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Original article

Developmental time at which spontaneous, X-ray-induced and EMS-induced recessive lethal mutations become effective in *Drosophila melanogaster*

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Summary – Allozyme markers phosphoglucomutase (*Pgm*) and alcoholdehydrogenase (*Adh*) were used to determine the developmental time at which recessive lethal mutations of *Drosophila melanogaster* become effective. X-ray and EMS (ethyl-methane-sulfonate) induced mutations proved effective at earlier developmental stages, on the average, than natural lethal mutations of spontaneous origin. In competition experiments with X-ray induced lethal chromosomes, kept in balance with lethal marker chromosomes, late lethal mutations proved superior to early lethal mutations. For EMS-induced lethal mutations this effect was not observed. Reasons for and consequence of these observations are discussed.

lethal mutation / genetic load / *Drosophila melanogaster* / chromosomal arrangement

Résumé – Moment du développement auquel les mutations létales récessives, spontanées ou induites par rayons X ou par EMS, deviennent effectives chez *Drosophila melanogaster*. *Des marqueurs enzymatiques (phosphoglucomutase et alcooldéshydrogénase) ont été utilisés pour déterminer le moment du développement auquel les mutations létales récessives de* Drosophila melanogaster *deviennent effectives. Les mutations induites par rayons X ou par EMS (éthyl-méthane-sulfonate) se sont montrées en moyenne effectives à des stades de développement plus précoces que les mutations létales d'origine spontanée. Dans des expériences de compétition entre des chromosomes porteurs de létaux induits par rayons X, maintenus en équilibre avec chromosomes porteurs de marqueurs létaux, les mutations létales tardives se sont montrées supérieures aux mutations létales précoces. Cet effet n'a pas été observé pour les mutations létales induites par EMS. Les raisons et les conséquences de ces observations sont discutées.*

mutation létale / fardeau génétique / *Drosophila melanogaster* / arrangement chromosomique

* Correspondence and reprints

INTRODUCTION

The effects of radiation and chemical mutagens have been studied intensively in many respects (see Lee, 1976; Sankaranarayanan and Sobels, 1976). For the description of mutation risk it was especially important to know the exact dose-effect relations. However, other parameters such as the quality of induced mutations and their dominance in heterozygous individuals must also be known to obtain a reliable risk estimation. With respect to population genetics, a number of problems, such as soft *vs* hard selection, balancing selection etc, still appear to be unsolved, and the meaning of the so-called genetic load for the composition of natural populations is still a matter of discussion (see Wallace, 1991).

Drosophila has been used as a good model organism for investigation of radiation and chemical mutagen risks. However, in almost all cases only the quantity and specificity of the induced mutations has been considered. Little information is available about the time at which new mutations start to display their negative effects. This is most probably due to methodological reasons. Studies by Hadorn and Chen (1952) on the respective time of death caused by different recessive lethal mutations of *Drosophila melanogaster* had shown that a phase specificity exists in most cases. However, the technique they used in these investigations was to count directly, at various periods of development, the number of all surviving descendants of parents heterozygous for the same recessive lethal mutation. From the difference between the counts the effective time of the lethal effect could be deduced. This approach is very laborious and appears to leave some uncertainty. No genetic markers were available at that time that could be recognized at all stages of development and that could be used to distinguish directly surviving homozygous lethal genotypes from the other genotypes. Now the technique of allozyme electrophoresis has opened up a new possibility in this respect. Many of the enzyme loci are expressed at all developmental stages and allozyme variants can be easily identified at all larval, pupal and imaginal stages after starch gel electrophoresis. The present study on the phase specificity of spontaneous and natural lethal mutations, X-ray induced lethal mutations and EMS-induced lethal mutations has taken advantage of these new technical possibilities.

The main purpose of the investigations was to compare X-ray-induced, EMS-induced and natural lethal mutations with respect to the time of lethality. This appeared to be of biological importance because lethal genotypes dying very early do not consume food resources nor do they create problems for the populations other than their lethality.

MATERIALS AND METHODS

Drosophila melanogaster strains

The 1 510 wild flies of *D melanogaster* used in these experiments were collected in a garden in Tübingen in the summer and fall of 1988.

The standard strain "Oregon" was used as reference strain for standard gene arrangements for all chromosomes. As a 2nd chromosome balancer a Cy L chromosome with the inversions In (2L) Cy + In (2R) Cy was used. The Cy L chromosome

was kept in balance over a Pm chromosome (Cy L/Pm strain; see Ashburner (1989); p 533, 537). The Cy L chromosome carries the *Adh F* allele, the Pm chromosome carries the *Adh S* allele (see next paragraph). As a 3rd chromosome balancer the Me chromosome of the TM1 strain was used (Me/Ser strain; see Ashburner, 1989, p 539). Both chromosomes carry the *Pgm F* allele. All marker chromosomes (Cy L, Pm, Me and Ser; for description of mutants see Lindsley and Grell, 1968) are lethal in the homozygous condition. Crossing over is prevented by inversions. For all test crosses (see below) only Cy L or Me chromosomes were used respectively (see next paragraph).

Choice of lethal chromosomes

Electrophoretically fast (F) or slow (S) allozyme variants of alcohol dehydrogenase (*Adh*) and phosphoglucomutase (*Pgm*) were used as markers in larval and pupal stages for chromosome II and III respectively. Chromosomes carrying spontaneous lethal mutations from a wild population in Tübingen were screened for the presence of the slow (S) allele of either locus. All those lethal chromosomes that carried the fast allele (F) were discarded since Cy L and Me carry *Adh F* or *Pgm F* respectively.

Lethal-free strains homozygous for the S allele of *Adh* or *Pgm* derived from the Tübingen wild population were irradiated or treated with EMS to induce lethal mutations in linkage with *Adh S* (chromosome II) or *Pgm S* (chromosome III), respectively.

X-irradiation and EMS treatment

Males, 3–5 d old, were irradiated with 5 000 rad in air. Irradiation was administrated at a rate of 900 rad/min from an X-ray source (Mueller, Typ RT 100) operated at 10 kV, 8 MA *via* a 10-cm tube with 1-mm Be filtration. For EMS treatment the method described by Lewis and Bacher (1968) was used. Males 2–4 d old were starved for 4 h and then transferred for 24 h to a vial containing filter paper moistened with 0.025 M EMS solution. The males were crossed immediately with virgin females of the marker strain (cross 1). After 3 (cross 2) and after 6 d (cross 3) the males were transferred to another vial and again crossed with new virgin females of the marker strain. Most of the lethal mutation used in this experiment were found in the third cross, *ie* from males > 5 d after EMS treatment. X-ray and EMS treatement was applied to obtain lethal mutations for the experiments only. These were terminated after the number of different lethal strains was sufficiently high. Induced mutation rate was clearly rather high but no reliable estimates can be deduced from the protocols. It cannot be excluded that some of the lethal mutations were double mutants. Allelism tests were made for EMS-induced lethal mutations only. Among the 68 test crosses for 2nd chromosome lethal mutations and the 93 for 3rd chromosome lethal mutations, only one case of allelism was observed. One of the 2 allelic strains was discarded.

Crossing procedures

Lethal chromosomes from a wild population or from the offspring of mutagenized flies were derived by the usual backcross-methods with respective marker strains.

Natural or treated wild type males ($+^A/+^B$) were crossed with virgin females of the respective marker strains (Cy L/Pm or Me/Ser). By using only single males (Cy L/+ or Me/+) from the offspring of these pairings for each of the backcrosses with Cy L/Pm or Me/Ser females it was ascertained that only one of the 2 + chromosomes ($+^A$ or $+^B$) of the male parent was present in each specific test cross (we will choose $+^A$ for further description). From the crosses Cy L/Pm × Cy L/$+^A$ or Me/Ser × Me/$+^A$, respectively, Cy L/$+^A$ or Me/$+^A$ genotypes could be obtained that were then intercrossed (Cy L/$+^A$ × Cy L/$+^A$ or Me/$+^A$ × Me/$+^A$). Among their adult offspring, $+^A/+^A$ genotypes are expected to appear if the $+^A$ chromosome does not carry a recessive lethal mutation and not to appear at all if they carry a lethal mutation. According to the usage in population genetics, only those chromosomes that in the actual test crosses give < 10% expected wild type flies are considered to be lethal. In this experiment, however, only those strains that gave no wild type flies at all were used.

All crosses, and all balanced lethal strains were kept constantly at 24°C to make sure that the visible marker Cy was phenotypically well expressed.

The same kind of crosses were used in principle for the determination of the time of lethal effects with the exception that electrophoretic (*Adh S* or *Pgm S*) and not morphological markers were used instead. Further explanations are provided together with the results.

Competition experiments

All lethal chromosomes (LX; X stands for any undefined lethal mutation) used in this experiment were kept as strains in the laboratory in balance over the Cy L or the Me chromosome respectively (Cy L/LX or Me/LX). These strains could be used later for competition experiments between different lethal chromosomes. A detailed description of these experiments is given with the results.

Electrophoresis

Horizontal starch gel electrophoresis was used for the determination of the *Adh* or *Pgm* genotypes. The normal *Drosophila* technique (Ayala *et al*, 1972) was sufficient to read the gels even for first instar larvae (fig 1).

Determination of larval stage

The larval stages can be recognized in *Drosophila* from the shape of the mouth hooks (see Ashburner, 1989) as shown in fig 2.

Preparation of chromosomes

Larvae were dissected in 0.9% sodium chloride solution and the salivary glands transferred for 5 min into 2% lacto–acetic–orcein for staining, covered with a coverslip and squashed.

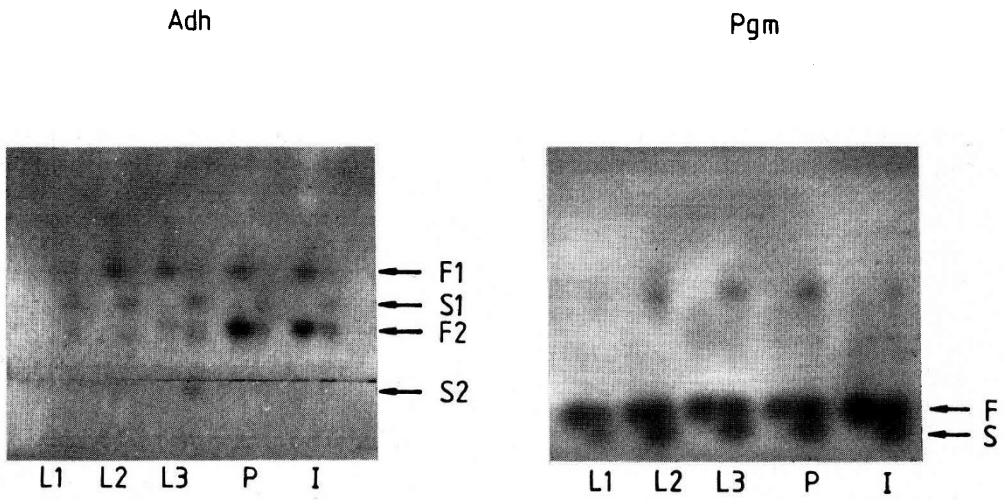

Fig 1. Allozyme pattern for *Adh*- (left) and *Pgm*- (right) genotypes using lst (L_1), 2nd (L_2), 3rd (L_3) instar larvae, pupae (P) or imagoes (I). For each developmental stage the F/F and the F/S allozyme pattern is shown. The allozyme pattern of genotypes homozygous for *Adh* F shows 2 bands (F_1, and F_2), that of heterozygotes *Adh* F/*Adh* S 4 bands (F_1, S_1, F_2 and S_2). Band S1 discriminates F/F from F/S genotypes even in the very faint pattern of L_1 larvae. Homozygotes F/F (1-banded pattern) and heterozygotes F/S (2-banded pattern) are easily recognizable in *Pgm*, even in L_1 larvae.

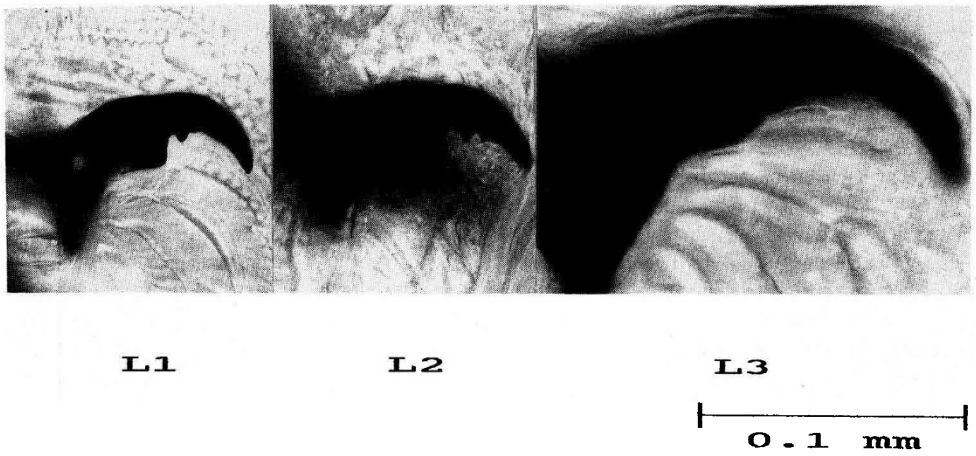

Fig 2. Mouth hooks of lst, 2nd and 3rd instar larvae. Photographs are in accordance with Strasburger (1935).

RESULTS

In the experiments described below, the allozyme marker *Adh* was used for second chromosome lethals and *Pgm* for third chromosome lethals. Both enzyme loci are expressed in all developmental stages from first instar larvae to adults (see fig 1). Electrophoretic analysis was always started with pupae. If lethal phenotypes were still present, the lethal was recorded as an "I"-lethal (effective before imaginal stage). Otherwise third, second or finally first instar larvae were tested electrophoretically. According to the last stage at which lethal homozygotes were absent for the first time, they were classified as I-, P-, L_3-, L_2-, or L_1-lethals.

Special population studies had to be made to characterize the spontaneous lethal mutations derived from nature in order to use only those lethal chromosomes that carry the S allele of *Adh* or *Pgm* respectively for the lethal test.

Among 694 wild second chromosomes tested, 625 (= 90.1%) carried the *Adh F* allele, and 69 (= 9.9%) *Adh S*. From 66 chromosomes with the *Adh S* allele, 10 (= 15.2%) were lethal. For third chromosomes the *Pgm* gene was used as an enzyme marker. In total 874 chromosomes were tested. Among those, 816 (=93.4%) carried the F allele, 57 (= 6.5%) the S allele and 1 (= 0.1%) a super-fast FF allele. Among 55 chromosomes with *Pgm S*, 10 (= 18.2%) were lethal. The 10 lethal 2nd and the 10 lethal 3rd chromosomes derived from these test crosses were used in the phase determination experiment.

An analysis of inversion polymorphism was performed for the 20 wild lethal chromosomes in addition. The results are shown in table I. Some of the frequent and cosmopolitan inversions (Ashburner and Lemeunier, 1976) were found in expected frequencies. One endemic inversion was present in addition on chromosome arm 3R. However, it should be noted that 6 of the independently derived 10 lethals of the second chromosome were on gene arrangement In (2L) t, whereas only standard arrangements were found among the 10 strains with lethal mutations on the 3rd chromosome. Since the frequency of In (2L) t in the natural population of *D melanogaster* from Tübingen was observed to range between 1–10% by Hadidy el Megid (1985), it might be assumed that lethals are hitchhiking on inversions In (2L) t in nature to some extent.

Most naturally occurring lethals are produced by spontaneous mutation events. Yet P-element activity (see Engels and Preston, 1984) might the another source of lethal mutations. Since the wild population from Tübingen is known to contain P and M cytotypes at the same time (Anxolabehere *et al* 1985) 20 iso-female strains from nature were screened for presence or absence of P-elements. From the observation of hybrid dysgenesis as well as from filter hybridization of a labeled P-element probe to *Hind* III digested total DNA from flies of the various strains (Kwon, 1990), 11 strains could be classified as P strains. The origin of some of the natural lethals by P-element mediated mutagenesis can consequently not be excluded.

One of the *Adh S* chromosomes and one of the *Pgm S* chromosomes from nature that proved lethal-free were used for the radiation and for the EMS experiments (see *Materials and Methods*). Induced lethals were extracted in the same manner as for wild lethals. For the phase determination experiment, 30 2nd chromosomes

Table I. Inversion gene arrangements in lethal strains from nature.

Chromosome	*Gene arrangement*	*No observed*	
		Among lethal II-chromosomes	*Among lethal III-chromosomes*
X	ST	10	10
2L	ST	4	10
	In (2L) t	6	0
2R	ST	9	10
	In (2R) NS	1	0
3L	ST	9	10
	In (3L) P	1	0
3R	ST	10	8
	In (3R) MO	0	1
	Endemic (83B-95E)	0	1

and 30 3rd chromosome X-ray induced lethals and 20 2nd chromosome and 30 3rd chromosome EMS induced lethals were available.

All the various chromosomes kept in balance were tested electrophoretically for the phase at which the lethal effect appears. In each specific test 30 individuals of each test cross were used for electrophoresis. Since it was found that the homozygous Cy L/Cy L as well as the homozygous Me/Me genotypes die before first instar larval stage, a 2:1 ratio for Cy L/lethal (= *Adh F/S*) or Me/lethal (= *Pgm F/S*) to lethal/lethal individuals (= *Adh* or *Pgm S/S*) was to be expected (*ie* 20:10 individuals). Only if all 30 individuals of a sample were heterozygous F/S it was it assumed that all lethal homozygotes had died before the respective stage of development used for electrophoresis. The results of all experiments are summarized in table II.

Among the 130 lethals tested, only 9 were "continuous", phase unspecific lethals, *ie* homozygotes for the lethal chromosome became less and less frequent from first instar larval to pupal stages. All other lethal chromosomes showed a more or less clear lethal phase effect, *ie* lethal homozygotes disappeared abruptly from one developmental phase to the other. None of the lethals was clearly biphasic, as observed by Hadorn and Chen (1952).

The distribution pattern of the lethal phase effects is graphically presented in figure 3. From there it can be seen that lethals from nature become effective later on the average than X-ray induced or EMS-induced lethals. Average time of lethal effect can be calculated from table II if continous lethals are excluded and the following time distances between the developmental stages are assumed: egg to L_1–24 h; L_1 to L_2–24 h; L_2 to L_3–24 h; L_3 to P–48 h; P to I–100 h (25°C). The average survival time of spontaneous lethals then becomes 120.0 ±16.1 h, X-ray-induced lethals 52.2 ± 5.9 h, and EMS-induced lethals 60.2 ± 7.5 h.

A χ^2 homogeneity test was performed to investigate whether the developmental phases of lethals (pooled into 3 classes: L_1+L_2, L_3+ P, I) were randomly distributed among the 3 types of lethals. The χ^2 values were highly significant between natural

Table II. Distribution of lethals arranged with respect to phase specificity.

Developmental[a] *stage*		*Lethals from nature (%)*	*X-ray-induced lethals (%)*	*ESM-induced lethals (%)*
II chromosome	L_1[b]	10	50	25
	L_2	30	13	5
	L_3	20	3	15
	P	10	23	20
	I	30	7	5
	C	0	3	30
	N[c]	10	30	20
III chromosome	L_1	0	60	47
	L_2	0	17	13
	L_3	40	23	27
	P	30	0	3
	I	30	0	3
	C	0	0	7
	N[c]	10	30	30

[a]Lethal phase is indicated by the first developmental stage at which no lethal homozygotes appeared among 30 individuals; [b]developmental stages: L_1 = first instar larva, L_2 = second instar larva, L_3 = third instar larva, P = pupa, I = imaginal stage, C = continuous lethals; [c]Total No of lethals tested.

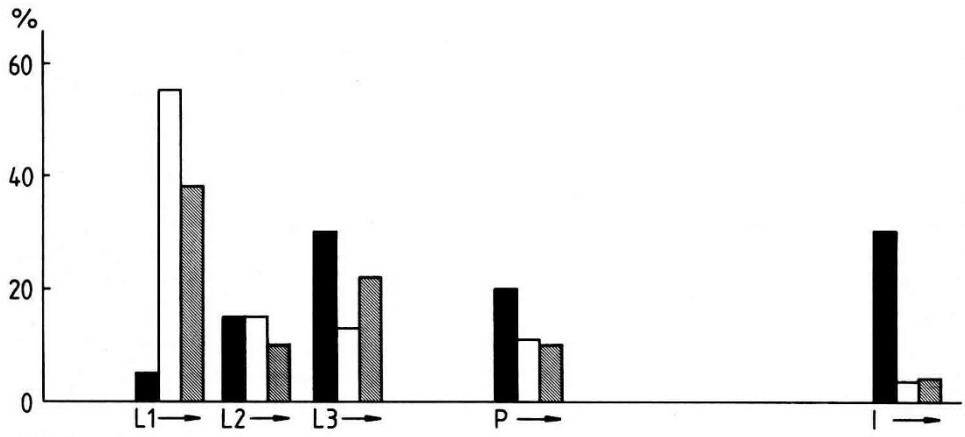

Fig 3. Frequency distribution of natural (black columns), X-ray induced (white columns), and EMS-induced (grey columns) lethal mutations with respect to developmental phase of the lethal effect. The heights of the columns correspond to the percentage at which the lethal class indicated at the abscissa is found. The distances between the developmental stages are proportional to time of development (see text for further explanations).

and both types of induced lethals, but not significant between X-ray-induced and EMS-induced lethals.

A cytological survey was performed for the X-ray- and EMS-induced lethals. It is wells known that an acute irradiation with a high X-ray dose over a rather short time induces a high proportion of chromosomal aberrations that are frequently lethal in the homozygous condition. EMS, on the other hand, induces mainly point mutations. Individuals from the strains, in which the induced lethal chromosomes were kept in balance over Cy L or Me, were crossed for that purpose to the standard strain Oregon (+/+, homozygous for *Adh F* and *Pgm F*). The F_1 offspring of these crosses is composed of 2 genotypes; one is heterozygous for the marker chromosomes (Cy L, *Adh F*/+, *Adh F*) or (Me, *Pgm F*/+*Pgm F*) and the other one is heterozygous for the lethal chromosome (let, *Adh S*/+, *Adh F*) or (let, *Pgm S*/+, *Pgm F*). Enzyme electrophoresis could consequently be used to discriminate between the 2 genotypes. The offspring larvae from the various crosses were dissected and their salivary glands used for chromosome preparations. The rest of the dissected larval body was then used for electrophoresis. Only chromosomes from heterozygous F/S larvae were investigated for chromosomal aberrations.

The results of the cytological examination are summarized in table III. Quite a number of induced chromosome aberrations could be detected among the X-ray-induced lethals, but only one among the EMS-induced lethals. Most of the aberrations were paracentric or pericentric inversions, but some deletions, transpositions and translocations were also observed (Kwon, 1990). It is also interesting to see from table III that the X-ray-induced aberrations were more frequently found among the early effective lethals than among the late effective lethals.

In the last series of experiments, the question was investigated of whether the late average lethal effect of the mutations from nature was due to a stronger selection against early effective lethals than against later effective lethals in heterozygous condition. Only strains with L_1 and L_3 lethals could be used for this purpose since the number of P or I lethal strains was too small for an experiment. Further, it was preferable to use only those lethals that were not kept in balance for too long a period in the laboratory. Since third chromosome X-ray- and EMS-induced lethals were more recently derived and tested, they were chosen for the study. Experimental population were constructed from the lines available. Each population was founded by 30 virgin females and 30 males from a strain containing an induced L_1 lethal in balance (Me/L_1) and 30 virgin females and 30 males containing an induced L_3 lethal in balance (Me/L_3). Each generation all females showing wild type phenotype ($= L_1/L_3$ genotype) were discarded in order to avoid recombination between L_1 and L_3 chromosomes. The fifth generation was used for a test cross in order to determine the relative frequencies of L_1 and L_3 chromosomes in the population. In each test 30 offspring flies were counted.

The results of the experiments using X-ray-induced lethals are shown in table IV, those of EMS-induced lethals in table V. Among the 12 populations of table IV, 4 (populations 2, 4, 5, 6) show a significant deviation from the 1:1 frequency ratio of L_1 to L_3 chromosomes; all of them deviate in favour of the respective late L_3 lethal. In one of the populations (population 7), a weakly significant deviation in favour of L_1 appears. All other populations have not changed their initial composition significantly. Yet, the total data from all populations together

Table III. Frequencies in percent of chromosomal aberrations in the X-ray- and EMS-induced lethal chromosomes arranged with respect to the time of lethal effect. Proportion of "No with aberration: Total No" in brackets.

Time of[a] *lethal effect*	*II Chromosome lethals*		*III Chromosome lethals*		*All lethals*	
	% with aberrations		*% with aberrations*		*% with aberrations*	
	rad	*EMS*	*rad*	*EMS*	*rad*	*EMS*
L_1	53 (8/15)	0 (0/ 5)	33 (6/18)	7 (1/14)	42 (14/33)	5 (1/19)
L_2	0 (0/ 4)	0 (0/ 1)	20 (1/ 5)	0 (0/ 4)	11 (1/ 9)	0 (0/ 5)
L_3	100 (1/ 1)	0 (0/ 3)	0 (0/ 7)	0 (0/ 8)	13 (1/ 8)	0 (0/11)
P	0 (0/ 7)	0 (0/ 4)	0 (0/ 0)	0 (0/ 1)	0 (0/ 7)	0 (0/ 5)
I	0 (0/ 2)	0 (0/ 1)	0 (0/ 0)	0 (0/ 1)	0 (0/ 2)	0 (0/ 2)
C	0 (0/ 1)	0 (0/ 6)	0 (0/ 0)	0 (0/ 2)	0 (0/ 1)	0 (0/ 8)

[a]Designation of developmental stages: see table II.

indicate once more that L_3 chromosomes are on the average less dominant with respect to fitness reduction (or more heterotic) in heterozygous condition than L_1 lethals. This phenomenon cannot be seen unambiguously in the populations with EMS-induced lethals (table V). Only 2 (populations 7, 12) of the 12 populations deviate significantly from the 1:1 expectation (both in favour of L_3). No significant departure occurs in the total data of all populations. However, it should be pointed out that the non-significant deviation of the total data is again in favour of L_3.

DISCUSSION

The fate of a mutation is basically determined by 3 different parameters: one that measures the effect on fitness (negative, positive or neutral), one that measures the mode of expression (recessive, dominant or heterotic), and one that measures the time at which the mutation becomes effective (in a certain stage of development or continously over life time). An amount of information has been gathered on fitness effects and dominance effects of mutations, but the time component of lethal effects has been almost completely neglected so far.

In the present investigation only recessive lethal mutations of *Drosophila melanogaster* have been studied to simplify the problem and to concentrate particularly on the effective time of lethality of homozygous carriers of a specific lethal mutation.

Our results show, in agreement with the earlier investigations of Hadorn and Chen (1952), that variability between different individual mutations exists. Some

Table IV. Selection experiments between X-ray-induced lethal chromosomes.

Population	*Lethal strains used*	*No of chromosomes found after 5 generations*		χ^2 *for deviation from 1:1*
		L_1	L_3	
1	A 48 × A 40	24	26	0.08
2	A 39 × A 40	11	39	15.68***
3	C 65 × C 90	18	29	2.57
4	C 23 × C 32	14	36	9.68**
5	C 65 × C 32	12	38	28.10***
6	B 07 × C 32	11	39	15.68***
7	B 79 × A 40	32	18	3.92*
8	B 79 × A 31	28	24	0.08
9	A 02 × C 32	25	25	0.00
10	A 88 × A 31	22	28	0.72
11	C 23 × A 87	23	27	0.32
12	A 02 × A 87	27	23	0.32
Total		254	352	19.18***

Each population was started with an L_1- (early effective) and an L_3- (late effective) chromosome in a 1:1 frequency ratio. $^{*}p0.05$; $^{**}p0.01$; $^{***}p0.001$.

Table V. Selection experiments with EMS-induced lethal chromosomes (for further explanation see table IV).

Population	*Lethal strains used*	*No of chromosomes found after 5 generations*		χ^2 *for deviation from 1:1*
		L_1	L_3	
1	C 22 × C 35	29	21	1.28
2	A 25 × A 40	24	22	0.09
3	A 35 × B 40	19	31	2.88
4	A 03 × A 25	23	27	0.32
5	A 42 × A 47	20	30	2.00
6	B 26 × A 47	29	21	1.28
7	B 18 × B 26	15	35	8.00**
8	B 59 × A 47	25	22	0.19
9	C 11 × B 46	27	23	0.32
10	B 22 × B 46	24	26	0.08
11	B 59 × C 47	26	22	0.31
12	C 17 × A 07	11	39	15.68***
Total		272	319	3.73

$^{**}p0.01$; $^{***}p0.001$.

of the lethal mutations become effective in early developmental phases, others in later phases; only a small percentage proves unspecific, continuously reducing the survival probability in all developmental phases equally. A probably general and new result of the present study is that lethal mutations extracted from natural populations become effective considerably later on the average than X-ray- and EMS-induced lethal mutations. Since natural mutations can be assumed to be of spontaneous origin, our observations could be interpreted in a broader sense even as evidence that spontaneous lethals become effective later on the average than induced lethals.

The weakest point in the general statement is certainly the assumption that lethal mutations extracted from nature are representative of newly arisen spontaneous mutations. Many of them have most probably persisted in the gene pool of the population for several generations and had therefore, been, exposed to natural selection. It must be expected that from the original spectrum of spontaneous lethal mutations those which persist mainly have less serious effects on the fitness of their heterozygous carriers; *ie* those that are less dominant. If it is assumed that a positive correlation between late effect of lethality and low dominance exists, then average natural lethals should display their lethal effects later than induced lethals that have not yet been exposed to natural selection. The competition experiments between L_1 and L_3 lethal mutations induced by X-rays (see table IV) and to a lesser degree those with EMS-induced mutations (table V) are very much in favour of this explanation. However, many more other observations must be considered in this context. A number of the natural lethals were found to be localized on gene arrangements with naturally occurring inversions (see table I). It can consequently not be excluded that some hitchhiking effects might be responsible for the persistence of those mutations in the gene pool, as has been observed on several occasions (Sperlich *et al*, 1982; Sperlich and Pfriem, 1986; Mestres *et al*, 1990). In addition, P elements were found to exist in the genomes of individuals of the population studied. Because of the coexistence of P- and M-cytotypes (Kwon, 1990; Anxolabehere *et al*, 1985), P-element mediated mutagenesis might be responsible for the origin of some of the lethal mutations studied. In conclusion, a generalization from the results presented here must be made with caution.

On the other hand, other differences between X-ray-induced, EMS-induced and natural lethal mutations evidently exist. As can be seen from table III, a very high proportion of X-ray-induced lethal mutations are combined with X-ray-induced chromosome aberrations, but only 1 single chromosome aberration was found among the EMS-induced lethal chromosomes. Furthermore, most of those X-ray-induced lethals that are combined with chromosome aberrations are early (L_1) effective lethals. These observations are certainly in favour of the assumption that lethal mutations that are associated with gross chromosomal rearrangements usually become effective early in development, whereas lethal effects caused by point mutations might be more easily compatible with survival to advanced developmental stages. Further investigations especially with new spontaneous lethal mutations are needed to clarify the situation.

Whatever the case might be, in the present study it was found that a great percentage of naturally occurring lethal mutations permits the survival of lethal homozygotes to later developmental stages. Whether this effect is also expressed in

lethal heterozygotes, in the sense that some dominant effects of the lethal mutation on the fitness of heterozygotes (Mukai *et al*, 1972) also become effective at the same developmental stage as in homozygotes, is not known. Yet, natural selection operating on the individual level will not act on the time component of a lethal mutation in any case. Only a significant positive correlation of phase specificity with the degreee of dominance of lethal alleles could be selectively effective. For the populations as a whole the situations might be different. Lethal genotypes dying very early in development do not consume any material from the resources available, but late dying genotypes do.

Especially if resources diminish and population sizes drop drastically recessive lethal mutations might become finally responsible for the extinction of the populations. This in turn could result in a kind of group selection (Boorman and Levit, 1974; Wade, 1978) against late effective lethals.

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