

LIFE-SPAN REDUCTION IN A *Drosophila melanogaster* STRAIN DEFICIENT IN EXCISION REPAIR

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ABSTRACT

The role played by excision repair in the life-span of *Drosophila melanogaster* was evaluated. The median and maximal life of virgin females and males carrying the X-linked excision repair deficient *mei-9^d* mutant were compared with those of repair proficient flies. Although the distribution of deaths of *mei-9^d* females and males exhibits a higher scattering than that of repair proficient flies, the four life-curves obtained are rectangular. Under the experimental conditions used this fact led us to conclude that in the populations analyzed death is due to senescence. It was also found that the median and maximal life of repair deficient flies are significantly lower than those of the control. The results obtained are attributed to the accumulation of unrepaired or incorrectly repaired DNA damage in *mei-9^d* flies and thus lend support to the view that DNA repair plays an important role in the determination of longevity.

INTRODUCTION

Genetic material has frequently been involved in theories of senescence (for reviews see Gensler and Bernstein, 1981 and Medvedev, 1984) and it has been hypothesized that the accumulation of DNA damage, with the consequent progressive loss of genetic information, could be the primary cause of biological aging.

Since the net accumulation of DNA damage is determined by the balance between its production and repair, increasing knowledge of the repair strategies evolved by different organisms, has stimulated the search for a possible relationship between specific repair mechanisms and aging.

Studies *in vivo* in the nematode *Tubatrix aceti* indicate a decline in DNA excision repair capacity with age (Targovnik *et al.*, 1984). In mammals a positive

correlation (Hall *et al.*, 1984) has been reported between life span and their excision repair efficiency, but other studies show only a partial (Francis *et al.*, 1981) correlation or none at all (Kato *et al.*, 1980). In cells from aged donors and from patients with progeria, a diminished (Epstein *et al.*, 1974; Plesko and Richardson, 1984; Licastro and Walford, 1986) as well as a preserved (Regan and Setlow, 1973; Ono and Okada, 1978; Hall *et al.*, 1981) capacity to repair DNA induced damage was found.

The ability of cells aged *in vitro* to repair induced damage has also been actively investigated. However, the results obtained are at best conflicting (Mattern and Cerutti, 1975; Hart and Setlow, 1976; Hasegawa *et al.*, 1984). However the question as to whether the declining repair capacity observed is one of the causes or a consequence of senescence remains open.

In more direct tests carried out to determine the role of excision repair in aging *in vitro*, it was found that cultured fibroblasts from xeroderma pigmentosum (XP) patients, known to be defective in excision repair (Cleaver, 1969) reached senescence at a passage number similar to cultured fibroblasts from normal individuals (Cleaver, 1984).

In an attempt to contribute to a better understanding of the relationship that might exist between DNA repair and life-span, a series of experiments with *Drosophila melanogaster* was undertaken. This organism is particularly suited to the study of senescence (Lamb, 1978) and the identification in the last few years of several repair deficient mutants (Boyd *et al.*, 1987) has opened interesting new perspectives and offers the possibility of a direct test *in vivo*.

In the experiments reported below, we have compared the life-tables of repair proficient females and males with those of a strain carrying the X-linked excision repair deficient *mei-9^d* mutation (Boyd *et al.*, 1976). Our results show that in both strains death results from senescence and that the excision repair deficient flies have a reduced life span.

MATERIALS AND METHODS

Drosophila melanogaster stocks carrying the X-linked mutations *y-me-9^d* (yellow, meiotic-9^d) or *y* (the original stock from which the *mei-9^d* mutant was isolated) were made isogenic for the second and third chromosomes. Each strain was also isogenic for its X-chromosome. This was accomplished by standard crosses with an *FM6/+; Cy (Curly)/B1 (Bristle); Ubx (Ultrabithorax)/Sb (Stubble)* stock. For description of all the symbols see Lindsley and Grell (1968) and for *mei-9^d* Baker *et al.* (1976).

Once established the stocks were maintained with similar larval density in culture bottles of 250 ml containing the yeast inoculated cornmeal-agar-sugar medium of this laboratory. Virgin females and males from both stocks were collected

simultaneously within 12 hours of emergence and kept separate (50 flies per 30 ml vial with 5 ml of culture medium and plugged with cotton). Throughout the experiments the flies were maintained at $25^0 \pm 0.5^0$ C in a 12 h light-12 h dark cycle.

Each experimental series consisted of 80 vials (20 vials of females and 20 vials of males of each strain). The flies were transferred without etherization every two days to vials containing fresh medium and the number of deaths was recorded every four days. The density of flies in each vial was maintained constant by replacing dead individuals with flies of the same age from other vials.

The median life span (age at which 50% of the flies survive), the maximal life span, the sample standard deviation and the coefficient of variation of the median were calculated for each group under analysis. Three replica experiments were carried out under similar conditions and Student's t-test was used to evaluate the statistical significance of the data.

RESULTS

Since there were no statistically significant differences between the three experimental series run, the data for each sex and genotype have been averaged.

The survival data for *y* and *y mei-9^a* flies (Table I) have been plotted to obtain the survival curves shown in Figure 1. The four curves are rectangular, with a plateau period during which only a few flies die (in the *y* strain the initial population undergoes only a 10% reduction during the first 60 days) followed by a rapid increase in the number of deaths in each interval.

The shape of the curves for females *y mei-9^a* and for females and males *y* is fairly similar, while that of *y mei-9^a* male exhibits a lower degree of rectangularity. To analyze this point further, the four survival curves were directly compared by taking the median life span observed as 100 per cent and all other data as percentage deviation (plus or minus) from these values (Pearl, 1940). The data obtained (Table I) have been plotted in Figure 2 with the median life of each curve at the same abscissal point. It can be seen that the maximal life span of *y* and *y mei-9^a* females and males extends beyond the median life span less than 40%, i.e. the ratio maximal life: median life for all curves analyzed is below 1.4 (Table I) fitting the model of rectangular curves described by Pearl (1940).

Table I also shows that females and males *y* have similar median and maximal life spans and that the median and maximal life spans of *y mei-9^a* males are significantly lower than those of females of the same genotype. When both strains are compared, the median and maximal life spans of *y mei-9^a* flies are significantly lower than those of *y*.

Table I - Life tables for *Drosophila melanogaster* females and males *y* and *y mei-9^a*. Average data of three replica experiments.

Days (x)	♀ <i>y</i>		♂ <i>y</i>		♀ <i>y mei-9^a</i>		♂ <i>y mei-9^a</i>	
	lx	xi	lx	xi	lx	xi	lx	xi
0-4	1.000	-100	1.000	-100	1.000	-100	1.000	-100
4-8	1.000	-94.6	1.000	-94.8	1.000	-94.0	1.000	-92.7
8-12	1.000	-89.3	1.000	-89.6	1.000	-88.1	1.000	-85.5
12-16	1.000	-83.9	1.000	-84.4	1.000	-82.1	1.000	-78.2
16-20	999	-78.5	1.000	-79.2	1.000	-76.1	982	-70.9
20-24	998	-73.2	1.000	-74.1	1.000	-70.1	964	-63.6
24-28	996	-67.8	1.000	-68.8	981	-64.2	944	-56.4
28-32	995	-62.4	1.000	-63.6	969	-58.2	930	-49.1
32-36	992	-57.0	993	-58.4	950	-52.2	903	-41.8
36-40	987	-51.6	982	-53.2	933	-46.3	881	-34.5
40-44	982	-46.3	971	-48.1	918	-40.3	838	-27.3
44-48	961	-40.9	960	-42.9	901	-34.3	804	-20.0
48-52	952	-35.6	941	-37.7	872	-28.4	701	-12.7
52-56	938	-30.2	923	-32.5	841	-22.4	598	-5.5
56-60	922	-24.8	908	-27.3	803	-16.4	512	+1.8
60-64	900	-19.5	882	-22.1	762	-10.4	340	+9.1
64-68	882	-14.1	860	-16.9	698	-4.5	220	+16.4
68-72	804	-8.7	839	-11.7	561	+1.5	144	+23.6
72-76	683	-3.4	788	-6.5	382	+7.5	64	+30.9
76-80	461	+2.0	561	-1.3	131	+13.4	0	+38.2
80-84	120	+7.4	404	+3.9	24	+19.4		
84-88	58	+12.8	202	+9.1	0	+25.4		
88-92	16	+18.1	109	+14.3				
92-96	0	+23.5	0	+19.5				
Med. life	74.5 ± 1		77 ± 2		67 ± 2		55 ± 2	
Max. life	90 ± 2		92 ± 3		82 ± 2		74 ± 2	
s	12.9		15.3		17.1		17.1	
C.V.	17.3		19.9		25.5		31.1	
Max. life/ Med. life	1.21		1.19		1.22		1.35	

(x) age interval; (lx) number of flies alive at the beginning of each interval; (xi) percentage deviation from median life; (s) sample standard deviation; (C.V.) coefficient of variation; Median and Maximal life: ♀ *y* vs. ♀ *y mei-9^a*; ♂ *y* vs. ♂ *y mei-9^a*; ♀ *y mei-9^a* vs. ♂ *y mei-9^a*; in all cases $P < 0.05$.

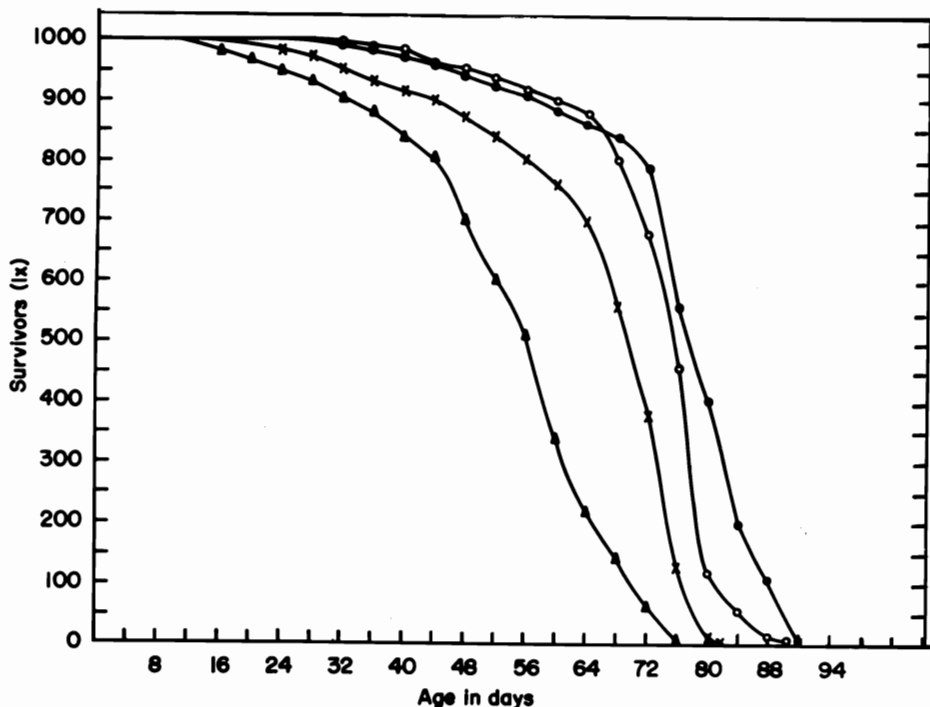


Figure 1 - Survival curves for four populations of *Drosophila melanogaster*. o ♀ y; ● ♂ y; x ♀ y mei-9^d; ▲ ♂ y mei-9^d.

DISCUSSION

Our results show that the survival curves of the four populations analyzed are typically rectangular that is, the median and maximum life span of the individuals are fairly close. This type of curve could be the consequence of senescence, an environmental lethal factor or the expression of the latent deleterious action of a gene(s) (Pearl, 1940). The latter possibility requires a homogeneous distribution of late acting lethal mutations in the tested populations which, to the best of our knowledge are not carried by our stocks. Since the experiments reported here were carried out under strictly controlled conditions, we believe that the death of y and y mei-9^d flies is due to senescence.

Our data further show that the longevity of y mei-9^d flies is significantly lower than that of y. The main genetic difference between the tested strains rests upon the constitution of their X-chromosome, because the stocks were made isogenic for the second and third chromosomes, which comprises approximately 80% of the genome.

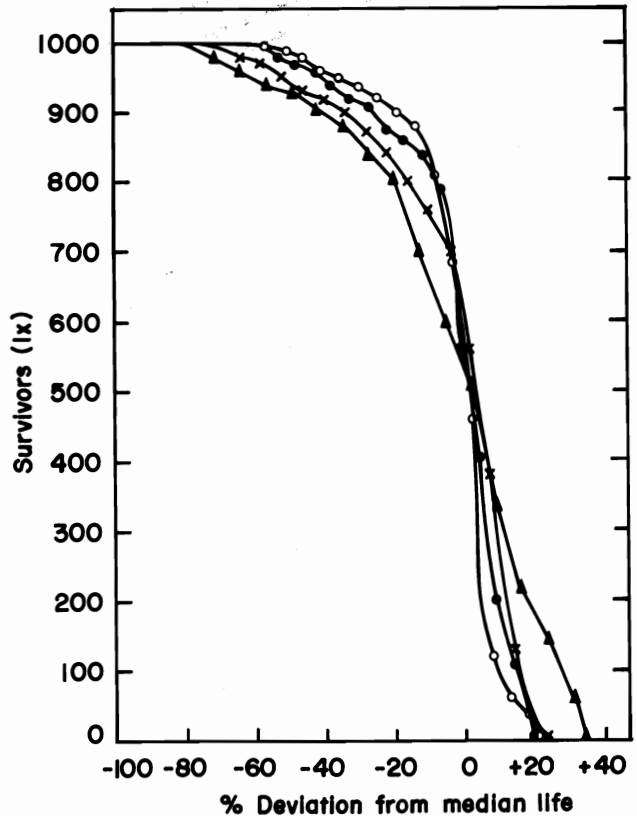


Figure 2 - Superimposed survival curves for *Drosophila melanogaster*, taking the median life of each population as 100% (abscissa) and all other ages as percentage deviation (plus or minus) from this value. \circ ♀ y; \bullet ♂ y; \times ♀ y *mei-9^d*; \blacktriangle ♂ y *mei-9^d*.

Thus, it seems reasonable to ascribe the differences found to an effect exerted by the X-linked *mei-9^d* mutation. A decrease in longevity is also exhibited by flies carrying the *mei-41^{DS}* mutation (Marquis *et al.*, 1983; Mayer and Baker, 1984) although in this case a different repair mechanism is affected, post-replication repair.

The *mei-9^d* mutation was originally isolated by its abnormal chromosomal behaviour during meiosis and subsequent biochemical analysis showed that somatic cells hemizygous or homozygous for this mutation are defective in excision repair. Mutant larvae exhibit hypersensitivity and are easily killed by a broad spectrum of mutagenic agents, and cytogenetic studies have shown that in nonmutagenized cells the frequency of chromosome breaks is higher than in the controls (Baker *et al.*, 1976; Baker *et al.*, 1978; Gatti, 1979). In *mei-9^d* flies the frequency of spontaneous (Hasson and Muñoz, 1988) as well as induced (Smith *et al.*, 1981; Ferro, 1983) mutation is also effectively increased. These results indicate that the normal functioning of the *mei-*

ϱ^d locus is required for the removal of a sizable proportion of spontaneous and induced DNA damage in somatic and germinal cells.

In the light of the foregoing evidence it is suggested that the life-span reduction observed in repair deficient *mei- ϱ^d* flies is due, at least in part, to the accumulation of unrepaired or wrongly repaired spontaneously arising DNA lesions. It is worth noting that in adult *Drosophila*, damaged cells cannot be replaced because in this organism cellular division is restricted to the germinal cell line (Lamb, 1978).

As would be expected for endogamic strains the life-curves of repair proficient flies exhibit a low variability (Figure 1) yet, the distribution of deaths of *y mei- ϱ^d* flies shows a higher scattering. This could be ascribed, owing to the stochastic nature of production of DNA damage, to an unequal accumulation of lesions in different individuals. The more pronounced effect observed in *y mei- ϱ^d* males relative to females, may result from the higher susceptibility to damage of *Drosophila* males, due to their hemizygous constitution for the X-chromosome.

As stated in the Introduction, the question as to whether DNA repair plays a role in the aging process has been a matter of much controversy. Our results offer direct evidence and seem to support the hypothesis that accumulation of DNA damage leads, through a progressive loss of accurate genetic information, to accelerated aging (Gensler and Bernstein, 1981).

Direct testing of the role played by excision repair in ageing has also been carried out in cultured fibroblasts from XP patients. The results obtained show that the number of cell doublings did not differ from the controls (Cleaver, 1984). These results suggest that the spontaneous lesions that these cells might not repair played no role in their *in vitro* aging.

Despite the difficulties of comparing the *Drosophila* life span with that of cultured human fibroblasts, Cleaver's (1984) results do not conflict with the data reported here, because although in both cases the mutants involved are defective in excision repair, they differ substantially. The *mei- ϱ^d* mutant shows hypersensitivity to a wide variety of DNA damaging agents. This has led to the suggestion that it may be affected in a controlling gene or in a late step of excision repair involving an exonuclease or a DNA polymerase, that may impede the removal of the damaged sequence regardless of the type of damage introduced (Nguyen and Boyd, 1977). This mutant is hypersensitive to killing by UV light, X-rays and γ -rays and defective in repair replication of DNA damaged by these agents (Baker *et al.*, 1976; Nguyen and Boyd, 1977; Dusenbery *et al.*, 1983), while in XP cells exposed to X-rays, repair replication (Cleaver, 1969; Kleijer *et al.*, 1970) and cell killing (Sasaki *et al.*, 1977, cited by Friedberg *et al.*, 1979) are within normal values. Furthermore, XP cells are only sensitive to one type of γ -ray induced damage in anoxia (Setlow *et al.*, 1976). The two mutant systems also exhibit differences in their response to chemical agents. The

mutation at the *mei-9^a* locus leads to a marked increase in sensitivity to methyl methanesulfonate, ethyl methanesulfonate and N-methyl-N-nitro-N-nitrosoguanidine (Boyd *et al.*, 1976; Nguyen *et al.*, 1979; Smith *et al.*, 1981; Dusenbery *et al.*, 1983), however, after treatment with these agents, XP cells are only marginally affected or not affected (Cleaver, 1971; Stich *et al.*, 1973; Fiedberg *et al.*, 1979; Heddle and Arlett, 1980). Moreover, in XP cells (Wolff *et al.*, 1975), unlike in *mei-9^a* cells (Baker *et al.*, 1976; Baker *et al.*, 1978; Gatti, 1979), the frequency of spontaneous chromosome aberrations is within control values.

Although the differences mentioned may be only a token of those actually existing, they suffice to show that some DNA damage is handled in different ways by these two mutants. Therefore, it is not surprising to find that *y mei-9^a* flies and XP cells differ in a trait that, like senescence, could be associated with the accumulation of unrepaired or incorrectly repaired DNA damage.

In view of the widespread occurrence of AP-sites, as a result of depurination and depyrimidation and their probable lethal effect in prokaryotes if not repaired before replication (Drake and Baltz, 1976), these lesions have been mentioned among those that might eventually play a role in aging (Gensler and Bernstein, 1981). Osgood and Boyd (1982) have identified in *Drosophila* an AP-endonuclease activity in cultured cells and larval brain ganglia. Indeed in extracts from *mei-9^a* flies this enzymatic activity is reduced. These authors suggest that a relationship may exist between a reduction in AP-endonuclease activity and the characteristics of treated and untreated *mei-9^a* mutants. The relevance of this finding to the decrease in longevity reported here cannot as yet be ascertained.

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RESUMO

O papel exercido pelo reparo de excisão sobre a longevidade de *Drosophila melanogaster* foi avaliado. A vida média e a máxima das fêmeas virgens e machos carregando o mutante ligado ao X deficiente para reparo de excisão *mei-9^a* foram comparadas com aquelas moscas com proficiência para reparo. Apesar da distribuição de mortes entre machos e fêmeas *mei-9^a* exibir uma dispersão maior do que aquelas de moscas com proficiência para reparo, as quatro curvas de sobrevivência são retangulares. Sob as condições experimentais usadas, este fato nos levou a concluir que nas populações analisadas a

mortalidade é devida a senescência. Também foi encontrado que a vida média e máxima das moscas com deficiência para reparo é significativamente menor que as do controle. Os resultados obtidos são atribuídos a um acúmulo de danos ao DNA não reparados ou reparados incorretamente em moscas *mei-9^a* e portanto apoiam o ponto de vista de que o reparo de DNA exerce um importante papel na determinação da longevidade.

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