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The effects of enhanced expression of elongation factor EF-1α on lifespan in *Drosophila melanogaster*

IV. A summary of three experiments

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Abstract

This paper summarizes three experiments on the genetic manipulation of fitness components involved in the evolution of lifespan through the introduction of an additional copy of the gene for elongation factor EF-1α into the genome of *Drosophila melanogaster*. The first experiment checked a prior claim that enhanced expression of elongation factor increased the lifespan of virgin male fruitflies. It used inbred stocks; three treatment and three control lines were available. The second experiment put one treatment and one control insert into different positions on the third chromosome, then measured the influence of six genetic backgrounds on treatment effects in healthier flies. The third experiment put six treatment and six control inserts into the genetic background whose lifespan was most sensitive to the effects of treatment in the second experiment, then measured the influence of insert positions on treatment effects in healthy flies.

The treatment never increased the lifespan of virgin males. It increased the lifespan of mated females in inbred flies reared to eclosion at 25°, reduced it in the positions experiment, and made no difference to lifespan in the backgrounds experiment. When it increased lifespan, it reduced fecundity. In inbred flies and in the positions experiment, the treatment reduced dry weight at eclosion of females. Marginal effects of gene substitutions on tradeoffs were measured directly. The results suggest that enhanced expression of elongation factor makes local changes within the bounds of tradeoffs that are given by a pre-existing physiological structure whose basic nature is not changed by the treatment.

Introduction

For decades, the fruitfly *Drosophila melanogaster* has been a model system for research on lifespan in which evolutionary and molecular paradigms have shaped the questions posed and the experiments done to answer them (Rose, 1991). The evolutionary view is that aging results from genes that have positive or neutral effects early in life and negative effects late in life (Williams, 1957). Such genes accumulate because selection is stronger on traits expressed earlier in life (Hamilton, 1966; Charlesworth & Williamson, 1975). A substantial tradition of selection experiments has led to the conclusion that lifespan in *Drosophila* trades off with components of fitness expressed early in life, but which early fitness component is involved has depended on the experiment. Both early fecundity (Rose & Charlesworth, 1980, 1981; Rose, 1984; Luckinbill *et al.*, 1984; Hillesheim & Stearns, 1992) and weight at eclosion (Hillesheim & Stearns, 1992) have been found to be negatively correlated with lifespan. Partridge and Fowler (1992) found that flies selected both for longer life and for increased fecundity late in life eclosed later and were slightly heavier at eclosion than flies selected for increased fecundity early in life.

Molecular manipulations of lifespan in *Drosophila* were first done by Shepherd *et al.* (1989). They inserted an additional copy of the gene for

elongation factor EF-1α into the genome of inbred flies on a P-element plasmid. They claimed that enhanced expression of elongation factor significantly increased the lifespan of virgin males; the difference in lifespan between the longest lived treatment line and the longest lived control line was a larger percentage of average lifespan at higher temperature - 33% at 29.5° and 18% at 25° ($p < 0.01$). This appeared to initiate a new approach to research on lifespan and life history evolution that would provide a substantial increase in the precision with which one can dissect the molecular and physiological causes of variation and covariation in fitness components. However, there were two reasons to be skeptical of the claim.

First, the original experiment was not well controlled and not replicated at all. The insert was flanked by the promoter and termination sequences for heat shock protein so that the additional copy of elongation factor would be expressed at high temperature. This was done to provide an internal control for the effects of different insert positions. The 'control' lines constructed with the same P-element plasmid differed from the treatments in four respects, not one: lack of elongation factor, insert position, length of insert, and slightly different genetic background (the lines were derived from an inbred stock but were not homozygous). The only unconfounded comparison allowed by such a manipulation is the two-way treatment × temperature interaction effect in a design that accounts for three-way line × treatment × temperature effects through adequate replication. The original experiment compared just one treatment line with one control line, a design without replication that does not allow one to separate the effects of treatment, position, length of insert, and genetic background. The design used by Shepherd *et al.* has one treatment (29.5°) and one control (the same flies measured at 25°), for the control line does not count. Building in the internal control provided by temperature-sensitive expression of the gene at the same insert position was a good idea, but when line × treatment × temperature interaction effects are significant, it can only be exploited if several treatment inserts are compared with several control inserts.

Second, implicit in such genetic manipulations is the assumption that single genes can have large effects on lifespan. Otherwise there would be no point in doing the manipulation. While evolutionary theory does not explicitly rule out such genes, lifespan, like other fitness components, behaves in selection experiments as though it were determined by many genes each of which has small phenotypic effects. If enhanced expression of elongation factor were sufficient, by itself, to produce a significant effect on lifespan, then the gene for elongation factor would have larger phenotypic effects than the average gene thought to affect a quantitative trait.

We do not know the function of the 'average gene thought to affect a quantitative trait', but the function of elongation factor is known. Elongation factor is present in all organisms and as a gene family in eukaryotes. It docks the incoming aminoacyl-tRNA correctly on the ribosome and catalyses the transition in which one amino acid is added to the peptide (Webster & Webster, 1982; Darnell, Lodish & Baltimore, 1990). Thus elongation factor is necessary for protein synthesis. In *Drosophila melanogaster* the gene exists in two copies: F2, which is translated mostly in pupae, and F1, which is thought to be a housekeeping gene needed in all growing cells. There is only one copy of F1 present in normal cells (Hovemann *et al.*, 1988). The synthesis of EF-1α protein sharply decreases with age in *Drosophila* and precedes the decrease in total protein synthesis by a few days (Webster & Webster, 1983). So far as is known, EF-1α exists as a single, fixed, homozygous allele. Such a fixed housekeeping gene would not contribute to quantitative variation.

Thus the manipulation of a gene like elongation factor should alter the physiological processes associated with lifespan and might not produce the sort of genetic variation present in selection experiments. Therefore we thought it would be worth repeating and extending the work of Shepherd *et al.* to make sure they were observing a real effect and, if they were, to try to understand better the conditions under which such effects can be elicited. The details are presented in three papers; this article summarizes them and makes points best drawn from comparisons of them.

We posed the following questions: (1) Does enhanced expression of elongation factor really increase lifespan in the lines used by Shepherd *et al.* (1989) when their experiments are repeated with adequate replication of lines (Stearns, Kaiser, & Hillesheim, 1993)? Does it also affect other fitness

components, especially fecundity, age at eclosion, and weight at eclosion? If it does, are the effects larger than would be expected for a gene contributing to a quantitative trait? (2) Do the effects of elongation factor on lifespan - and other fitness components - depend upon the genetic background into which the plasmid is inserted and upon the position of the insert (Kaiser & Stearns, 1993)? (3) What impact does enhanced expression of elongation factor have on tradeoffs between lifespan and other fitness components (Stearns & Kaiser, 1993)?

Methods

We first describe the methods common to all three experiments, then those only used in certain experiments. Treatment lines consisted of *D. melanogaster* transformed with a P-element containing the ry^+ marker plus the *F1* copy of the gene for elongation factor *EF-1*α flanked by initiation and termination sequences for the heat shock protein. Control lines had been similarly manipulated but lacked the gene for elongation factor; their insert was shorter and at a different position. All measurements were made at 25° and at 29.5° to allow us to compare traits with and without the enhanced expression of elongation factor at the higher temperature. Flies were reared from first instar larvae gathered within eight hours of hatching and then kept at standard density. Ages were calculated from the mid-point of egg-laying, which lasted four hours. When experiments had to be run in sequence rather than parallel, treatment and control lines were run together, so that differences among sequences were absorbed into the effects of replicates (individuals or vials) within lines. To measure the longevity of virgins, ten vials per line and sex were established with ten two-day-old flies and given one drop of fresh yeast. For mated females, ten vials per line were established with ten two-day-old virgin females and 15 two-day-old males. Three times a week the flies were transferred to new vials and the number of dead flies was recorded until the last fly died. In vials with mated females, males were replaced if there were fewer males than females in the vial.

To measure developmental time and dry weight at eclosion, ten vials per line each received 12 larvae. Three days later the number of newly pupated larvae was recorded every four hours. When all larvae had pupated, the vials were placed in an eclosion fractionator (Stearns *et al.*, 1987) that collected freshly eclosed flies at six hour intervals. After their sex was determined, they were dried at 50° for three h and weighed to 0.01 mg.

To measure lifetime egg production, 30 vials per line were established with one female and two males per vial. The laying surface was replaced daily by a new one with a drop of fresh yeast. Dead males were removed and replaced by a young virgin male from the same line. The number of eggs laid by each female in 24 h was counted daily until the last female died. Fecundity early in life was defined as the number of eggs laid per day from the 4th to the 14th day after eclosion. This was 13-23 days after birth at 25° and 11-21 days after birth at 29°. Fecundity late in life was defined as the number of eggs laid from the 15th to the 5th day before the death of the last female. This was 32-42 days after birth at 25° and 23-33 days after birth at 29.5°.

Experiment I – Inbred flies

Three treatment and three control lines that Shepherd *et al.* had produced were adapted to our laboratory conditions for four generations. Because one control was lost at high temperature because of high preadult mortality, comparisons of lifespans and fecundities were made with three treatments and two controls. Tests for other traits used all six lines. The lines used by Shepherd *et al.* (1989) are labelled E3 (experimental) and C3 (control) in these experiments. In this experiment flies used to measure fecundity and longevity were held at 25° until eclosion to make our results comparable to those of Shepherd *et al.* and because larval mortality was high at 29.5° in these inbred flies. Longevity and fecundity were measured with 40 flies per line, age and weight at eclosion with 24 flies per line.

Experiment II – Effects of backgrounds

The treatment and control lines were obtained from a jumpstart cross in which one arranges for P elements already inserted in the genome to jump to new insert positions (Cooley, Kelley & Sprading, 1988; Robertson *et al.*, 1988; Bellen *et al.*, 1989). The use of balancers ensured that the only surviving flies carried the plasmid at a random position on the third chromosome (Fig. 1). After one fly with the desired marker combinations was crossed again with the balancer stock, a brother-sister cross be-

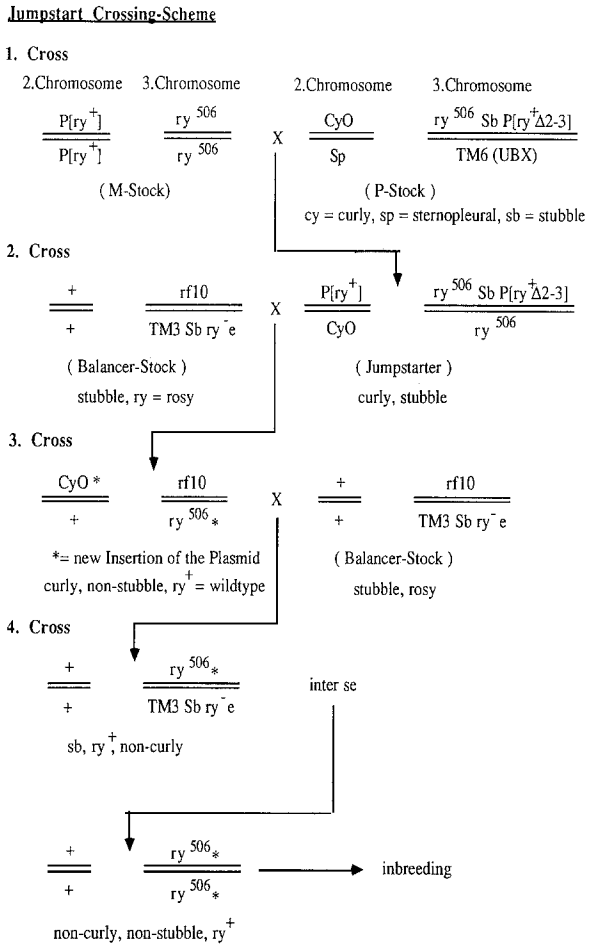

Fig. 1. The jumpstart crossing scheme. In the flies tested, half the genome came from the jumpstart cross. Its first chromosome was the same in all flies to the degree that inbreeding had made it homozygous; its second chromosome was the same in all flies; its third chromosome was the same in all flies except that the treatment flies had the EF-insert and the control flies had the control insert. In the other half of the genome, the chromosomes were the same within genetic backgrounds to the degree that brother-sister mating since 1987 had made them homozygous. They differed among genetic backgrounds.

tween flies with the correct markers yielded flies homozygous for the plasmid construct. The test lines were obtained by outcrossing the one inbred treatment and one inbred control line with six different inbred lab stocks to yield six pairs of outbred treatment and control lines with the genetic backgrounds to be tested. Genetic backgrounds were provided by isofemale lines held in the laboratory since 1987 and maintained by full-sib mating for about 60 generations. In the experiments on backgrounds and positions larvae and pupae were reared at the same temperature as the adults.

Experiment III – Effects of insert positions

The test flies were obtained by outcrossing the six inbred treatment and six inbred control lines from the jumpstart cross with one inbred lab stock to yield six pairs of heterozygous treatment and control lines with the positions to be tested. Thus all flies tested had the same genetic background, Line 5, whose lifespan had been most sensitive to the effects of enhanced expression of elongation factor in the previous experiment. All inserts were on the third chromosome, but the control inserts were not in the same positions on the third chromosome as the treatment inserts.

Comparison of experimental designs

Table 1 compares the four experiments that have been done on the effects of enhanced expression of elongation factor on fitness traits in *Drosophila*. The major differences among the experiments are these: (a) Replication - one control and one treatment line in Shepherd's experiment, three controls (one was lost for longevity and fecundity at 29.5°) and three treatments in our experiment on Shepherd's lines, six controls and six treatments in our experiments on backgrounds and positions. (b) Dosage of the additional gene for elongation factor - two additional copies in Shepherd's experiment and our experiment on his lines, one additional copy in the backgrounds and positions experiments. (c) Flies tested - Shepherd tested virgin males only, we tested virgin males and females and mated females in all three experiments. (d) Genetic backgrounds - different to an unknown but probably small degree in every line in Shepherd's experiment and our experiment on his lines, six pairs of backgrounds matching treatment and control lines in the backgrounds experiment, one background in the positions experiment. (e) Insert positions - different in every line in Shepherd's experiment and our experiment on his lines with some on Chromosome II and some on Chromosome III; one treatment position and one control position in the backgrounds experiment, both on Chromosome III; six treatment positions and six control positions in the positions experiment, all on Chromosome III. (f) Genetic state of the flies - inbred in Shepherd's our experiment and our experiment on his lines, outbred in the backgrounds and positions experiments. (g) Temperature at which the larvae and pupae were reared - 25° in Shepherd's experiment and our ex-

Table 1. Design of experiments on the effects of elongation factor on fitness components in *Drosophila melanogaster.*

Design element	Paper			
	Shepherd	Expt. I Repeat	Expt. II Backgrounds	Expt. III Positions
Control lines	1	3 (2 at 29.5 °)	6	6
Treatment lines	1	3	6	6
Flies tested:	Virgin males	Virgin males, virgin females, and mated females		
Backgrounds	2	6	6	1
Insert positions	2	6	1	6
extra *EF – 1α*	2 copies	2 copies	1 copy	1 copy
Larvae and pupae	25 °	25 °	25 & 29.5 °	25 & 29.5 °
Sample size per line:				
Longevity	300	40	100[1]	100[1]
Fecundity	-	40	30	30
Eclosion	-	24	120[2]	120[2]
Sample size total:				
Longevity	300	1160	7200	7200
Fecundity	-	411	720	720
Eclosion	-		2384[3]	2268[3]

[1] For each class: virgin males, virgin females, and mated females.
[2] Number starting.
[3] Number of larvae surviving to eclosion out of 2400.

periment on his lines, 25° and 29.5° in the backgrounds and positions experiments. (h) Total sample sizes - small in Shepherd's experiment and our experiment on his lines, large in the backgrounds and positions experiments.

Statistical analysis

In all experiments, comparisons of treatment and control lines had to take into account variation among replicates and differences in average lifetime between 25 °C and 29.5 °C. We used two methods to analyse that variation: factorial ANOVAs on time to pupation, time to eclosion, dry weight at eclosion, fecundities, and lifespans; and Cox regressions (Cox, 1972) on time to pupation, time to eclosion, and lifespans. Cox regressions measure differences in rates. When the two methods differed on level of significance, we have reported the less significant level. The statistical model had three main factors - treatment, temperature, and background (or position) - and all interactions. Thus where i: 1..2 indexes treatment α, j: 1..2 indexes temperature β, k: 1..6 denotes background (or position) γ, and l: 1..n denotes either vials (n = 10 per line per treatment, temperature, and background) or individuals (n ≈ 700 for fecundity, ≈ 2400 for lifespan), the model was:

$$y = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \epsilon_{ijkl}$$

In all three experiments the only test for treatment effects that was not confounded by the effects of position or length of insert is the comparison of treatment with control for *differences* in the expressions of the traits at 25° and 29.5°. This is tested by the treatment × temperature interaction effect (Fig. 2). Because that effect should measure a percentage rather than an absolute difference, and to normalize distributions, all data were log-transformed for the ANOVAs.

The ANOVAs for lifespan and fecundity were done using individual MS as the denominator for the F-test. In measuring lifespan, flies were reared in groups of ten individuals per vial, but as deaths occurred, flies were moved each day to hold densities at ten per vial insofar as possible. Vial MS was inappropriate for testing effects on lifespan because flies had been moved among vials. For fecundity,

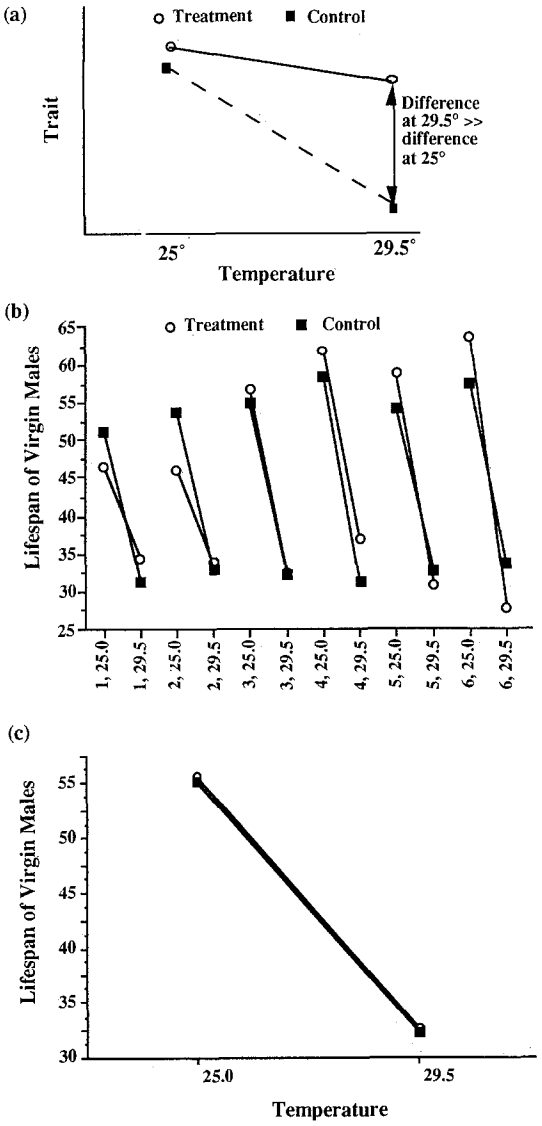

Fig. 2. The importance of line × treatment × temperature interaction effects in these experiments. (a) Increased expression of elongation factor at higher temperature might lengthen life, but all flies have shorter lives at higher temperature. One could test for the effects of treatment by comparing the difference between treatment and control at 29.5° with that at 25°; (b) That simple test is inappropriate, for different lines have different reactions to the change in temperature. These produce three-way interaction effects, here depicted for position × treatment × temperature effects on the lifespan of virgin males; (c) The line effects cancel each other out in this two-way treatment × temperature interaction.

with one female per vial, individual and vial MS were equivalent. For time to pupation, time as pupae, time to eclosion, and weight at eclosion, flies were grouped 12 to a vial with ten vials per line. The ANOVAs were done with vials nested within treatment, temperature, and line, and vial MS was used as the denominator in the F test.

In the experiment on backgrounds, lifespans were tested for flies that lived more than eight days, for the distribution of lifespans was bimodal with a small group of flies that died early in life (5-6% of the total).

Line × treatment × temperature interaction effects are the key to interpreting these experiments. While increased expression of elongation factor at higher temperature might lengthen life, all flies have shorter lives at higher temperature. The simplest situation, apparently the implicit assumption of Shepherd *et al.* (1989), is that there is a small difference in the lifespan of treatments and controls at 25°, a larger difference at 29.5°, and one can test for the effects of treatment by comparing the difference between treatment and control at 29.5° with that at 25° (Fig. 2a). However, that simple test is inappropriate for two reasons. First, in some lines treatments and controls have different lifespans at 25° (there appears to be some 'leakage' in the expression of elongation factor at 25°). Second, different lines have different reactions to the change in temperature. These produce three-way line × treatment × temperature effects. Fig. 2b depicts the position × treatment × temperature effects on the lifespan of virgin males in the positions experiment ($p = 0.0001$ in the ANOVA on log-transformed data). If one worked only with one pair of positions, one could reach any of the possible conclusions. For example - Line 1: treatment has shorter life at 25°, longer life at 29.5°; conclusion: increased expression of elongation factor lengthens lifespan. Line 6: Treatment has longer life at 25°, shorter life at 29.5°; conclusion: increased expression of elongation factor shortens lifespan. Line 3: not much difference at either temperature; conclusion: treatment has no effect. In this particular case, the line effects cancel each other out in the mean treatment × temperature interaction (Fig. 2c); the correct conclusion: the treatment effect is not significant ($p = 0.1894$). For this reason, we present the details on interactions for the most significant effects.

Results

Treatment effects

The treatment × temperature interaction effect cap-

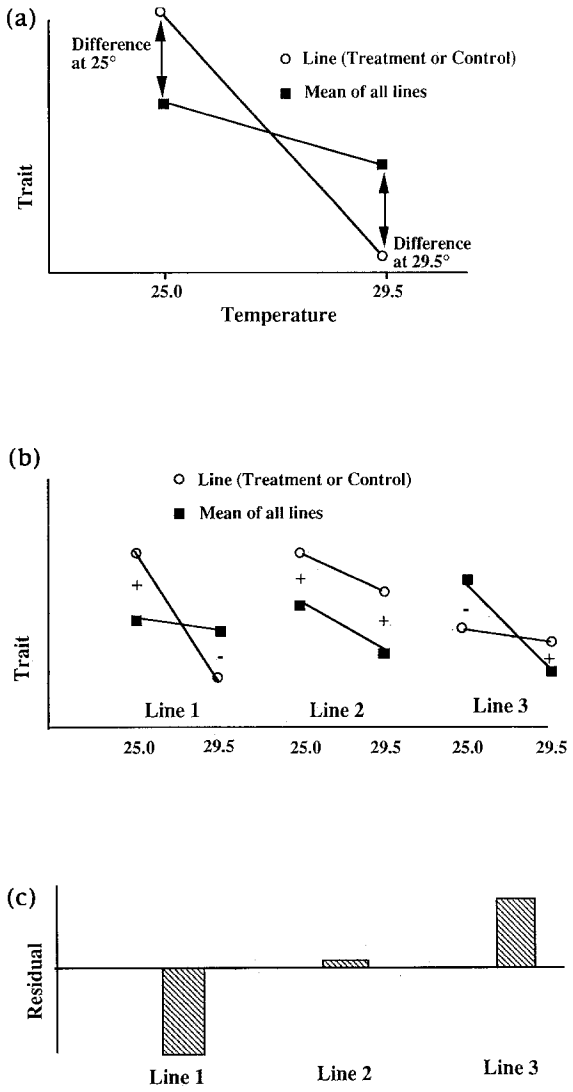

Fig. 3. The calculation of the residuals. (a) First the deviation of each line from the overall mean was calculated at each temperature; (b) These deviations express line × temperature interaction effects. Line 1 was larger than the mean at 25° and smaller at 29.5°; the effect of increased temperature was a larger reduction in the value of the trait in Line 1 than was experienced on average. Line 2 was consistently larger than the mean at both temperatures, just slightly more so at 29.5°. Line 3 was smaller than the mean at 25° and larger at 29.5°; (c) The residuals are calculated by subtracting the difference at 25° from the difference at 29.5°. If a residual is negative, increased temperature reduced the trait. If a residual is positive, increased temperature increased the trait, relative to the overall mean. These residuals isolate the temperature effect on each line. The average of the residuals for the treatment lines is the overall response of treatment to increased temperature; the average of the residuals for the control lines is the overall response of the control lines to increased temperature. If the difference between the average treatment and average control effect is significant, that will be expressed in the treatment × temperature interaction effect in the ANOVA.

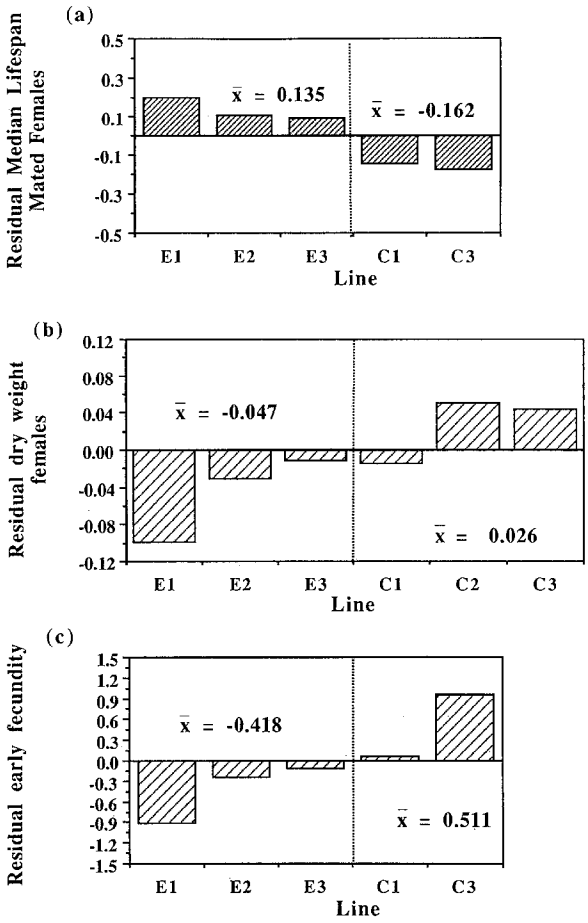

Fig. 4. Plots of residuals for significant treatment × temperature effects in Expt. I. Treatment lines are on the left and are labelled E1, E2 ... Control lines are on the right and are labelled C1, C2 ... The significance of the treatment × temperature interaction effect is reported: (a) Median lifespan of mated females ($p < 0.0001$ by ANOVA, $p < 0.0001$ by Cox regression); (b) dry weight at eclosion of females ($p < 0.0004$ by ANOVA); (c) early fecundity ($p < 0.0001$ by ANOVA).

tures the significance of an increase or decrease in the value of a trait at higher temperature and corresponds to the purported enhanced expression of elongation factor at higher temperature. For the major significant treatment × temperature effects, plots of residuals are given depicting both the magnitude of the effects in treatment and control and the variation among lines. Fig. 3 describes how these residuals were calculated.

Table 2 reports the magnitude and direction of significant treatment × temperature interaction effects in all three experiments. In Expt. I on inbred flies, the treatment affected females but not males. It increased the lifespan of mated females by 30.0%

Table 2. Magnitude and direction of the treatment × temperature interaction effects in the three experiments. * Not significant when tested by 3-way MS but significant when tested by vial or individual MS.

Trait	Expt. I Biocentre	Expt. II Backgrounds	Expt. III Positions
Time to pupation	NS	NS	NS
Time as pupae			
Males	NS	NS	NS
Time to eclosion			
Males	NS	(- 1.1%)*	NS
Females	NS	NS	NS
Dry weight at eclosion			
Females	- 7.3%	NS	(- 8.0%)*
Fecundity			
Lifetime	- 77%	NS	NS
Early	- 93%	NS	NS
Lifespan			
Virgin males [1]	NS	(+ 6.8%)*	NS
Mated females	+ 30.0%	NS	NS

[1] The relatively longer lifespan at higher temperature resulted from a decrease in the absolute lifespan of the treatment flies at lower temperature with no difference at higher temperature.

(Fig. 4a), decreased their dry weight at eclosion by 7.3% (Fig. 4b), reduced their lifetime fecundity by 77%, and reduced their early fecundity by 93% (Fig. 4c).

In the backgrounds experiment on heterozygous flies, the treatment reduced time to eclosion of males by 1.1% but had no clear effects on lifespan or fecundity. In the positions experiment, the treatment reduced the dry weight at eclosion of females by 8% (Fig. 5).

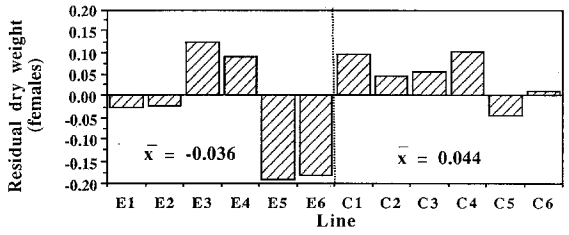

Fig. 5. Plots of residuals for significant treatment × temperature effects in Expt. III (effects of insert position). Treatment lines are on the left and are labelled E1, E2 ... Control lines are on the right and are labelled C1, C2 ... The significance of the treatment × temperature interaction effect is reported: dry weight at eclosion of females ($p = 0.0001$ by ANOVA).

The relative magnitude of the effects of treatment

The relative magnitude of the different effects on pupation rate, eclosion rate, and mortality rate can be estimated from the coefficients in the Cox regressions. Those coefficients weight the impact of each effect on instantaneous rates. Table 4 compares the largest treatment × temperature interaction effects in all three experiments on those rates, using temperature, background, and position effects as the standard of comparison where appropriate. Effects of enhanced expression of EF-1α, as detected in the treatment × temperature interaction, were largest on lifespan, where they ranged from 16% to 136% of the temperature effect. Effects of enhanced expression of EF-1α on time to pupation ranged from 2.1% to 2.5% of the temperature effect. The effect of enhanced expression of EF-1α on time to eclosion was 3.5% of the temperature effect in Expt. II. Effects of treatment were always smaller than effects of background (7% to 18%) or position (13% to 16%).

Summary of treatment effects on individual traits

In no case did the treatment increase the lifespan of virgin males. The relative increase in lifespan at higher temperature in the backgrounds experiment in fact resulted from an absolute decrease in treatment lifespan at lower temperature and no difference at higher temperature. When the treatment increased the lifespan of mated females in Expt. I, it reduced weight at eclosion and fecundity. When it reduced weight at eclosion in the positions experiment, it also reduced lifespan.

Table 3. Relative magnitude of the treatment × temperature interaction effect inferred from the absolute value of the coefficients in the Cox regression.

Expt	Trait	Effect expressed as % of T° effect	% of Back effect	% of Position effect
I	Lifespan of mated females	136%		
II	Time of pupation	2.5%	6.7%	
II	Time to eclosion (females)	3.5%	18%	
III	Lifespan of mated females	16%		13%
III	Time to pupation	2.1%		15%

Table 4. Analysis of the effects of treatment on relationships between pairs of traits calculated from the line means. All the effects reported were significant at the $p<0.01$ level by the Tukey-Kramer test on the difference in slopes; SAS Type II regressions. The only cases reported are those in which the relationship was negative at both temperatures or in which the treatment changed a positive relation to a negative one (*).

Expt	Relation	Slopes		Diff
		Cont or 25 °	Treat 29.5 °	
a)	Slopes of relations compared between treatment and control (lumping temperatures).			
II	Weight at eclosion vs. late fecundity	-3847.23	-4888.10	-1040.86
b)	Slopes of relations compared between treatment and control within temperature.			
II	Early fecundity vs. lifespan at 25 ° *	6.07	-3.14	-9.21
	Age at eclosion vs. late fecundity at 25 ° *	428.65	-59.92	-488.57
	Weight at eclosion vs. late fecundity at 29.5 °	-2786.77	-785.64	2001.13
III	Weight at eclosion vs. late fecundity at 25 °	-3870.41	-1810.62	2059.79
	Early fecundity vs. lifespan at 29.5 ° *	5.19	-17.43	-22.62
	Weight at eclosion vs. late fecundity at 29.5 °	-2378.19	-1239.46	1138.74

Effects of mating and temperature

In all three experiments, both mating and temperature shortened the lives of females, but the effects were not additive. Mated females did not suffer as great a reduction in lifespan at high temperature as did virgin females (reduction in median lifespans of females from 25° to 29.5° in Expt. I: virgins -15 days = 41% of lifespan at 25°, mated -1 day = 6%; in Expt. II: virgins -35 days = 56%, mated -11 days = 29%; Expt. III: virgins -13 days = 28%, mated -7 days = 16%).

Background effects

Expt. II revealed significant effects of genetic background (Kaiser & Stearns, 1993). Backgrounds varied significantly in time to pupation; time spent as pupae by males; time to eclosion (developmental time); dry body weights; lifetime, early, and late fecundity; mortality rates; and mean and median lifespans. To compare traits measured on different scales (e.g. milligrams and days), we converted the range among backgrounds into a percentage of the appropriate mean. This range was 3.0% of time to pupation, 3.3% of time spent as pupae by males, 4.0% of time spent as pupae by females, 2.1% of time to eclosion for males, 2.7% of time to eclosion for females, 12% of dry body weight at eclosion for males, 8.8% of dry body weight at eclosion for females, 39% of total lifetime fecundity, 33% of early fecundity, 79% of late fecundity, 19% of virgin male lifespan, 13% of virgin female lifespan, and 32% of mated female lifespan. For all traits except female dry weight at eclosion, background effects were larger than treatment effects as judged by the ratio of the mean squares. The ratio of background to treatment mean square was 0.83-2.65 for traits measured early in life (time to pupation, time spent as pupae, time to eclosion, dry weight at eclosion), but for fecundity (2.40-29.57) and lifespan (4.52-372.30) it was much higher.

The background × treatment, background × temperature, and background × treatment × temperature interaction effects were significant in 21 of 39 cases.

Position effects

Expt. III revealed significant position effects (Kaiser & Stearns, 1993). Lines varied significantly for time to pupation, time spent as pupae by females (not males), time to eclosion (developmental time) for both sexes, dry body weights of females (not males), lifetime (but not early or late) fecundity, mortality rates and mean lifespans. The range among insert positions was 2.9% of time to pupation, 3.2% of time spent as pupae by males, 5.6% of time spent as pupae by females, 2.7% of time to

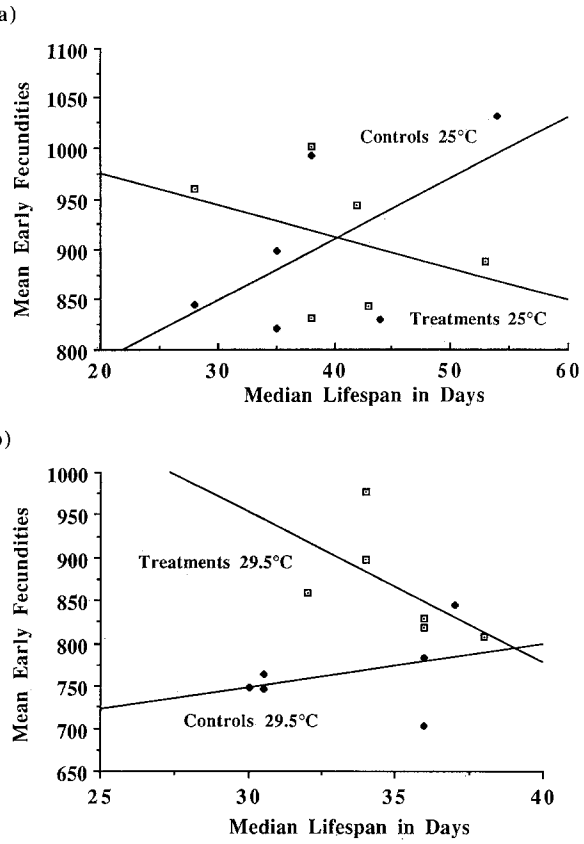

Fig. 6. Two of the four cases in which the treatment changed a relationship from positive to negative, creating a tradeoff involved in lifespan where none had existed in the control: (a) early fecundity vs. lifespan at 25° in Expt. II; (b) early fecundity vs. lifespan at 29.5° in Expt. III.

eclosion for males, 4.7% of time to eclosion for females, 4.0% of dry body weight at eclosion for males, 6.5% of dry body weight at eclosion for females, 22% of total lifetime fecundity, 16% of early fecundity, 52% of late fecundity, 14% of virgin male lifespan, 7.0% of virgin female lifespan, and 24% of mated female lifespan. For time spent as pupae by females (ratio = 4.21), lifespan of virgin males (ratio = 4.51), and lifespan of mated females (ratio = 1.39), position effects were larger than treatment effects as judged by the ratio of the mean squares. That ratio was 0.09-0.53 for the other traits.

The position × treatment, position × temperature, and position × treatment × temperature interaction effects were significant in 23 of 39 cases.

Tradeoffs

There are six tradeoffs between components of fitness expressed early and late in life and measured in these experiments that bear on the evolution of lifespan: (1) early fecundity vs. lifespan, (2) age at eclosion vs. lifespan, (3) weight at eclosion vs. lifespan, (4) early fecundity vs. late fecundity, (5) age at eclosion vs. late fecundity, (6) weight at eclosion vs. late fecundity. Several criteria could be used to assess the significance of these six tradeoffs. They include:

First criterion. Did the treatment significantly increase one trait and significantly decrease the other as measured in the residuals that are tested by the treatment × temperature interaction effect? By this criterion, only a few tradeoffs were found. In Expt. I, the treatment significantly increased the lifespan of mated females, reduced weight at eclosion, and reduced lifetime and early fecundity. In Expt. III, it significantly reduced the lifespan and the dry weight at eclosion of mated females but did not change lifetime, early, or late fecundity. In Expt. I weight at eclosion and fecundity covaried positively (both were reduced). No tradeoffs of lifespan with fitness components were detected in Expt. II.

Second criterion. Did the effects of treatment significantly change the slope of the regression of one trait on the other? This question can be asked at both temperatures and in the two-dimensional plots of residuals. All combinations of lifetime fecundity, early fecundity, late fecundity, lifespan, age at eclosion, and weight at eclosion were examined in mated females. All combinations of age and weight at eclosion and lifespan were examined in virgin males and females. Relations were compared (a) between treatment and control (lumping temperatures), (b) between temperatures (lumping treatments), (c) between treatment and control within each temperature, (d) between treatment and control in the residuals expressing treatment × temperature interaction effects. Only the effects reported in Table 4 were significant at the $p < 0.01$ level by the Tukey-Kramer test on the difference in slopes. We chose 0.01 as the significance level to compensate for the effect of multiple unplanned comparisons.

In four cases the treatment made a positive relation negative, establishing a tradeoff in the treatment where none had existed in the control: age at eclosion vs. late fecundity (Expt. III, lumping tem-

peratures), early fecundity vs. lifespan at 25° (Expt. II) (Fig. 6a), age at eclosion vs. late fecundity at 25° (Expt. II), and early fecundity vs. lifespan at 29.5° (Expt. III) (Fig. 6b).

Third criterion. Did the changes from control to treatment within line all lie within an envelope of variation describing a tradeoff in the residuals? This can only be properly applied in Expt. II, on backgrounds, where there was one treatment insert and one control insert. The analysis is best described by an example, the tradeoff between mean lifetime fecundity and median lifespan. The pairs of controls and treatments can be connected by arrows (Fig. 7a). Three of these arrows (from controls 4, 5, and 6 to the corresponding treatments) describe a tradeoff in one direction: the treatment increased residual lifespan and decreased residual fecundity. Three (from controls 1, 2, and 3 to the corresponding treatments) represent changes in the other direction: the treatment reduced residual lifespan and increased residual fecundity.

There are two methods of summarising these changes; both are depicted in Fig. 7b, where the

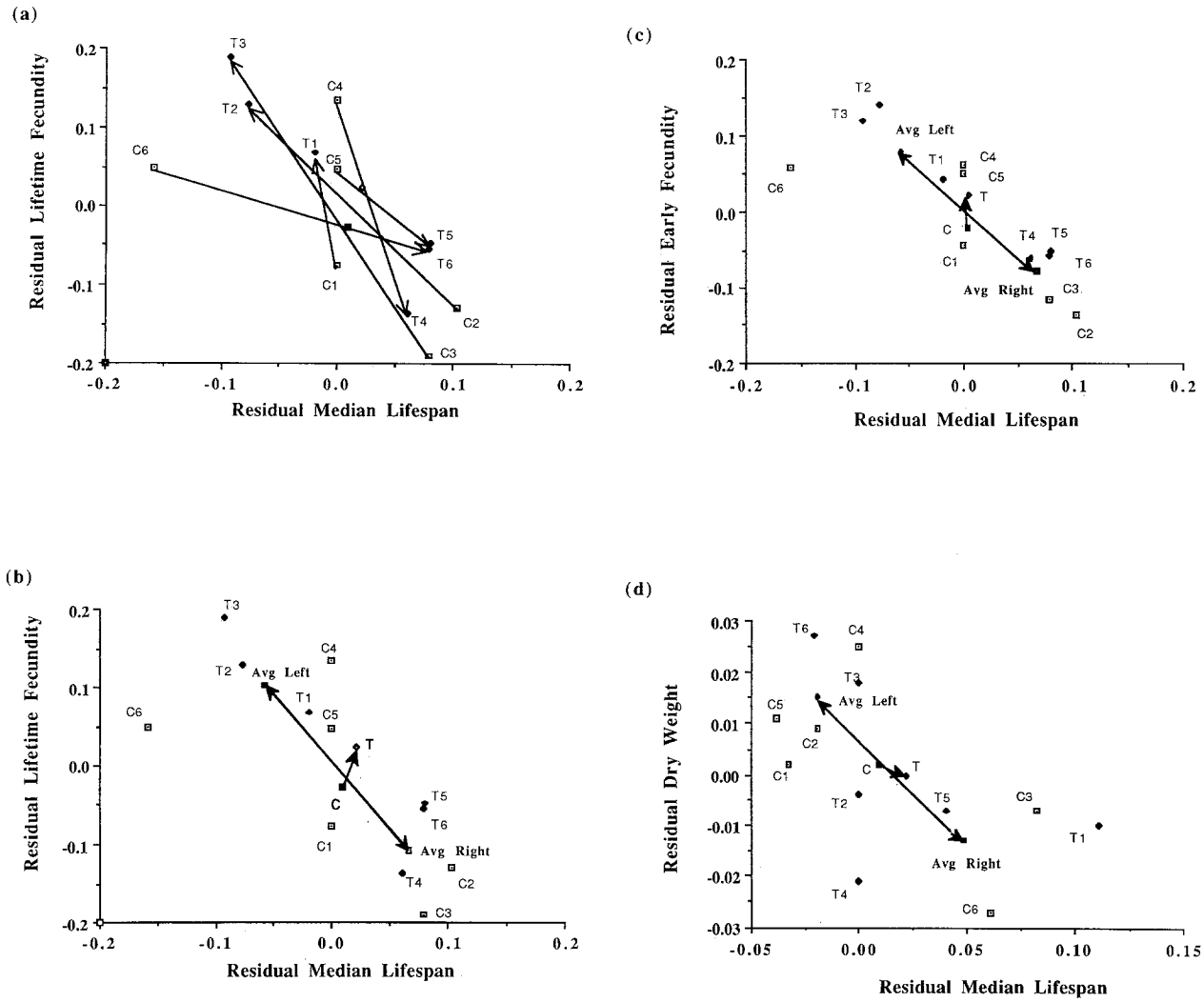

Fig. 7. Analysis of the details of tradeoffs in the residuals for Expt. II (a) The changes ftom control to treatment are plotted for each line; (b) There are two methods of summarising the changes. The first - the change from mean control to mean treatment - is misleading, for it produces the short arrow pointing in a direction in which none of the changes occurred. The second - the change from the mean left end of an arrow to the mean right end - expresses the pattern in the data much better; (c) Similar efffects for the tradeoff early fecundity vs. lifespan in mated females; (d) Similar effects for the tradeoff dry weight at eclosion vs. lifespan in virgin males.

short arrow pointing up and to the right describes the change between the average control residual and the average treatment residual. It misses the consistency of the changes in all six backgrounds, which are all contained within an envelope running from the upper left to the lower right. The longer line running from the upper left to the lower right describes the change from the average left end to the average right end of an arrow in Fig. 7a, irrespective of treatments and controls. The left average residual lifespan is significantly smaller than the right one, and the upper average residual fecundity is significantly larger than the lower one.

The problem with the analysis of means becomes clear: there are two legitimate senses in which a difference between treatment and control can describe a change corresponding to a tradeoff - reduced lifespan and increased fecundity, or increased lifespan and reduced fecundity. When both types of change are present, they counteract each other, and adding all changes together wipes out significant differences and leads to the misleading conclusion that the treatment had no effect on the tradeoff. It would be more accurate to say: all effects of treatment were constrained to occur within the broader limits of a tradeoff, but the direction of the change caused by the treatment depended on the genetic background.

One can judge the significance of such a relation by two criteria: (a) t-tests on the differences between the left and right and upper and lower ends of the lines connecting treatment and controls, and (b) the significance of the treatment × line interaction for each trait. In the background experiment, two tradeoffs were significant for the first criterion but not for the second: lifespan of mated females (t-test, $p = 0.0074$; treat ×line, $p = 0.1357$) vs. total fecundity (t-test, $p = 0.003$; treat × line, $p = 0.0004$) (Fig. 7b) and lifespan of virgin males (t-test, $p = 0.0118$; treat × line, $p = 0.2013$) vs. dry weight at eclosion (t-test, $p = 0.006$; treat × line, $p = 0.0001$) (Fig. 7d). Only one tradeoff was significant by both criteria: lifespan of mated females vs. early fecundity (t-test, $p = 0.0024$; treat × line, $p = 0.0038$) (Fig. 7c). Thus in the backgrounds experiment, where no tradeoffs associated with lifespan were detected by other methods, there were changes occurring in such tradeoffs, but they were hidden in the summary statistics because the effects cancelled each other out.

Discussion

Does enhanced expression of elongation factor increase lifespan?

Enhanced expression of elongation factor increased the lifespan of mated females by 30% in Expt. I on inbred flies reared to eclosion at 25°. It had no effects on lifespan in Expt. II (backgrounds), where the flies were heterozygotes with half the genome derived from a wild population. It reduced the lifespan of mated females by 15% in Expt. III (positions), which was done in the genetic background whose lifespan was most sensitive to the effects of elongation factor in Expt. II. In no case did enhanced expression of elongation factor increase the lifespan of virgin males. Thus the genetic manipulation of EF-1α extended the lifespan of mated females in inbred flies reared to eclosion at 25° and had either no effect on (Expt. II) or reduced (Expt. III) the lifespan of mated females in outbred flies reared to eclosion at both temperatures. These results do not rule out an as yet unanalyzed interaction between the genetic state of the flies - inbred or outbred - and whether or not the flies were reared to eclosion at 25° or at both temperatures.

Does enhanced expression of EF-1α effect other fitness components?

The treatment reduced time to pupation in Expt. II and time to eclosion in both sexes in Expt. II and in males in Expt. III. It decreased weight at eclosion in females in Expts. I and III. In Expt. I it reduced early and lifetime fecundity. In Expt. I it increased the lifespan of mated females; in Expt. III it reduced that lifespan. Overall, the effects of elongation factor on age and weight at eclosion were more consistent than its effects on lifespan or fecundity. This genetic manipulation produced interesting effects on the components of fitness that are most important in life history evolution and the evolution of lifespan. Further applications of recombinant DNA methods to the analysis of the causes of lifespan and of tradeoffs among fitness components would certainly be justified.

Were treatment effects large?

The treatment × temperature interaction effects on fitness components were quite large in inbred flies - 30% differences were observed for lifespan, up to 93% for fecundity - and smaller but still impressive

for single gene effects in outbred flies - about 1-2% for measures of developmental time, up to 8% for dry weight at eclosion, up to 15% for lifespan of mated females.

Comparisons to temperature, background, and position effects

There is another approach to the question about the magnitude of treatment effects: one can compare the changes attributed to treatment with those attributed to temperature, genetic background, and insert position (cf. Table 4). Effects of enhanced expression of EF-1α on lifespan were quite large in comparison to temperature effects - from 16% to 136% of the change in lifespan caused by the difference between 25° and 29.5°. Any genetic manipulation of a fitness component in a fruitfly that has as large an effect as temperature must be considered as having very significant impact. Effects of treatment were consistently smaller than effects of backgrounds and positions. This has two implications, one for experimental design and one for the spread of such an insert as a mutant allele in a natural population.

The implication for experimental design is that such experiments must contain adequate representation of genetic backgrounds and insert positions, for if the effect of treatment is usually smaller than the effect of background or position, its significance can only be detected and properly assessed with adequate replication. This comment is strengthened by the observation of pervasive interaction effects. The degree to which a trait responded to enhanced expression of EF-1α at higher temperature depended very strongly on the genetic background and the position of the insert. Such interaction effects make it impossible to conclude whether the treatment has any effect at all in an experiment done with only one insert position in only one genetic background (cf. Shepherd *et al.*, 1989).

The implication for the spread of such an insert as a mutant allele is that its interactions with genetic backgrounds, generated by mating and recombination, and its movement into new positions in the genome, generated by transposon jumping, will have effects on its fate that are as large as or larger than its direct effect on fitness components. This makes it virtually impossible to predict from experiments like these what would be the fate of a genetically manipulated fruitfly in a natural population, for in such a case much would depend on interaction effects with unknown genetic backgrounds and unknown insert positions. This has rather pessimistic implications for our ability to predict the consequences of releasing genetically manipulated organisms into natural populations.

Tradeoffs

Tradeoffs between fitness components expressed early and late in life are central to the evolutionary theory of lifespan. In this series of experiments, several were detected. Which traits traded off depended on the experiment and the criterion used to detect the tradeoff.

First criterion. The most straightforward criterion is significant, opposite effects of treatment on two traits. Where treatment effects are judged by the significance of the treatment × temperature interaction, that criterion detected a tradeoff between the lifespan of mated females and their total and early fecundity in Expt. I, no tradeoff associated with lifespan in Expt. II, and a positive association, not a tradeoff, between lifespan of females and weight at eclosion in Expt. III.

Second criterion. The treatment also had significant effects on the slopes of relationships between two traits. Those relationships can be calculated for treatment and control lumping temperatures together, for each temperature separately, and in the residuals that expression the treatment × temperature interaction effects. In all three experiments combined, there were 108 such relations that could be tested (Stearns & Kaiser, 1993). Enhanced expression of elongation factor had significant effects on relations that could be described as measured in seven cases (Table 4).

In three cases the treatment changed a positive relation in the control into a negative relation in the treatment. Those cases involved age at eclosion and early fecundity on the one hand and late fecundity and lifespan on the other, and all occurred in Expt. II or Expt. III. This suggests that in healthy flies increases in lifespan associated with enhanced expression of elongation factor entail costs that must be paid in age and weight at eclosion and early fecundity. However, the fact that the treatment also erased some tradeoffs that had existed in the control

makes clear that the total impact of treatment on tradeoffs is complex. Whether the treatment would increase the fitness of flies in a natural population could only be assessed in an experiment that followed the frequency of the treatment insert in a population under natural conditions.

The correlations across lines in Expt. II are genetic correlations generated by the epistatic interactions between one treatment insert position, one control insert position, and six genetic backgrounds. Thus they are not additive genetic correlations. Nevertheless, we found it interesting that a manipulation that is equivalent to introducing a single mutant into a population (Expt. II) can change such genetic correlations from positive to negative and from negative to positive. We did not expect that epistatic genetic correlations would be so labile that fixing a single new mutation could change their sign.

Third criterion. Recall that no tradeoffs relevant to lifespan were detected in Expt. II in the treatment × temperature interaction effects (Table 2). When we analyzed the changes in each genetic background from control to treatment in the residuals, we discovered that they were sometimes constrained to lie within an envelope that appeared to be determined by processes on which the treatment itself had little effect. This was not true for all relations that we examined; in some of them the changes occurred in a confusing variety of directions. But there were three relations in the residuals in which most of the differences between control and treatment lines described changes in one direction or the other along a tradeoff (cf. Fig. 7a): total fecundity of mated females vs. lifespan, early fecundity of mated females vs. lifespan, and dry weight at eclosion of virgin males vs. lifespan. In these cases, changes were occurring in tradeoffs due to epistatic interactions between treatment inserts and genetic backgrounds, but those changes were hidden in the summary statistics because the effects cancelled each other out.

Similar effects may be hidden in the other experiments but cannot be detected because the logic of the experimental design does not allow one to connect a single treatment with a single control line in either Expt. I or Expt. III, where every treatment insert was at a different position from every control insert. We suspect that such effects may exist in other organisms; this is, to our knowledge, the first experiment done that could have detected them.

The physiology and genetics of tradeoffs. Several research groups working on lifespan in *Drosophila* have concluded that interactions between genetics and physiology may be the next thing that sheds light on the evolution of lifespan (M. Rose, L. Partridge, pers. comm.). Two aspects of the results reviewed here suggest that genetic changes are carried out within the bounds of a physiological framework that determines the tradeoffs among fitness components. The origin and causes of that framework may have to be understood before we can understand the evolution of lifespan itself.

The first point can be seen by comparing tradeoffs in Expt. I with those in Expt. III. In Expt. I, increased lifespan of mated females was associated with reduced fecundity in the residuals. In Expt. III, reduced lifespan of mated females was associated with reduced dry weight at eclosion in the residuals. The trait with which lifespan was significantly associated changed with the type of fly tested. The second point can be seen in the analysis of changes from control to treatment in the residuals in Expt. II, which revealed hidden, compensatory changes in tradeoffs. Both suggest that the tradeoff existed independent of the treatment, which could make a change within an existing structure but could not modify the structure itself. We think that structure is physiological.

A difference between the experiments on inbred and outbred flies suggests that temperature physiology may also be involved in the generation of tradeoffs. In the experiment on inbred flies, the larvae and pupae used to measure fecundity and lifespan were all reared at 25° so that the measurements would be comparable with those made be Shepherd *et al.* (1989), who had reared all flies to eclosion at that temperature because pre-eclosion mortality was high in those inbred flies at 29.5°. In the backgrounds and positions experiments, flies were reared at both temperatures for all traits. In the first experiment, effects on fecundity and lifespan were much larger than in the latter two experiments, but effects on larval and pupal traits were not. In the latter two experiments, effects on time to pupation, time as pupae, and time to eclosion were significant, but there were no effects on fecundity, and effects on lifespan were less dramatic.

Table 5. The experiments in which tradeoffs associated with aging have been detected in *Drosophila melanogaster*.

Tradeoff detected	Experiment						
	Luckinbill[1]	Rose[2]	Partridge[3]	Hillesheim[4]	Expt. I	Expt. II	Expt. III
Early fecundity/ lifespan	yes	yes	no	yes	yes	yes [6,7]	yes [6]
Early fecundity/ late fecundity	yes	yes	no	yes	no	yes [7]	no
Size at eclosion/ lifespan	- [5]	- [5]	yes	yes	no	yes [7]	no
Developmental time/ lifespan	- [5]	- [5]	yes	- [5]	no	no	no
Larval competitive ability/lifespan	- [5]	- [5]	yes	- [5]	- [5]	- [5]	- [5]
Developmental time/ late fecundity	- [5]	- [5]	no	- [5]	no	no	yes [6]
Weight at eclosion/ late fecundity	- [5]	- [5]	no	- [5]	no	yes [8]	yes [8]

[1] Luckinbill *et al.* (1984). [2] Rose (1984). [3] Partridge & Fowler (1992). [4] Hillesheim & Stearns (1992). [5] Did not look. [6] Change of sign of tradeoff caused by treatment. [7] Tradeoff detected in the detailed analysis of residuals. [8] Tradeoff present, treatment reduced slope, which remained negative.

Comparison with other experiments on tradeoffs involved in lifespan. Table 5 compares the tradeoffs that have been found in four selection experiments on traits involved in lifespan and in these three experiments. On balance, these experiments support the view that early fecundity and lifespan do trade off, as suggested by Luckinbill *et al.* (1984), Rose (1984), and Hillesheim and Stearns (1992). However, they also support the idea that age and size at eclosion are involved in tradeoffs on lifespan, as suggested by Partridge and Fowler (1992). The general conclusion remains: early and late fitness components do trade off, but the ones involved depend on the experiment and how the tradeoffs are measured.

Strategies of molecular manipulation of life histories. Two problems with the interpretation of the results of these experiments suggested improvements in the design of molecular manipulations. First, the treatment insert was 2 kb longer than the control insert. This confounded the treatment effects and led to particular difficulties in interpreting the direct effects of treatment in the positions experiment. A better control would be created by *in situ* deactivation of the treatment insert, resulting in a control insert of precisely the same position and length and almost the same sequence as the treatment insert. This method is available in yeast but not yet in *Drosophila*.

Second, interaction effects are hard to interpret. They make the statistical analysis more complicated and harder to communicate. It would be better to design inserts that would be expressed at one lower temperature, within the normal physiological range of *D. melanogaster*; and vary the insert dosage for a sample of insert positions, e.g. one and four inserts, treatment and control, on each of three third chromosomes (three positions, same for treatment and control, achieved by *in situ* mutation) inserted into each of four genetic backgrounds. That would yield 48 lines to be tested, the same number that was tested here in both experiments combined, and it would give more complete information on position × background interaction effects.

Conclusion

Genetic manipulation of a gene of known function can significantly affect lifespan in *Drosophila*, and it can lead to surprisingly large changes in other fitness components, including age and weight at eclosion and fecundity. Such manipulations also provide promising new information on the nature of tradeoffs.

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References

Bellen, H. J., C. J. O'Kane, C. Wilson, U. Grossniklaus, R. K. Pearson & W. J. Gehring, 1989. P-element-mediated enhancer detection: a versatile method to study development in *Drosophila*. Genes and Development 3: 1288-1300.

Charlesworth, B. & J. A. Williamson, 1975. The probability of the survival of a mutant gene in an age-structured population and implications for the evolution of life-histories. Genet. Res. 26: 1-10.

Cooley, L., R. Kelley & A. Sprading, 1988. Insertional mutagenesis of the Drosophila genome with single P-elements. Science 239: 1121-1128.

Cox, D. R., 1972. Regression models and life-tables (with discussion). J. Roy. Stat. Soc. B 34: 187-220.

Darnell, J., H. Lodish & D. Baltimore, 1990. Molecular cell biology. 2nd Ed. W. H. Freeman, New York.

Hamilton, W. D., 1966. The moulding of senescence by natural selection. J. Theor. Biol. 12: 12-45.

Hillesheim, E. & S. C. Stearns, 1992. Correlated responses in life history traits to artificial selection for body weight in *Drosophila melanogaster*. Evolution 46: 745-52.

Hovemann, B., S. Richter, U. Walldorf & C. Cziepluch, 1988. Two genes encode related cytoplasmic elongation factors EF-1a in *Drosophila melanogaster* with continuous and stage specific expression. Nucleic Acids Res. 16: 3175-94.

Johnson, T. E., 1990. Increased life-span of *age-1* mutants in *Caenorhabditis elegans* and lower Gompertz rate of aging. Science 249: 908-912.

Kaiser, M. & S. C. Stearns, 1993. Effects on fitness components of enhanced expression of elongation factor EF-1α in *Drosophila melanogaster* II. Genetic backgrounds and insert positions. Under review.

Luckinbill, L., R. Arking, M. G. Clare, W. C. Cirocco & S. A. Buck, 1984. Selection for delayed senescence in *Drosophila melanogaster*. Evolution 38: 996-1003.

Partridge, L. & K. Fowler, 1992. Direct and correlated responses to selection for age at reproduction in *Drosophila melanogaster*. Evolution 46: 76-91.

Robertson, H. M., C. R. Preston, R. W. Phillis, D. M. Johnson-Schlitz, W. K. Benz & W. R. Engels, 1988. A stable source of P-element transposase in *Drosophila melanogaster*. Genetics 118: 61-470.

Rose, M., 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution 38: 1004-1010.

Rose, M. R., 1991. Evolutionary biology of aging. Oxford University Press, Oxford.

Rose, M. R. & B. Charlesworth, 1980. A test of evolutionary theories of senescence. Nature 287: 141-142.

Rose, M. R. & B. Charlesworth, 1981. Genetics of life history in *Drosophila melanogaster*. II. Exploratory selection experiments. Genetics 97: 187-196.

SAS Institute, 1985. SAS User's Guide: Statistics. SAS Institute, Cary, North Carolina, 957 p.

SAS Institute, 1991. SAS Technical report P-127. SAS/STAT Software: The PHREG procedure. Version 6. SAS Institute, Cary, North Carolina. 59 p.

Shepherd, J. C. W., U. Walldorf, P. Hug & W. J. Gehring, 1989. Fruit flies with additional expression of the elongation factor EF-1α life longer. Proc. Natl. Acad. Sci. USA 86: 7520-1.

Sokol, R. R. & F. J. Rohlf, 1981. Biometry. 2nd Ed. W. H. Freeman, San Francisco.

Stearns, S. C., T. Diggelmann, M. Gebhardt, H. Bachmann & R. Wechsler, 1987. A device for collecting flies of precisely determined post-hatching age. Drosophila Information Service 66: 167-169.

Stearns, S. C., 1992. The evolution of life histories. Oxford University Press, London. 248 p.

Stearns, S. C., M. Kaiser & E. Hillesheim, 1993. Effect on fitness components of enhanced expression of elongation factor EF-1α in *Drosophila melanogaster*. I. The contrasting approaches of molecular and population biologists. Am. Nat. (in press).

Stearns, S. C. & M. Kaiser, 1993. Effects on fitness components of enhanced expression of elongation factor EF-1α in *Drosophila melanogaster*. III. Analysis of tradeoffs between fitness components. Under review.

Webster, G. C. & S. L. Webster, 1982. Effects of age on the post-initiation stages of protein synthesis. Mech. Aging Dev. 18: 369-378.

Webster, G. C. & S. L. Webster, 1983. Decline in synthesis of elongation factor on (EF-1) precedes the decreased synthesis of total protein in aging *Drosophila melanogaster*. Mech. Aging Dev. 22: 121-128.

Williams, G. C., 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11: 398-411.