

# Adult life span in *Drosophila melanogaster* populations selected for long and short developmental period

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## ABSTRACT

The relationship between pre-imaginal developmental period and adult life span was studied in *Drosophila melanogaster* populations not-selected and selected for long ( $\bar{x} = 386$  h) and short ( $\bar{x} = 226$  h) developmental period during 433 and 650 generations, respectively. An opposite and statistically significant life span difference between sexes was observed in the selected populations. In the slow population, females showed a significantly longer life span while the males had the shortest longevity. Thus, a slight positive correlation was observed only in females of the slow population which however had decreased fecundity. This may account for their greater longevity. The apparently random results in backcrosses suggest that life span is a complex character and many factors such as nuclear and mitochondrial genes, sex, and physiological, ethological and environmental traits interact for its determination.

## INTRODUCTION

The developmental theory of ageing (Lints, 1978, 1988) predicts a positive correlation between developmental period and adult longevity (Miquel and Fleming, 1988). Burcombe and Hollingsworth (1970) by altering the breeding temperature and Lints and Lints (1971) by changing larval density at a constant temperature, demonstrated that a longer developmental period was associated with a longer adult life span in *Drosophila melanogaster*. However, Yonemura *et al.* (1991a), employing Oregon-R *D. melanogaster* selected over 13 generations for long- and short-life span, verified that early emergers were often long-lived and suggested that the function of the longevity genes begins at the larval or pupal stage before emergence, since no significant relationship was observed between hatching

time and adult life span or between hatching and emerging time. Zwann *et al.* (1991, 1992) concluded that pre-adult developmental time is not a causal factor for the determination of adult longevity in *D. melanogaster*. In our laboratory we have selected flies for time of development over a period of 27 years, which has resulted in special genomic sets (Oliveira, 1979). These confer on the selected populations: 1) incipient reproductive isolation due to hybrid inferiority involving late females (Loreto and Oliveira, 1988a); 2) different electrophoretic patterns of genic activation chronology and general staining intensity for eight enzymatic systems (Oliveira and Cordeiro, 1982a, 1982b, 1984, Loreto and Oliveira, 1988b); and 3) hormonal imbalance (Loreto *et al.*, 1988; Jung, 1992). Moreover, two major genes probably control developmental time, and a recessive one is responsible for slow development (Oliveira and Cordeiro, 1981). This long developmental period gene was mapped on II. 63.8 (Oliveira *et al.*, 1991) and the main precocious gene at III. 62.38 (Correa and Oliveira,

unpublished results). In the present study we used these selected populations to analyze the relationship between hatching, larval and pupal time and adult life span in order to clarify the discrepant correlations found between pre-adult developmental period and adult life span.

## MATERIAL AND METHODS

Oregon-R *D. melanogaster* populations selected over 650 and 433 generations for short and long developmental period, respectively, and a non-selected population were used as the basic populations. They were named F = fast population (short developmental period), S = slow population (long developmental period) and C = control population.

Before selection, the egg-adult cycle at 25°C was 10 days. At this temperature, after selection, the F population adults started eclosion on the 7th day after oviposition, whereas the S population flies began only on the 13th day. To maintain the fast development (F) cultures, we bred the first flies that eclosed, especially those that hatched on the 8th and 9th days, let the flies age for two days and then prepared three culture bottles of 1/4 capacity, containing about 100 individuals each, that were transferred to new bottles every three hours. For the maintenance of the slow population (S) we bred only the last flies that eclosed, on the 13th day or later. These cultures were transferred every six hours, a rate that maintained about the same level of larval competition as in the more viable first instar larvae of the F population. For the C population we used flies collected from the 8th to the 15th day after oviposition. The culture medium used in this experiment consisted of 15 g agar, 1000 ml distilled water, 150 ml honey, 100 g yeast, Ponceau dye and 3 ml propionic acid.

Reciprocal hybrids (F female x S male = FS, and S female x F male = SF) were backcrossed with males and females of the S population. Only the S population was used because, as observed by Oliveira and Cordeiro (1981), the main gene for fast development is dominant over the genes for slow development and so it may be impossible to discriminate slow individuals in the backcross with the F population. After four days the females of the backcrosses were placed on oviposition medium for 30 minutes, and the observation of embryo hatching was started 16 hours later.

Each hatched embryo (first instar larva) was placed individually in bottles with the same oviposition medium, and its ontogenetic development was observed until adult eclosion. The eclosed adults were then placed in couples (one male plus one female) in

another bottle and periodically transferred until death. The same procedure was used for the analysis of the original F and S populations, and of the unselected control population (C). The entire experiment was carried out at 25°C in the constant presence of light.

Differences between sexes and life spans were tested by the Wilcoxon-Mann-Whitney method. The comparisons between crosses were made by the non-parametric Kruskal-Wallis ANOVA followed by multiple comparisons in a fashion paralleling the Tukey type test (Zar, 1984). This test was chosen because the distribution of developmental time was not normal in the populations. The correlations between pre-imaginal stages and life span were evaluated by means of the Spearman rank correlation coefficient.

## RESULTS

Among the selected (F) and (S) and nonselected (C) populations, F showed the shortest development time at all stages, S showed the longest time and the control was intermediate. In the backcrosses the descendants generally were intermediate (Table I).

The males of the C population had the longest longevity while those of the S population had the shortest longevity (Table I). There were opposite and statistically significant life span differences between sexes in the selected populations. In the control, the life span difference between sexes was very high. In the backcrosses, sex differences practically disappeared and the life span was close to that of the early males and late females. The only backcross showing a significant difference between sexes was S female x FS male, where males lived on average, four days less than females.

The life spans of males of all four backcrosses did not differ significantly. The descendants of SF female x S male had the same life span as the F population and the offspring of S female x SF male showed averages similar to those of the control population. In the backcrosses with the late population no male offspring showed life spans similar to those of the short life span population.

Among females, the S population descendants showed a significantly longer life span than the F and C populations, which did not differ significantly from each other. Among the backcrosses, no significant differences in female life span averages were observed and all of them were close to the S parental population.

The correlation analysis between life span and pre-adult developmental period is presented in Tables II (males) and III (females).

**Table I** - Means and standard deviations of pre-adult development period (in hours) and adult longevity (in days) for males and females of *Drosophila melanogaster* populations selected and non selected for short and long developmental period and their backcrosses.

Crosses and comparison between sexes		N	Developmental stage				
			E	L	Pu-A	E-A	LO
1. F x F	Female	60	16.9 ± 1.0	113.6 ± 10.6	96.6 ± 6.7	227.0 ± 15.2	30.0 ± 13.7 <sup>b(2)</sup>
	Male	60	17.1 ± 1.8	117.5 ± 8.8	92.5 ± 7.1	225.0 ± 13.0	24.3 ± 10.1 <sup>z</sup>
			NS (1)	NS	P < 0.05	NS	P < 0.05
2. C x C	Female	79	21.9 ± 2.4	131.5 ± 16.0	123.9 ± 9.4	277.3 ± 21.4	41.7 ± 13.0 <sup>d</sup>
	Male	79	21.6 ± 2.1	131.6 ± 14.0	115.8 ± 9.0	268.0 ± 18.0	19.0 ± 4.6 <sup>z</sup>
			NS	NS	P < 0.001	P < 0.01	P < 0.001
3. S x S	Female	68	24.8 ± 1.2	219.6 ± 12.8	142.1 ± 9.0	386.5 ± 15.2	21.9 ± 8.1 <sup>a</sup>
	Male	68	24.9 ± 2.0	223.1 ± 12.5	135.4 ± 11.4	383.4 ± 13.5	30.6 ± 10.8 <sup>x</sup>
			NS	NS	P < 0.001	NS	P < 0.01
4. SF x S	Female	87	18.6 ± 1.4	172.2 ± 12.8	108.6 ± 12.8	254.4 ± 18.2	33.6 ± 13.5 <sup>bc</sup>
	Male	87	18.7 ± 1.3	136.0 ± 16.6	106.2 ± 11.2	260.9 ± 19.5	32.9 ± 11.5 <sup>x</sup>
			NS	P < 0.01	NS	P < 0.05	NS
5. S x SF	Female	88	19.6 ± 2.3	132.1 ± 22.5	101.7 ± 8.5	253.4 ± 25.8	38.0 ± 14.7 <sup>cd</sup>
	Male	88	19.0 ± 2.3	135.6 ± 24.6	97.1 ± 7.5	251.7 ± 24.6	35.6 ± 11.8 <sup>x</sup>
			NS	NS	P < 0.001	NS	NS
6. FS x S	Female	84	21.0 ± 2.9	114.7 ± 11.6	114.9 ± 12.9	250.6 ± 19.4	32.5 ± 11.7 <sup>bc</sup>
	Male	84	20.8 ± 3.0	116.8 ± 13.9	112.9 ± 11.2	250.4 ± 20.2	32.2 ± 10.6 <sup>x</sup>
			NS	NS	NS	NS	NS
7. S x FS	Female	85	20.8 ± 1.9	137.7 ± 29.2	113.3 ± 10.6	269.7 ± 34.4	32.9 ± 12.6 <sup>bc</sup>
	Male	85	20.5 ± 1.8	151.0 ± 35.2	101.9 ± 9.5	273.3 ± 39.9	37.1 ± 13.6 <sup>x</sup>
			NS	P < 0.01	P < 0.01	NS	P < 0.05

F = fast population; S = slow population; C = control population. N = number of observed individuals. (1) = significance of the Wilcoxon-Mann-Whitney test comparing the two sexes in each cross; (2) = means indicated by same letter do not differ significantly (0.05 level of significance) among crosses; males and females analysed separately. E = egg-first instar larva; L = first instar larva-pupa; Pu-A = pupa-adult; E-A = egg-adult; LO = longevity. M = male; F = female.

Although a homogeneous population is not suitable for division into groups, in the present study we found no overlapping in egg-adult developmental time when we analyzed the selected populations. We observed that while the F population needed less than 290 hours (242 hours for males and 238 hours for females) to reach full pre-imaginal development, the S population needed more than 290 hours (402 hours for males and 397 hours for females) for egg-adult development. In the C population male flies took on average 299 hours and females took 286 hours, while in backcrosses the average was 282 hours for males and 285 hours for females. Nevertheless, when we observed the

individual eclosion of backcrosses we found flies that eclosed before and after 290 hours because the gene for fast development is dominant over those for slow development (Oliveira and Cordeiro, 1981) and in backcrosses we had heterozytes for fast development and homozygotes for slow development. Flies in these two groups were expected to occur in the control population. Thus in this analysis the flies were separated into two groups according to their egg-adult developmental period: flies that needed less than 290 hours to reach full development were considered "precocious" and those that needed 290 hours or more were termed "late".

No correlation between life span and duration of pre-imaginal period was observed in males of the selected populations. However, in the control individuals that eclosed after 290 hours, a significant positive correlation between life span and the larva-pupa period and a significant negative correlation with the pupa-adult period were found (Table II). These opposing findings in successive developmental stages led to the lack of a significant correlation between life span and the length of the pre-imaginal period (Table II,  $P > 0.20$ ). In the backcrosses, significant positive correlations were found only in the following crosses: the egg-larva period of S female x SF male and the larva-pupa period of S female x FS male (Table II).

Among the females of the selected populations, only those belonging to the S population showed low but significant positive correlations between longevity and both the larva-pupa and egg-adult periods (Table III).

In the backcrosses the same positive correlations were found in the precocious offsprings of the

SF female x S male and S female x FS male for egg-adult crosses only (Table III). In the S female x SF male cross, however, a low but significant negative correlation with length of the pupa-adult stage was detected (Table III).

## DISCUSSION

The relationship between longevity and genetic and/or environmental factors has been extensively studied over the last years (for a review see Collatz and Sohal, 1986; Lints and Soliman, 1988 and Finch, 1990). However, a clear picture of these relationships has not yet been presented. The present investigation was carried out to correlate developmental period with life span. From the data in Table I the effectiveness of selection for developmental period is clear because the F population developed much faster than the S population, while the control population presented intermediate values at all developmental stages.

**Table II** - Correlation coefficient between adult longevity and pre-adult development period for males of *Drosophila melanogaster* populations selected and non selected for developmental period.

Crosses	Female	Male	N	Pre-adult development				
				Egg-adult development	Egg-first instar larva	First instar larva-pupa	Pupa-adult	Egg-adult
1. F x F	x	F	60	< 290 h	-0.052	0.081	0.050	0.004
			0	≥ 290 h	-	-	-	-
2. C x C	x	C	57	< 290 h	-0.086	-0.143	-0.031	-0.131
			22	> 290 h	0.163	0.557**	-0.525*	0.132
3. S x S	x	S	0	< 290 h	-	-	-	-
			68	> 290 h	-0.059	-0.037	0.078	-0.033
4. SF x S	x	S	84	< 290 h	0.116	-0.087	0.014	-0.064
			3	> 290 h	-0.065	-0.762	0.918	-0.434
5. S x SF	x	SF	76	< 290 h	-0.066	-0.162	0.027	-0.140
			12	> 290 h	0.606*	-0.033	-0.058	0.571
6. FS x S	x	S	84	< 290 h	-0.171	-0.028	-0.065	-0.111
			0	> 290 h	-	-	-	-
7. S x FS	x	FS	61	< 290 h	0.213	0.144	0.168	0.208
			24	> 290 h	0.029	0.436*	0.134	0.335

F = fast population; S = slow population; C = control population. N = number of observed individuals. (F x F) = fast population cross; (C x C) = control population cross; (S x S) = slow population cross; (SF x S; S x SF; FS x S; S x FS) = backcross between fast and slow population. \* = significant at 0.05 level; \*\* = significant at 0.01 level.

**Table III** - Correlation coefficients between adult longevity and pre-adult development period for females of *Drosophila melanogaster* populations selected and non selected for long and short developmental period.

Crosses	Female	Male	N	Pre-adult development					
				Egg-adult development	Egg-first instar larva	First instar larva-pupa	Pupa-adult	Egg-adult	
1.	F	x	F	60	< 290 h	0.138	0.139	0.168	0.103
				0	≥ 290 h	-	-	-	-
2.	C	x	C	71	< 290 h	0.197	-0.092	0.045	0.091
				8	> 290 h	-0.102	0.437	0.323	0.336
3.	S	x	S	0	< 290 h	-	-	-	-
				68	> 290 h	-0.028	0.270*	0.177	0.288*
4.	SF	x	S	79	< 290 h	0.113	0.258*	0.129	0.353*
				8	> 290 h	-0.093	-0.100	-0.198	0.366
5.	S	x	SF	77	< 290 h	-0.045	-0.050	-0.226*	-0.092
				11	> 290 h	-0.545	0.433	-0.057	-0.198
6.	FS	x	S	84	< 290 h	-0.202	-0.007	-0.116	-0.147
				0	> 290 h	-	-	-	-
7.	S	x	FS	48	< 290 h	0.164	0.160	0.184	0.328*
				37	> 290 h	-0.168	-0.009	-0.012	-0.017

F = fast population; S = slow population; C = control population. N = number of observed individuals. (F x F) = fast population cross; (C x C) = control population cross; (S x S) = slow population cross; (SF x S; S x SF; FS x S; S x FS) = backcross between fast and slow population. \* = significant at 0.05 level.

The longevities we found for males and females were generally lower than those in the literature. This may be due to our experimental setup with sexes combined in couples. Studies carried out by Partridge (1986) have demonstrated that reproductive activity has a negative effect on life span. When male reproductive activity is manipulated by experimentally altering the rate of supply of virgin females to males, it is shown that higher levels of mating activity do reduce life span (Partridge and Farquhar, 1981). In *D. melanogaster* there have been several demonstrations that in general virgin females live longer than inseminated females (Malick and Kidwell, 1966). Service (1988) showed that virgin *D. melanogaster* of both sexes lived longer than mated flies, but differences in longevity between populations that had been selected for early-life or late-life fitness were not affected by mating status.

In our experiment we observed differences in longevity between single and coupled flies, with single flies living longer than mated flies (unpublished results). However, we measured the life span of flies in couples

because we think that this situation is more similar to field conditions and when we take into account that, from a biological viewpoint, what is important is the offspring that an individual leaves for the next generation it seems most reasonable for us to analyze flies in couples.

The contrast among versus within selected population, when we analyzed concomitantly the sex longevity performance, showed that genetic variation must occur among populations and this should have important biological implications. The opposed and statistically significant life span differences found in the populations account for the maintenance of these genes. In our experiment the results showed that populations with distinct pre-imaginal developmental period need to have concomitant sex longevity differences to survive. It is possible that regulatory genes responsible for these traits act in coadaptive clusters. A concomitant selection for developmental period and longevity probably would resolve this question. A similar pattern of sex-specific effects on the relationships of egg-to-

adult developmental time to adult longevity has been noted, for the abnormal abdomen system, in *Drosophila mercatorum* by Templeton *et al.* (1993).

As the response to developmental period is asymmetric (Clarke *et al.*, 1961; Oliveira, 1979), being somewhat easy to select individuals for a long period, which is not the case for a short period; it appears that marginal phenotypes are present but need to carry sets of genes which enable them to survive and transmit their genes to the next generation, insuring species variability. The effect of natural selection declines with ageing after the onset of reproduction (Charlesworth, 1980). This means that any decrease in a fitness character (e.g. fecundity or longevity) has a greater influence on lifetime fitness when it occurs earlier in a life cycle. In our S population Oliveira and Cordeiro (1981) showed that females have low fecundity, productivity and egg-adult viability close to semilethal conditions. These may account for the low but positive correlation with life span observed in slow females.

The negative genetic correlation between early fecundity and longevity indicates that, in agreement with the antagonistic pleiotropy hypothesis, reduction in longevity could evolve by correlated genetic responses to selection acting on early reproduction. Several selection experiments have indicated genetic responses of increased longevity, some of which were accompanied by changes in lifetime fecundity (Luckinbill *et al.*, 1984). A delay in senescence tends to be related to a reduction in early fecundity (Tanaka, 1993).

Rose (1984) propagated long-lived strains by breeding from older adults and found that early fertility declines and longevity increases. This finding suggests that one of the main ways in which female longevity can be increased is by a drop in early fertility which is therefore evidence that female reproductive activity does reduce longevity. As our slow females decreased their fecundity this accounts for their greater longevity. The general picture seems to be that various aspects of reproduction do have a cost in terms of survival.

When analyzing the relationship between life span and pre-imaginal development period, Yonemura *et al.* (1991a) found no correlation between embryonic hatching time and adult life span and suggested that the developmental period during the embryonic stage is not influenced by the longevity genes, which were localized by Yonemura *et al.* (1989, 1990) and Luckinbill *et al.* (1988) on the three main *D. melanogaster* chromosomes. This lack of correlation is corroborated by our data for the pre-hatching embryonic stage of the selected populations. However, the observation that early adult emergers are often long-lived (Yonemura *et al.*, 1991a) was not confirmed in our experiment; on the

contrary, our late females were long-lived. This result corroborates the recent suggestion of Buck *et al.* (1993) about the relationship between developmental period and adult longevity. In this case these two traits do not present simple relationships as claimed by Yonemura *et al.* (1991a). Our experiment, however, showed correlation among sex, developmental period and adult life span. One population that was not submitted to selection for extremes of developmental period maintained a counterbalanced genotypic set for the ontogenetic needs of the species, showing a low frequency of extremely fast and slow individuals. Unfortunately, the study of the action of pre-adult developmental period on the adult life span of *D. melanogaster* has been limited thus far to hybrid genotypes, so that the true contribution of this biological variable to life span, has not yet been clarified.

Another biological aspect which seems to be related to life span and developmental period is the maternal effect. Yonemura *et al.* (1991b) found a cytoplasmic factor which is presumed to influence the expression of the nuclear longevity genes. This factor showed a typical maternal inheritance and was considered to be an extranuclear gene, probably a mitochondrial gene. In the late selected populations, Oliveira and Cordeiro (1981) found a maternal factor which plays a role in decreasing the viability of first instar larvae of this population. Since the backcrosses had a life span similar to that of the maternal line but a faster developmental time, and since there was no difference between backcrosses with S as mothers and fathers, apparently this maternal factor seems not be related to life span. However, our late population is characterized by the presence of a fast IDH-NADP allele, whereas the control (C) population and the population selected for fast development (F) have a slow IDH-NADP allele (Loreto and Oliveira, 1988c). Surprisingly, descendents of reciprocal crosses between the two selected (F and S) populations, later selected again for fast and slow developmental time, for many generations, showed that, to be developmentally slow, flies must always carry the fast allele for IDH-NADP (Oliveira, 1989). This is not due to linkage because the IDH-NADP gene is located on the third chromosome (Fox, 1971), while the gene for late development is located on the second chromosome (Oliveira *et al.*, 1991). The oxidative decarboxylation of isocitrate is catalyzed by isocitrate dehydrogenase inside mitochondria, and because of the close association between a specific IDH-NADP allele and the late population the fact that this late population carries a strong maternal effect and 34% of IDH-NADP is found in the mitochondrial fraction (Fox *et al.*, 1972)

has permitted the interconnection between IDH-NADP pattern, late development and life span. Additional biochemical characterization of fast and slow IDH-NADP allele products is needed to confirm this suggestion.

Wallace (1992) suggests that oxidative phosphorylation declines with age; the expression of the mitochondrial DNA oxidative phosphorylation genes requires functional mitochondrial replication, transcription and translation systems and the polypeptides for these processes are encoded by the nuclear DNA. Thus, the biogenesis of oxidative phosphorylation requires many nuclear and cytoplasmic genes. One likely mechanism of malfunction in oxidative phosphorylation is the accumulation of damage to mitochondrial DNA by oxidation and the progressive increase of deleted mtDNAs, because of their tendency to replicate more rapidly than full-size mtDNA. Richter (1988) proposed that oxidative generated DNA fragments will escape from the mitochondria and become integrated into the nuclear genome. Time-dependent nuclear accumulation of mitochondrial DNA fragments may progressively change the nuclear information content and thereby cause aging.

As can be seen, the ageing process is very complex, and nuclear as well as cytoplasmic factors probably play an important role. Our results for females selected for late development over a very long time (433 generations) corroborate data reported by Burcombe and Hollingsworth (1970) and Lints and Lints (1971), but the lack of correlation between developmental period and longevity for males and the low consistency of the results obtained when the genotypes are split in the backcrosses and a new genetic background is built indicate that the relationship between longevity and developmental period is not simple and linear as proposed by these authors and by Yonemura *et al.* (1991a). Some other factors may be related to life span and for this reason more integrative experiments considering cytoplasmic and nuclear factors in addition to sex and physiological, ethological and environmental conditions known to play a role in the determination of life span are necessary for a better understanding of the relationship between developmental period, longevity and the ageing process.

## RESUMO

O relacionamento entre velocidade de desenvolvimento pré-imaginal e longevidade do adulto foi estudado em populações de *Drosophila melanogaster* não selecionadas e selecionadas para desenvolvimento rápido ( $\bar{x}$  = 226 hs) e desenvolvimento lento ( $\bar{x}$  =

386 hs) durante 650 a 433 gerações respectivamente. Observou-se uma diferença de longevidade estatisticamente significativa entre os sexos nas populações selecionadas. Entre as fêmeas a população lenta mostrou longevidade significativamente maior enquanto os machos tiveram a menor longevidade. Assim uma correlação positiva baixa foi observada somente nas fêmeas da população lenta as quais, todavia, diminuem sua fecundidade e isto pode explicar sua maior longevidade. Os resultados aparentemente ao acaso dos retrocruzamentos sugerem que a longevidade é um caráter complexo e muitos fatores como genes nucleares, herança materna, sexo e características fisiológicas, etológicas e ambientais interagem para sua determinação.

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