked lethal may be detected. For not only are there disturbances in the sex-ratio resulting from the death of males, but one class of each pair of male contrary classes is markedly smaller than the other. Thus a measure of the rate of lethal mutation in the X-chromosome may be obtained. (MULLER and ALTENBURG 1919).

TABLE 1

A. The original lethal culture, w^evf ♀ × w^evf ♂. A deficiency in the male classes with the eosin end of the maternal w^evf chromosome is shown.

w^evf	+	w^e	vf	w^ev	f	w^ef	v
♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂
0 8	1 44	0 10	0 19	0 4	0 9	0 0	0 2

The original culture gave, as table 1A shows, an unexpected count of one female and 96 males. Moreover, there seemed also to be discrepancies

in the male classes receiving the eosin end of the eosin-vermilion-forked chromosome of the mother. The one female was sterile, for she produced no offspring by either an eosin-vermilion-forked sib or by a fresh wild type male. Such sterility is frequent in the lethal strain. The explanation of the deficiencies shown by the male classes receiving the eosin end of the mother's eosin-vermilion-forked chromosome is not clear. They are probably not purely "fortuitous". That they are not due to the heterozygous presence in the mother of a sex-linked *semi*-lethal in this region is shown by the fact that four cultures, producing grandsons of the eosin-vermilion-forked males did not show such deficiencies. The totals show the following male contrary classes: 85 wild type, 60 $w^e vf$; 23w^e, 30vf; 29$w^e v$, 22f; 5$w^e f$, 6v; 262 females were produced. That the discrepancies in the eosin-containing male classes of the first lethal culture are not necessarily correlated with the production of the low female count is demonstrated by the following: (1) The lethal strain was derived from a non-crossover wild type male of this culture. (2) Later lethal counts were obtained from crosses of eosin-vermilion-forked males by females one of whose X-chromosomes contained eosin, vermilion and forked (the other X-chromosome was wild type), and these counts did not present comparable deficiencies in the male classes containing eosin. This is shown by table 1B.

B. Further crosses of $w^e vf$ ♀ × $w^e vf$ ♂ *which give lethal counts. The lethal has been introduced from the lethal strain. No significant deficiency is shown in the male classes with the eosin end of the maternal* $w^e vf$ *chromosome.*

	$w^e vf$		+		w^e		vf		$w^e v$		f		$w^e f$		v	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
	8	25	3	35	1	17	2	18	1	5	1	8	0	2	0	0
	0	21	1	21	0	7	0	9	0	2	0	6	0	1	0	2
Total	8	46	4	56	1	24	2	27	1	7	1	14	0	3	0	2

The most plausible explanation of the irregularities in the contrary classes of table 1A involves the assumption that a new sex-linked lethal, quite a different lethal from that which kills females, arose in the mother of the culture. This new male-affecting lethal was supposedly located in the eosin end of the eosin-vermilion-forked chromosome and was not present in all of the unreduced eggs. The latter would necessarily be true since those of the surviving eosin-vermilion-forked sons which were tested did not transmit such a lethal. Sex-linked lethals of this type are ordinarily kept by breeding the heterozygous females. The procedure was impossible in this case because these females were killed by the maternally inherited

lethal. That is to say, the only individuals which would be expected to survive and show evidence of such a sex-linked lethal were killed by another lethal. It is obvious that this hypothesis remains tentative, since of necessity it is not amenable to test.

It should be noted concerning the cultures of table 1B that the lethal counts given are of F_2 offspring of a cross of an eosin-vermilion-forked female from the mutation rate experiment mentioned above, by a lethal male. The grandmother consequently is from the strain from which the female-killing lethal originally was derived. The interpretation of maternal inheritance employed later in the paper implies that if out-crosses are made of lethal males to non-lethal strains and the progeny are inbred, the female-deficient counts should not be produced until the F_3 individuals are obtained. However, if both parents should contribute the lethal, such counts might appear before F_3. Since the grandmother of the individuals included in table 1B was from the strain from which the lethal was derived, it is reasonable to assume that she may have been heterozygous for one or more genes concerned. If so, these cultures show no unexpected results. Of the 256 cultures which exhibit the lethal count (included in figure 1) these two alone show characteristics at first sight (and only at first sight) not compatible with the interpretation of maternal inheritance.

The exact conditions under which the lethal originally arose are unknown. Judging by its subsequent behavior, which shows that the mother of a lethal count must be homozygous for a second chromosome recessive, it seems most probable that this recessive was already present in the eosin-vermilion-forked stock which appears in the pedigree of the strain. If so, the several intervening generations produced the genetic combination conducive to the appearance of the female-deficient ratio.

CHARACTERISTICS OF THE LETHAL

The most outstanding feature of the lethal is, of course, the low proportion of females produced. The total number of flies from all cultures known to exhibit the lethal ratio gives 3,982 females and 21,821 males; thus the average sex-ratio is 1 female : 5.5 males. However there is a great variation in the sex-ratio produced by different cultures; for although a considerable proportion of the cultures produce very few females, or none, in others the sex-ratio more nearly approaches equality. This variation is shown by figure 1.

It should be stated concerning the cultures included in figure 1 that there has been an appreciable increase since the discovery of the mutant in the number of females produced per bottle. Of the cultures producing no

daughters, a relatively larger proportion appeared early in the work; the large number of cultures giving 20 to 30 percent females came late. Since the environment was the same at these two periods the effect may be attributed to selection. It is obvious that the strain would be perpetuated by matings from those bottles which, although showing definite lethal ratios, gave female offspring. Modifiers would thus be selected for whose tendency would be partially to counteract the lethal effect.

A few cultures producing sex-ratios intermediate between 1 female : 2 males and 1 female : 1 male were excluded from the totals and from figure 1 because, although several of these were shown by breeding tests to contain the lethal (there were about 6 of these; they would not substantially alter the sex-ratio of 1 female : 5.5 males), others were not so tested, and some of these latter undoubtedly did not represent lethal counts. Experience with non-lethal lines demonstrates that occasional fortuitous ratios of this type

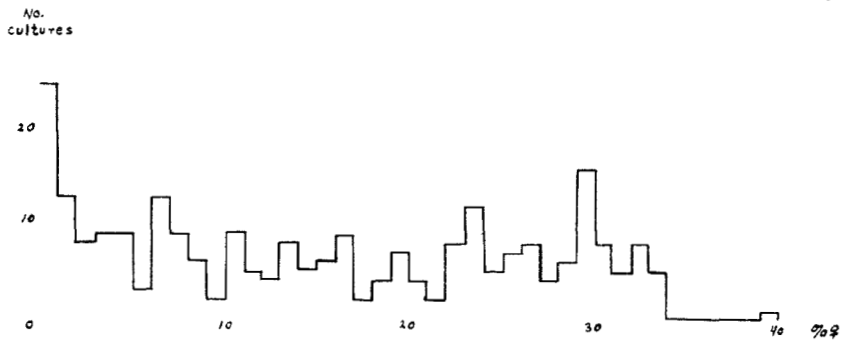


FIGURE 1. Variation in the female percentage of the total number of flies in cultures exhibiting the lethal count.

are produced, particularly when the number of offspring is not large. The tendency of such of these cultures as may have been lethal slightly to alter the sex-ratio would, moreover, be at least largely offset by the inclusion of cultures undoubtedly representing lethal counts which were excluded on the basis of their very low production.

Especially unfortunate attributes of the lethal strain are its high sterility and its low productivity. Large numbers of individuals produce no offspring whatever, and this seems to be due to sterility in both sexes. When offspring are produced, the numbers frequently are very small. Broods are produced, however, as an examination of the tables shows, which are large in size. The factors of low productivity and of high sterility have made the collection of data difficult. Indeed it is probable it would not have been considered worth while to preserve the strain, had it not been for a peculiarity of behavior indicating maternal inheritance.

Other characters of the lethal strain include various morphological abnormalities. Chief among these is the presence in all lethal cultures, of broken abdominal bands (at least since this character was discovered very early in the work). There is considerable variation in the extent to which this character is exhibited. Most, if not all, broken bands seem to be dependent upon a tendency to reduction in the dorso-lateral plates; in some individuals one of these plates is completely suppressed. Probably at least some of the sterility of the strain is correlated with these broken bands, for frequently the external genitalia of broken banded individuals are abnormal. The ratio of broken to non-broken is not appreciably different in the two sexes. The broken bands are found not only in bottles exhibiting the lethal count; they are also found in cultures derived from the lethal strain which do not show a lethal ratio. Individuals known to be heterozygous for the second chromosome recessive involved may have broken bands. The exact relation of the broken bands to this recessive lethal gene is not known; they may be dependent upon other genes. The matter is probably complicated by an environmental effect.

Other abnormalities occurring in the lethal strain with many times the frequency shown by normal stocks include: (1) a slight roughening of the eye, (2) a stunting of one wing or its complete absence; this is usually accompanied by a deformity of the thorax on the same side, (3) a reduction in the size of one or both eyes, and (4) a shrivelling or absence of one leg. Recently these characters, excepting the first, have largely disappeared. It seems probable that they were not definitely correlated with the presence of the lethal gene, but were independent characters present in masked condition in the stock and were brought out by the intensive inbreeding which was practised. It may be noted, however, that abnormalities of this type are common in individuals in which the developmental process is in any way radically upset.

Departures from the normal sex-ratio in *Drosophila melanogaster* are commonly due to an elimination of males by lethal or semi-lethal genes in the X-chromosome. However, sex-ratios exhibiting a deficiency in the number of females produced, although rare, have previously been reported. Subjection to a temperature of 31.5°C. during pupation has been found (MANN 1923) slightly to increase the number of males. Moreover, mutant characters are known, as truncate (ALTENBURG and MULLER 1920), which are somatically somewhat more pronounced in the female than in the male, and which in consequence lead to a somewhat lower relative viability in females. A sex-limited, sex-linked lethal was reported by THOMPSON (abstract, 1921) at the 1920 meeting of the AMERICAN SOCIETY of ZOO-

LOGISTS. This lethal killed all females homozygous for it; males with the lethal were perfectly viable, but held the wings erect. Crosses of heterozygous females with lethal males gave sex-ratios of 1 female : 2 males.

A similar lethal has more recently been described by BONNIER (1923). This lethal kills homozygous females, but has no apparent effect on the males containing it. As in the above case, it gives sex-ratios of 1 female : 2 males. BONNIER has shown how by its means sex-dimorphism can be simulated. He also predicts the finding of autosomal lethals which kill one or the other sex. This prediction is verified by the present lethal.

There is recorded in a recent paper on attached X-chromosomes (L. V. MORGAN 1925) a single culture (No. 184) producing a relatively large number of males. This is from a strain of peculiar behavior (being investigated by T. H. MORGAN) in which the ratio of males to females is variable but high.

Of a different type were the sex-ratios of the unisexual broods reported in 1910 by QUACKENBUSH. One of these cultures produced 135 males and no females, a ratio much like those given by the lethal now reported. It seems reasonable, as has been suggested by STURTEVANT (1920), that the sex-ratios obtained by QUACKENBUSH were due to crosses between *Drosophila simulans* and *Drosophila melanogaster*, which two species were at that time not distinguished (STURTEVANT 1919). The derivation of the parents from mass cultures is suggestive. The sterility of the flies constituting these unisexual broods and their rudimentary gonads, as well as the sex-ratios produced, are all characteristic of crosses of these species, as STURTEVANT'S work shows. In regard to the culture mentioned above which gave a count showing no females, it may be stated that the cross of *Drosophila simulans* female by *Drosophila melanogaster* male, but not the reciprocal cross, gives a greatly lowered proportion of females; the reciprocal cross gives all females.

Striking similarities are shown between a lethal in *Drosophila funebris* reported by MOHR and STURTEVANT (1919) and the present lethal. These investigators found a brood consisting of one female and 87 males. Sex-ratios showing the excess of males were repeated in later generations, but as in the *melanogaster* lethal, there was considerable variation in the sex-ratio, for there was a range from cultures producing no females to cultures in which the sexes were approximately equal in number. Broken abdominal bands were present, but unlike those occurring in *melanogaster* were only rarely found in males. It was believed that those individuals with extreme broken bands did not emerge from the pupa cases. When the strain was crossed to unrelated races the F_1 sex-ratio was 1 female : 1 male, and

broken abdominal bands did not occur. Both characters reappeared in F_2. This lethal does not, therefore, show maternal inheritance, and in this respect also it is different from the *melanogaster* mutant.

PROOF OF THE MATERNAL INHERITANCE OF THE LETHAL

In demonstrating the maternal inheritance of the lethal it will be shown that the reciprocal out-crosses produce different results. This, however, is true in out-crosses of ordinary sex-linked mutants. The further demonstration is then necessary that the character reappears for the first time in F_3 rather than in F_2. It will be shown also that the presence in the mother in homozygous condition of a second chromosome gene is necessary in order that the offspring exhibit the lethal count.

It may be stated at the outset that this second chromosome recessive is apparently not the only necessary element involved. This is indicated particularly by the fact that individuals of the same genetic composition with regard to this gene do not always give the same breeding results. That is, cultures sometimes do not give lethal counts which are expected to do so. The converse is not true. The accessory factors are not known; it is possible that environment plays a role.[1] There are indications that modifying or complementary genes exist. This statement is based upon: (1) the relative difficulty with which lethal counts are again obtained once the strain is out-crossed, even when the second chromosome is traced, and (2) the fact that a given female once having given a lethal ratio continues to do so when she is transferred to new bottles. This is strikingly brought out in the work on determining the time of death of the daughters, when it was necessary to make daily transfers of the parents. It has not been considered worth while to attempt a genetic analysis of these modifying genes. Their presence in no way invalidates the conclusion that the lethal effect is maternally inherited and is dependent upon a second-chromosome recessive.

Out-crosses of lethal females

Table 2 shows the immediate results of crosses of females from the lethal strain with males of the stocks indicated. The following genes, in addition to those previously enumerated, are involved. In chromosome II are: star, *S*, 0.0; black, *b*, 46.5; purple, p_r, 52.5; curved, *c*, 73.5 (BRIDGES and MORGAN 1919 and BRIDGES 1921). The curly used is a second chromosome

[1]Rather extensive attempts to vary the environment by means of acid and temperature have not yielded significant results, chiefly because of the difficulty of breeding this strain under con ditions not optimum.

TABLE 2

Crosses exhibiting the lethal count; the female parent in each case is from the lethal strain, the male parent of the type indicated is of different stock.

A. The female parent is known to be virgin.

	P_1♂	♀	♂	♂ per 1 ♀
	w^e *v f*	16	110	6.9
	w^e *v f*	1	69	69.0
	b pr c	17	98	5.8
	b pr c	1	126	126.0
	b pr c	11	71	6.5
	b pr c	26	106	4.1
	b pr c	1	121	121.0
	b pr c	16	126	7.9
	b pr c	36	128	3.6
	b pr c	0	49	. .
	b pr c	9	46	5.1
	b pr c	0	35	. .
	b pr c	38	105	2.8
	b pr c	3	39	13.0
	b pr c	10	53	5.3
	b pr c	1	124	124.0
	b pr c	8	129	16.1
	b pr c	22	50	2.3
	b pr c	6	96	16.0
	b pr c	0	83	. .
	b pr c	38	164	4.3
	b pr c	2	162	81.0
	IIIple	18	48	2.7
Total		280	2138	7.6

*B. The female parent is not known to be virgin; for cultures marked * she is known not to be virgin.*

	P_1 ♂	♀	♂	SHOWING DOMINANT CHARACTERS ♀	SHOWING DOMINANT CHARACTERS ♂	♂ per 1 ♀
	S D	2	57	0	28	28.5
	S D	0	90	0	63	. .
	**S D*	18	158	7	82	8.8
	**S D*	38	166	2	13	4.4
	S D	27	65	4	38	2.4
	S D	5	44	1	30	8.8
	Curly	1	56	1	27	56.0
	Curly	5	83	1	14	16.6
Total		96	719	16	295	7.5

stock of the composition $C_{CL}C_yc_n^2C_{CR}/+$. C_y is the gene for curly wings which is generally lethal in homozygous condition, c_n^2 is an allelomorph of cinnabar, and C_{CL} and C_{CR} prevent crossing over (WARD 1923). In this paper "curly" and "non-curly" offspring of the cross of curly stock by the lethal will be mentioned; the former designates offspring containing the C_y-chromosome of the curly stock, the latter may or may not (as will be made clear in the proper place) designate offspring containing the other chromosome. Dichaete, *D*, 40.0, is a third chromosome mutant. The IIIple stock used includes the following third chromosome recessives: roughoid, r_u, 0.0; hairy, *h*, 26.5; scarlet, s_t, 43.8; peach, p^p, 48.0; spineless, s_s, 58.5; sooty, e^s, 70.7 (BRIDGES and MORGAN 1923). It may be further noted that star and dichaete are both dominants and are both lethal when homozygous.

The cross of a female from the lethal strain by an unrelated male may, then, produce the lethal count, and this is true of all the stocks from which males were adequately tested. The lethal counts are not obtained among the F_2 progeny, but may reappear in F_3. The cultures included in table 2B are of particular interest. The female parent of the cultures starred was in each case purposely crossed first to a sib and later to a star-dichaete male. The fact that considerably less than three-fourths of the offspring exhibit one or both of the dominant characters indicates that the female produced offspring by both males. The same is undoubtedly true of the female parent of the last culture (in which cross the expectation is somewhat more curly than non-curly due to the greater relative viability of curly), although she was not intentionally double-mated. In these cases the lethal count is exhibited by the offspring showing dominant characters; moreover after the subtraction of the expected number of normal offspring (assuming that she was mated with the dominant male alone) from the actual number of normal offspring, the remainder also exhibits the lethal count. These crosses show, therefore, that not only may a female from the lethal strain produce a lethal count when mated to a male of unrelated stock, but a single female may be crossed in succession to two different types of male, and the lethal count be shown among the offspring of both males. This is to be expected in the case of a maternally inherited character.

Out-crosses of lethal males

Table 3 shows the results of the cross of males from the lethal strain with females from unrelated stocks. The sex-ratio in F_1 is 1 : 1. The same ratio is shown by the offspring of inbred F_1; it was thought unnecessary

to tabulate these results. In the F_3 obtained from these F_2 the lethal counts reappear, as the table shows.

TABLE 3

Crosses not exhibiting the lethal count; the female parent in each case is from the stock indicated, the male parent is from the lethal strain. As shown, offspring of these cultures exhibit the lethal count in the third generation.

	P_1 ♀	♀	♂	♂ per 1 ♀	F_1 lethal counts		
					♀	♂	♂ per 1 ♀
	S D	64	64	1.0	1	84	84.0
	S D	17	18	1.1	0	42	..
	S D	34	38	1.1	0	75	..
	..	..	..	..	2	54	27.0
	S D	60	52	.9	3	42	14.0
	Curly	49	50	1.0	0	146	..
	..	..	..	..	8	113	14.1
	Curly	10	11	1.1	11	152	13.8
	..	..	..	..	0	131	..
	..	..	..	..	0	106	..
	Wild-type	110	104	.9	2	98	49.0
	..	..	..	..	11	51	4.6
	Curly	64	49	.8	26	90	3.5
	..	..	..	..	0	106	..
	b pr c	111	117	1.1	17	98	5.8
	..	..	..	..	1	126	126.0
	..	..	..	..	11	71	6.5
	..	..	..	..	26	106	4.1
	..	..	..	..	1	121	121.0
	..	..	..	..	16	126	7.9
	..	..	..	..	36	128	3.6
	..	..	..	..	0	49	..
	..	..	..	..	9	46	5.1
	..	..	..	..	0	35	..
	..	..	..	..	38	105	2.8
	..	..	..	..	10	53	5.3
	..	..	..	..	1	124	124.0
	..	..	..	..	8	129	16.1
	..	..	..	..	22	50	2.3
	..	..	..	..	6	96	16.0
	..	..	..	..	2	162	81.0
	b p_r *c*	21	28	1.3	3	39	13.0
	IIIple	49	55	1.1	18	48	2.7
	IIIple	47	36	.8	0	83	..
	..	..	..	...	38	164	4.3
	$\frac{S \quad D}{\text{Curly} \quad +}$	39	37	.9	1	28	28.0
Total		675	659	1—	Total 327	3327	10.2

Reciprocal crosses, then, produce entirely different results in F_1. The disappearance of the lethal ratio in the cross of lethal male by unrelated female is purely temporary, since the peculiarity reappears in F_3.

Crosses from the curly-lethal strain

It was found in crosses between flies from the lethal cultures and flies from curly stock, that when the progeny were inbred, lethal counts might be obtained from non-curly females but they were never obtained from curly females. This is illustrated by tables 4 and 5. The parents of the two sets of cultures shown here differ, so far as known, only as regards one chromosome. For the male parents of both sets are curly and are from the inbred curly-lethal strain; the female parents of table 4 are non-curly

TABLE 4

Crosses exhibiting the lethal count; the parents are from the strain derived by crossing curly stock with the lethal. The female parent is non-curly; the male parent curly.

	♀	♂	♂ per 1 ♀
	0	131	..
	15	64	4.3
	4	50	12.5
	6	54	9.0
	24	74	3.1
	1	96	96.0
	20	133	6.7
	2	76	38.0
	6	67	11.2
	27	58	2.2
	7	127	18.1
Total	112	930	8.3

and from the curly-lethal strain, the female parents of table 5 are curly from this strain. The former females produce lethal counts, the latter do not. The latter are heterozygous for the second chromosome received from the lethal stock. The former, it is assumed, have become homozygous for this chromosome. Corresponding to the fact that not all non-curly individuals would be expected to be homozygous for this chromosome (since some of them must contain the homologue of the chromosome containing curly in the curly stock), is the fact that not all such non-curly females give the lethal count. That the curly female parents of the cultures of table 5 contained the lethal, although they did not show it, is demonstrated by the fact that their non-curly daughters again produced the lethal counts, as shown. In other words, to produce the lethal count, a

female must be homozygous for the second chromosome received from the lethal strain; that is, she must be homozygous for a second chromosome recessive.

TABLE 5

Crosses not giving lethal counts; both parents are curly and are from the strain derived by crossing curly stock with the lethal. From the non-curly daughters lethal counts were again obtained, as indicated.

				Lethal counts from F_1 non-curly ♀♀			
	♀	♂	♂ per 1 ♀		♀	♂	♂ per 1 ♀
	84	50	.6		11	152	13.8
	..	..	..		0	131	....
	..	..	..		0	106	....
	91	75	.8		0	146	....
	88	87	1—		26	90	3.5
	..	..	.		0	106	....
	74	70	.9		39	103	2.6
	..	..	...		30	67	2.2
	105	99	.9		24	83	3.5
	96	102	1.1		12	63	5.3
	..	..	..		32	91	2.8
	52	62	1.2		9	59	6.6
	58	52	.9		1	96	96.0
	47	60	1.3		21	164	7.8
	43	29	.7		37	113	3.1
	..	..	..		21	103	4.9
	145	162	1.1		10	60	6.0
	119	130	1.1		6	67	11.2
	109	112	1.0		27	58	2.2
	76	74	1—		28	59	2.1
	..	..	..		20	52	2.6
	101	86	.9		2	36	18.0
Total	1288	1250	1—	Tota	356	1905	5.4

Figure 2 represents a typical pedigree chosen to illustrate the genetic behavior of the lethal. To avoid irrelevant complexity only cultures are included which are in the line of ancestry of lethal counts. As is shown, a lethal male was mated to a female from curly stock, and this cross produced the offspring of culture A which showed a 1 : 1 sex-ratio. Two lines were established from this culture, in one the curly offspring of A were chosen as parents, in the other the non-curly offspring. The mating of curly individuals, B, gave a sex-ratio as expected of 1 female : 1 male. It is to be noted that the only non-curly chromosomes of the second pair contained in this line were necessarily from the lethal strain. Non-curly individuals

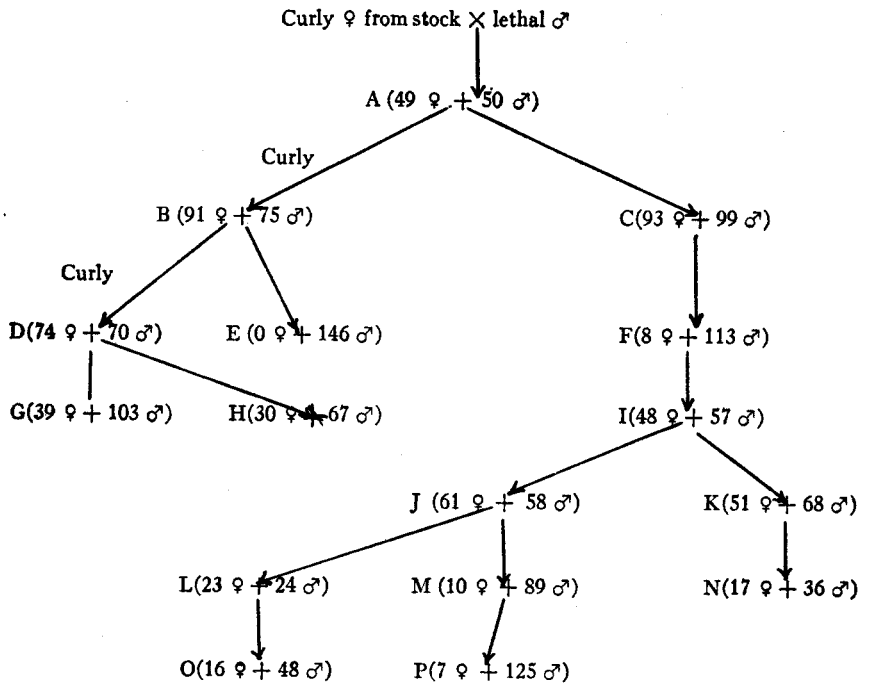
would be expected to produce lethal cultures. This expectation is verified by the cultures E, G and H.

The second line established, which was from the non-curly offspring of A, arose from the culture C. The parents of this latter culture are heterozygous and are expected not to give a lethal count; C shows a 1 : 1 sex-ratio. But the culture established from offspring of C might involve a mother homozygous for the lethal, correspondingly F does show the lethal count (these are the F_3 of the original cross). Not all of the females of this strain would be expected to give lethal counts, and they do not. All of these results are explicable on the basis of the presence of a second chromosome recessive. All other pedigrees involving the lethal may be interpreted on this same basis, although some of the results indicate the influence of modifying genes, as has been previously explained.

Since it seems unlikely on the basis of the breeding difficulties involved that the mutant will be of particular value in future experiments, the exact locus of the recessive gene in the second linkage group has not been determined. Incomplete data suggest a position near purple (52.5).

FIGURE 2

A typical pedigree showing the genetic behavior of the lethal.



CROSSES WITH A STRAIN WITH ATTACHED X-CHROMOSOMES

Whether the Y of the male, whose sisters die, is responsible *per se* for his survival is a question of interest. If this were true, females containing one extra chromosome, a Y, should be quite as viable as their brothers. To investigate the problem double yellow was used; both sexes of this strain contain a Y chromosome. The X-chromosomes of the female are more or less permanently attached (L. V. MORGAN 1922, 1925) and both contain the recessive gene yellow (*y*, 0.0). A female of this stock, then, obtains her X-chromosomes from her mother alone, she receives also a Y from her father. The male, on the other hand, receives his X from his father and his Y from his mother. It is to be expected that if the lethal is crossed to this double yellow stock, and if the presence of a Y chromosome is responsible for the survival of flies containing it, then succeeding generations should never yield lethal sex-ratios. But in such crosses lethal ratios (accompanied by broken abdominal bands) were obtained, as is shown by table 6; and in the expected proportion of cultures.

TABLE 6

Crosses giving the lethal count; the female parent of each is double yellow.

	Super ♀	♀	♂	♂ per 1♀
	0	43	87	2.0
	0	28	61	2.2
	0	27	67	2.5
	1	31	67	2.2
	0	37	75	2.0
	0	20	50	2.5
	0	43	89	2.1
	1	37	79	2.1
	0	26	55	2.1
	0	4	45	11.3
	0	0	77	....
Total	2	296	752	2.5

These results, however, are possibly not to be regarded as significant, for it was learned that a lethal somewhat similar in behavior is present in the attached-X stocks and sporadically gives female deficient ratios. This lethal is being studied by T. H. MORGAN. The lethal counts shown in table 6 are not, therefore, known with certainty to be due to the second-chromosome maternally inherited lethal.[1] Hence they do not prove, as

[1] Data from the crosses with double yellow have not been included in figures or tables other than table 6.

might offhand be concluded, that the Y is not responsible for the greater male viability. The question still remains open.

These crosses with the double yellow stock have bearing, however, on another problem. It might conceivably be held (although in view of considerations later to be presented, this is illogical) that the fact that the female zygotes do not appear is due to their never having been formed. Such an interpretation implies a gametic incompatibility in the lethal strain between an egg from a homozygous mother and the sperm which carries *X*. Now in the strain derived from the cross of lethal by double yellow, the sex resulting from such a combination is male (instead of female as is normally the case). If gametic incompatibility holds, therefore, these crosses will yield male-deficient sex-ratios rather than the female-deficient ratios present in the pure lethal strain. This is not the case; in the crosses involving double yellow no such reversed sex-ratio was obtained. It is believed that this fact probably cannot be attributed in each case to the exact counteracting (or overbalancing) effect of the presence of MORGAN'S lethal. It may be stated, therefore, that this evidence indicates that gametic incompatibility is not responsible for the female-deficient sex-ratio. The conclusion is strengthened by further evidence, as will be shown.

TIME OF DEATH OF FEMALES

On the assumption that gametic incompatibility is responsible for the abnormal sex-ratios it would be expected in the pure lethal strain that practically all eggs from homozygous mothers would be fertilized by Y-containing sperm, since X-containing sperm would be relatively impotent as regards fertilization. The number of males resulting, therefore, would be twice as large as in cultures from the same strain raised under similar conditions and showing non-lethal counts. This apparently is not true.

The fact that the number of offspring in lethal cultures is on the whole approximately half that of non-lethal cultures of the same strain, further shows that the females have not been "transformed" into males. The absence of intersexes tends also to preclude this possibility.

The above reasoning leads to the conclusion, therefore, that the female zygotes are formed but die. Dissection of pupae about ready to hatch shows lethal sex-ratios; death has consequently occurred before this stage.

In order to determine exactly when these zygotes die females were isolated and transferred daily. Each vial in which the females were kept was provided (according to the method of J. C. LI) with a narrow glass slide upon which was placed a slightly smaller strip of dark colored

blotting paper impregnated with fermented banana. Eggs were laid on the banana and showed up clearly against this background. The eggs were counted daily; after the elapse of several days the strip was again examined and the number of empty egg cases counted; this number is equivalent to the number of larvae which hatched (empty egg cases are more readily counted than burrowing larvae); a few days later the pupae were counted; as were the adults which emerged.

The conclusions which follow are based upon observations of the development of over 3,500 eggs from females producing lethal counts and of over 2,000 control eggs from a normal strain. Lethal females laid quite as many eggs as the control females. A marked discrepancy occurs, however, in the number of eggs which hatch—only 48 percent of the lethal eggs hatched as opposed to 73 percent of the normal eggs (this percentage is said to be low for normal eggs). Of the larvae from the lethal strain 70 percent pupate, of the larvae from the normal strain 86 percent. Of the pupae from the lethal strain 85 percent hatch into adults; of the normal pupae 95 percent. Thus it is obvious that although the embryo of the lethal strain has a lower viability throughout the entire developmental process, the difference in death rate is much more pronounced in the egg stage than at any other period. Although it cannot be stated definitely that all the females of the lethal strain which do not survive die as eggs, it seems probable that a large majority of these females die at that stage.

DISCUSSION

If a female from the lethal line is crossed to a male from an unrelated stock, the offspring immediately resulting may exhibit the lethal sex-ratio. But if the reciprocal cross is made, the F_1 never exhibit this ratio. Both crosses, alike, give lethal counts in F_3. The immediate offspring of the cross presumably, then, have the same genetic composition (whatever modifiers exist cannot be sex-linked); the only difference being that when the lethal is introduced from the mother most of the daughters die, but when it is introduced from the father they live. In other words, whether a given daughter lives or dies depends not upon her own genetic composition, aside from the fact of her being a female, but upon that of her mother.

We may draw further inferences regarding the nature of this maternal inheritance. It is obvious that the greatest difference between the female zygotes of the reciprocal crosses lies in the origin of their cytoplasm and shell. When the cytoplasm and shell are derived from the lethal line most of the daughters die, but when they are derived from the non-lethal line the daughters live. The cytoplasm or shell (or both) of the eggs from fe-

males of the lethal strain has (or have) been so affected that the combination with two X-chromosomes and the autosomes is very much less viable than the combination of non-lethal cytoplasm plus shell with two X-chromosomes and the autosomes, or than lethal (or non-lethal) cytoplasm and shell with X plus Y plus the autosomes. In this connection the reciprocal species crosses of *Drosophila simulans* with *Drosophila melanogaster* (STURTEVANT 1920) may be recalled. As with the present lethal the female offspring of these crosses are rarely able to develop if the egg cytoplasm and shell have been derived from one of the lines, but develop readily if they have been supplied by the other line. That the influence in the case of the lethal now reported is dependent in the final analysis upon the nucleus rather than upon independent cytoplasmic materials is shown by the fact that it is inherited as a second chromosome recessive.

If, in the lethal strain, the chromosomes of a given egg are assumed to be directly responsible for the effect on that egg, it seems probable that the effect becomes established previous to maturation, since, although half of the reduced eggs of a heterozygous female contain the second chromosome recessive, they do not show the lethal effect. However it is not necessarily the case that the chromosomes of the egg itself are responsible for the influence—the effect might conceivably result indirectly from the activity of the maternal chromosomes by means of substances circulating in the blood, or by absorbed materials from the interstitial cells of the ovary, or by means of the egg shell, or by other means. In case the influence is of this indirect type, it may or may not be impressed upon the egg after reduction.

CONCLUSIONS

1. There has been found in *Drosophila melanogaster* a lethal effect the appearance of which is governed by the genetic composition of the mother.

2. The sex-ratio of cultures exhibiting the lethal ratio is variable; the average sex-ratio is 1 female : 5.5 males.

3. A female whose progeny show the lethal sex-ratio must be homozygous for a second chromosome recessive gene.

4. The death of the females which do not appear as adults, probably occurs chiefly in the egg stage. It may be attributed to an influence of the maternal genetic composition upon the eggs which will give rise to the offspring exhibiting the lethal ratio, previous to the time when these eggs leave the mother's body. The presence in such eggs of the female-determining chromosomal complex results in a much higher death rate during development than does the presence of the male-determining chromosomal complex.

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