Cytoplasmic influence on the expression of nuclear genes affecting life span in *Drosophila melanogaster*

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In earlier studies we have found that the difference between short and long life spans of two inbred strains of *Drosophila melanogaster* is controlled by nuclear major genes. The present study has revealed a cytoplasmic factor that influences the expression of the nuclear longevity genes. The factor shows a typical maternal inheritance and is considered to be an extranuclear gene, such as mitochondrial DNA (chondriome). This paper marks the discovery of two basic forms of inheritance, nuclear and extra-nuclear, in the genetics of life span of *D. melanogaster*. These findings suggest that further studies, including genetic engineering, on longevity and aging might enable direct manipulation of these characters.

Keywords: *Drosophila melanogaster*, extranuclear genes, gene expression, life-span, nuclear major genes.

Introduction

From the results obtained in a number of studies, it has been concluded that life span is under polygenic control. Genetic contribution to variation of life span is not large; environmental conditions such as temperature or food are major determinants. Heritability of life span has been calculated as 0.133–0.442 in *Drosophila* (Maynard Smith, 1959; Tantawy & Rakha, 1964), 0.2–0.3 in nematodes (Johnson & Wood, 1982), and 0.21–0.36 in mice (Storer, 1966).

Goodrick (1975), however, obtained high heritability (0.47–0.787) of life span in mice, and his calculations indicated that the inheritance of life span in mice was controlled by a single gene. The present authors have undertaken mating experiments with two purebred strains of *Drosophila melanogaster* (*D.m.*) and have demonstrated that major genes at two loci, autosomal and X chromosomal, determined their life spans. These longevity genes were analysed for their mode of inheritance and were named Jm (Yonemura *et al.*, 1989, 1990). Luckinbill *et al.* (1988) also succeeded in selection of life span in *D.m.*, and demonstrated that the difference in life span in their stocks was mainly determined by a few genes on chromosome 1 (X chromosome) and chromosome 3 (autosome).

The present work reports the discovery of a new genetic influence on life span of *D.m.* involving extra-nuclear genes as well as nuclear genes, Jm A and Jm X. Thus, two fundamental modes of inheritance, nuclear and extranuclear, are shown to influence life span.

Materials and methods

Two short-lived purebred strains of *D.m.* were used as the parental stocks, P₁ and P₂. These were mated according to the combinations listed in Table 1 to produce reciprocal F₁ hybrids, F₂ and all backcross generations. Flies were bred at 27°C on food containing 8 per cent dry yeast and 5 per cent sugar. The dead were counted every day and were removed from the culture vials. The P₁ strain was established from a wild type stock of *D.m.* through 13 or more generations of sib-mating; their genes are presumed to be homozygous at 95 per cent or more loci. This strain is identical with the P₁ generation (the short-lived parent) used in previous work (Yonemura *et al.*, 1989, 1990). The P₂ strain in the present work is a purebred line established by the same procedure from Oregon R. Details of culture and handling methods were the same as in an earlier report (Yonemura *et al.*, 1989).

Results

The means and standard deviations of the parental stocks, the F_1 and F_2 generations, and the reciprocal backcross generations are given in Table 2. These data ignore the obvious differences between the sexes or between different crosses in the same generations. However, the procedure is necessary to enable the following calculations.

If we take the phenotypic variance in the F_2 as V_P , the genotypic variance as V_G , and the environmental variance as V_E , then $V_P = V_G + V_E$. V_E is given by $(V_{P_1} + V_{P_2} + 2V_{F_1})/4$ using variances of P_1 , P_2 and F_1 . From Table 2 it can be calculated that $V_P = 289.00$ and $V_E = 153.05$. Heritability in the broad sense, h^2b , is given by V_G/V_P . Then h^2b can be calculated as $h^2b = 0.4704$. V_G is divided into additive variance (V_A), dominance variance (V_D) and interaction variance (V_I): $V_G = V_A + V_D + V_I$. V_A is given by $2V_{F_2} - V_B$, which is calculated from Table 2 as $V_A = 305.75$. This figure is larger than $V_G = 135.95$ calculated before, indicating that V_D and V_I take no part in V_G . In such a genetic model the number of loci of the contributing genes can be calculated as $N = R^2/8V_A = 0.03$ (where R is the difference of mean life span between P_1 and P_2).

Table 1 Notation of generations in reciprocal crosses

Mating	Progeny
$P_1 \sigma \times P_2 \varphi$	F_{1-1}
$P_1 \varphi \times P_2 \sigma$	F_{1-2}
$F_{1-1} \sigma \times F_{1-1} \varphi$	F_{2-1}
$F_{1-2} \sigma \times F_{1-2} \varphi$	F_{2-2}
$P_1 \sigma \times F_{1-1} \varphi$	B_{1-1}
$P_1 \sigma \times F_{1-2} \varphi$	B_{1-2}
$P_1 \varphi \times F_{1-1} \sigma$	B_{1-3}
$P_1 \varphi \times F_{1-2} \sigma$	B_{1-4}
$P_2 \sigma \times F_{1-1} \varphi$	B_{2-1}
$P_2 \sigma \times F_{1-2} \varphi$	B_{2-2}
$P_2 \varphi \times F_{1-1} \sigma$	B_{2-3}
$P_2 \varphi \times F_{1-2} \sigma$	B_{2-4}

Table 2 Sample number in each generation and the mean life span. Values are means $\pm$ s.d. (days)

Generation	Number	Life span
$P_1(\sigma + \varphi)$	641	36.5 ± 5.6
$P_2(\sigma + \varphi)$	640	28.1 ± 3.1
$F_1(\text{total})$	1297	48.1 ± 16.9
$F_2(\text{total})$	1303	43.4 ± 17.0
$B_1(\text{total})$	2539	47.0 ± 15.8
$B_2(\text{total})$	2582	39.8 ± 16.3
$B(\text{total})$	5121	43.4 ± 16.5

These results show that the inheritance of life span in this experiment was controlled by few major genes.

On the other hand, the scaling test of Mather (1949) requires the following calculations, taking the means of B_1 , B_2 and F_2 generations as $\overline{B_1}$, $\overline{B_2}$ and $\overline{F_2}$ and those of P_1 , P_2 and F_1 as $\overline{P_1}$, $\overline{P_2}$ and $\overline{F_1}$: $A = 2\overline{B_1} - \overline{P_1} - \overline{F_1}$, $B = 2\overline{B_2} - \overline{P_2} - \overline{F_1}$, $C = 4\overline{F_2} - 2\overline{F_1} - \overline{P_1} - \overline{P_2}$.

If $A \equiv B \equiv C \equiv 0$, genes in the model are considered to have only additive and dominance effects and to show no epistatic effect; epistatic effect is an interaction between gene loci. Calculations based on the data in Table 2 result in $A = 9.4 \pm 0.814$, $B = 3.4 \pm 0.804$ and $C = 12.8 \pm 2.12$, which are significantly larger than 0 ($P < 0.05$). This shows interaction variance is large. This is inconsistent with the above conclusion that V_G solely consists of V_A . Consequently, the quantitative genetic analysis is invalid.

The distribution of life spans of the different generations of *D.m.* studied here are shown in Fig. 1 (P , F_1 and F_2 generations) and Fig. 2 (backcross generations).

The progenies showing the shortest mean life spans were P_2 and generations originating from P_2 females: F_{1-1} , F_{2-1} , B_{1-1} , B_{2-1} , B_{2-3} , B_{2-4} . The exception to this general observation was B_{1-3} . It is pointed out that, although P_2 females in the parental generation initiated the B_{1-3} generation, the females in the backcross (P_1) contained no contribution (either nuclear or cytoplasmic) from P_2 females.

In the progenies where the females used for breeding did not have a contribution from P_2 females, F_{1-2} , F_{2-2} , B_{1-2} , B_{1-4} , B_{2-2} , long-lived flies were observed. Such long-lived flies were not observed among the parental strains, including P_1 which, similarly, did not have a contribution from P_2 females.

Discussions and conclusions

The striking observation in this study is the marked difference in the life spans of progenies from reciprocal crosses. For example, F_{1-1} and F_{1-2} ; F_{2-1} and F_{2-2} (Fig. 1). A difference between the mean life spans of male F_1 flies from reciprocal crosses might be accounted for by differences in genes located on the X and Y chromosomes of the parental stocks. However, such an explanation could not account for the difference in the mean life spans of female F_1 flies observed here (F_{1-1} and F_{1-2} , Fig. 1). However, an extranuclear influence appears to be involved. All progenies where the cytoplasm of the zygote originated from the P_2 parental strain, P_2 , F_{1-1} , F_{2-1} , B_{1-1} , B_{2-1} , B_{2-3} , B_{2-4} , showed a unimodal, short, life span. On the other hand, progenies where the cytoplasm of the zygote originated from the P_1 parental strain, but the nuclear genetic material contained a contribution from the P_2

Fig. 1 Life spans of stocks of *Drosophila melanogaster*, where day of emergence is given as day 0.

Distributions of mortality rates in parent generations (P_1 , P_2) and their reciprocal crosses (F_1 , F_2). The solid line is for males and the dotted line is for females. Genotypes corresponding to each peak are shown in the figure. Jm *A1* and Jm *A2* are autosomal longevity genes; Jm *X1* and Jm *X2* are X-chromosomal genes; Jm *Y1* and Jm *Y2* are genes assumed on the Y chromosome; Jm *E1* and Jm *E2* are extranuclear genes, influencing life span.

parental strain, F_{1-2} , F_{2-2} , B_{1-2} , B_{1-3} , B_{1-4} , B_{2-2} , showed a wide range of life spans, including long life spans not observed in the parental stocks.

Consequently, the results indicate that P_2 possesses a cytoplasmic factor which has a life-shortening effect which can over-ride the nuclear longevity genes, and which follows maternal inheritance accurately. This is the reason why the variance in the F_1 ($V_{F_1} = V_{F_{1-1}} + V_{F_{1-2}}$) increased, and why the above quantitative genetic analysis is invalid.

In generations where P_2 females made no contribution to the cytoplasm of the zygote, longevity graphs appeared nearly identical with those in the previous reports and their genotypic values could be likewise assigned (Yonemura *et al.*, 1989, 1990). The nuclear genes which influenced life span to lengths far greater than those of the parental stocks, are concluded as being from the P_2 parents, in which their effects were countered by the life-shortening cytoplasmic factor. In order to account for the life spans of the flies observed here, we have to add the cytoplasmic factor, or extranuclear gene, to the Jm longevity gene systems observed and studied in our previous reports (Yonemura *et al.*, 1989, 1990).

The cytoplasmic factor, or extranuclear gene, is denoted as *E1* for P_1 strain and *E2* for P_2 . In the previous reports (Yonemura *et al.*, 1989, 1990), the nuclear longevity genes for P_1 were assigned as Jm *A1* (autosomal) and Jm *X1* and Jm *Y1* (sex chromosomal). In the present study, nuclear genes for P_2 were denoted Jm *A2*, Jm *X2* and Jm *Y2* (although identity of these genes with those of the P_2 strain in the previous reports is not certain). From the present experimental results, extranuclear genes are to be added to the genotypes as below:

P_1 males: $A1A1X1Y1 \cdot E1$,

P_1 females: $A1A1X1X1 \cdot E1$,

P_2 males: $A2A2X2Y2 \cdot E2$,

P_2 females: $A2A2X2X2 \cdot E2$.

Genotypes of other generations may be simply assigned. In Figs 1 and 2, the genotypes for corresponding peaks are indicated (except for F_2 , to avoid congestion in the figure).

All generations possessing *E2* were short-lived, irrespective of their other Jm genotypes. The shortest mode of the mortality peak in males was 27 days in the P_2 , and the longest was 32 days in the F_{1-1} ; in females, the peak commonly appeared a little later. In the generations with *E1*, the observed segregation of life span was according to the other Jm genotypes; genotypic values could be estimated from the data in Figs 1 and 2. Taking the mode of the mortality peak as the

Fig. 2 Life spans of stocks of *Drosophila melanogaster*, where day of emergence is given as day 0. Distributions of mortality rates in backcross generations. The solid line is for males and the dotted line is for females. Genotypes corresponding to each peak are shown. Jm A1 and Jm A2 are autosomal longevity genes; Jm X1 and Jm X2 are X-chromosomal genes; Jm Y1 and Jm Y2 are genes assumed on the Y chromosome; Jm E1 and Jm E2 are extranuclear genes, influencing life span.

genotypic value, genotypes possessing E1 showed the following values.

$$A1A1X1Y1 = A1A1X1Y2 = 37 \text{ days } (P_1: 35, B_{1-2}: 39, B_{1-3}: 36, B_{1-4}: 36). \quad (1)$$

$$A1A1X1X1 = 42 \text{ days } (P_1: 39, B_{1-3}: 43, B_{1-4}: 43). \quad (2)$$

$$A1A1X1X2 = A1A1X2Y1 = 50 \text{ days } (B_{1-2}: 48, B_{1-3}: 48, B_{1-4}: 53). \quad (3)$$

$$A1A2X1Y1 = A1A2X1Y2 = A2A2X1Y2 = 59 \text{ days } (F_{1-2}: 57, B_{1-2}: 61, B_{1-3}: 57, B_{1-4}: 60, B_{2-2}: 58, 60). \quad (4)$$

$$A1A2X1X1 = 66 \text{ days } (B_{1-1}: 65, B_{1-4}: 66). \quad (5)$$

$$A1A2X2Y1 = A1A2X2Y2 = A1A2X1X2 = A1A2X2X2 = A2A2X2Y2 = A2A2X1X2 = A2A2X2X2 = 72 \text{ days } (F_{1-2}: 69, B_{1-2}: 72, B_{2-2}: 74, B_{1-3}: 68). \quad (6)$$

From these equations, genotypic values (in days) were calculated as follows:

$$Y1 = Y2 = 0,$$

$$A1A1 = 32,$$

$$A1A2 = 52,$$

$$A2A2 = 52,$$

$X1 = 5$ (to $A1A1$) and 7 (to $A1A2$ and $A2A2$),
 $X2 = 18$ (to $A1A1$) and 20 (to $A1A2$ and $A2A2$).

$Y1$ and $Y2$ show no effect of longevity genes. $A2$ is dominant to $A1$. $A1A1$ presents 32 days of basic life span, while $A1A2$ and $A2A2$ give 52 days. $X1$ adds 5 days of supplemental life to $A1A1$ and 7 days to $A1A2$ and $A2A2$; this effect shows a dosage effect since homozygous females live longer than hemizygous males. $X2$ has a higher additive effect than $X1$, adding 18 days to $A1A1$ and 20 days to $A1A2$ and $A2A2$. In long-lived generations where segregation of life span is observed, the short-lived trait originates from P_1 and the long-lived trait from P_2 . These data clearly demonstrate that the short-lived P_2 possesses nuclear longevity genes for a long life span, but an extranuclear factor suppresses their expression. Table 3 shows all the genotypic values under the control of extranuclear factor $E1$, calculated from the above data.

The life span mode of P_1 males, the flies with the shortest life span in the presence of $E1$ is 35 days, and the longest mode under the control of $E2$ is 32 days. This indicates that the life-shortening effect of $E2$ influences not only long-lived genotypes but also short-lived genotypes. That is to say, all Jm nuclear longevity genes are influenced by $E2$.

The present study has demonstrated a typical maternal inheritance of short life span, indicating the presence of cytoplasmic factor which influences the expression of Jm longevity genes. It is inappropriate to assume that this factor is a product of the mother, such as a hormone or other metabolic substance, transmitted through cytoplasm of the ovum. If this was the case, it would be expected to produce an effect only in the F_{1-1} generation (P_2 female $\times$ P_1 male), but it could hardly leave an effect on all individuals in the F_{2-1} , the next generation from the F_{1-1} . Consequently, this factor can be assessed as a substance reproduced by the F_{1-1} and transmitted through the F_{1-1} females to the F_{2-1} , that is to say, a self-reproducing extranuclear gene, most probably part of the mitochondrial DNA (chondriome). We tentatively name the factor in P_1 as Jm $E1$ and in P_2 as Jm $E2$. Mitochondrial DNA in

D.m. has been noted to diminish with aging; Massie & Williams (1987) noticed an association between this quantitative variation and senility.

The present experiment has shown that the $E2$ gene has a strong suppressive effect on nuclear genes, since $A2A2X2Y2$ individuals, which have a life span of 73 days with $E1$, the longest life span of *D.m.* at 27°C, live only 30 days with $E2$, the shortest life span of *D.m.* This difference of life span is attributed to a qualitative difference between $E1$ and $E2$ in influencing life span, rather than a quantitative difference of mitochondrial DNA, as suggested by Massie & Williams (1987). It is possible that a large difference of life span among species is based on differences between extranuclear longevity genes rather than between nuclear genes.

In *D.m.*, developmental speed in the preimaginal stage has been found to have inter-relations with quantitative traits such as adult life span (Wattiaux, 1962; Burcombe & Hollingsworth, 1970; Lints & Lints, 1971). We have found that nuclear longevity genes control developmental speed in the preimaginal stage, from larval stage to emerging (Yonemura *et al.*, 1991). It will be important to determine whether the extranuclear genes disclosed by the present report also influence developmental speed. It seems easier to investigate the molecular mechanisms whereby longevity genes influence developmental speed rather than to demonstrate how they determine life span. Further, now that an extranuclear, and presumably mitochondrial, factor has been shown to have a large influence on life span, it appears feasible to undertake physical manipulations, including studies involving recombinant DNA technology, with a view to altering life spans of stocks on *D.m.*

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Table 3 Life span given by each nuclear longevity genotype (genotypic value in days) under the influence of Jm $E1$, an extranuclear gene

	—	X1Y1 X1Y2	X1X1	X1X2 X2X2
<i>A1A1</i>	32	37	42	50
<i>A1A2</i>	52	59	66	72
<i>A2A2</i>	52	59	66	72

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