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Expression of genetic and environmental variation during ageing

2. Selection for increased lifespan in *Drosophila melanogaster*

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Summary. A selection experiment with *Drosophila melanogaster* was carried out to test some theories of ageing by calculating genetic parameters for a reproductive fitness trait at different ages. Successful selection for increased lifespan showed that longevity is a trait under genetic control. Positive genetic correlations between early and late fitness were found. These results do not support the pleiotropy theory of ageing which predicts a negative genetic correlation. Both environmental and additive genetic variation clearly increased with age. Increased environmental variation probably reflects the individuals' difficulties in coping with environmental stress. The increase in additive genetic variation supports the mutation accumulation theory of ageing, as well as other theories that postulate increased additive genetic variation with age.

Key words: Genetic and environmental variation – Age changes – Selection for longevity

Introduction

There are several theories on the fundamental causes of ageing, but no general agreement among them. One way of discriminating between different theories is to collect and analyse data on reproductive fitness traits and lifespan. We decided to look at the problem from a quantitative genetic point of view. There is fairly good evidence in the literature, that ageing is under quantitative genetic control (for a review see Arking 1987). The calculation of genetic parameters, such as heritability, genetic correlations, genetic variance, and genetic response to selection for traits related to fitness and ageing, gives information that makes it possible to test these theories.

Williams (1957) suggested that genes with unfavourable effects on fitness at later ages could still be favoured by natural selection if they had beneficial effects at an earlier age. This pleiotropy theory predicts a negative genetic correlation between early and late fitness.

Most previous investigations have found negative genetic correlations between early and late fitness. Rose and Charlesworth (1981 b) estimated negative genetic correlations between early and late fitness using pedigree data. They also found that fecundity at an earlier age decreased when they selected for increased fecundity at a late age in *Drosophila melanogaster*, thus indicating a negative genetic correlation between early and late fitness. When Luckinbill et al. (1984) selected for late reproductive output, they found increased late fecundity and decreased early fecundity. Additionally, negative genetic correlations between early and late fitness were obtained in a study by Tucic et al. (1988) investigating fitness components in *D. melanogaster*.

Positive genetic correlations have also been reported. Giesel (1986) found positive genetic correlations between early and late fitness in a population of *D. melanogaster*. Engström et al. (1989) investigated genetic variation and genetic correlations between fitness traits at different ages in *D. melanogaster* to determine whether those parameters change consistently with age. Genetic correlations between the different age periods were positive in that study too.

Another main evolutionary theory of ageing is the mutation-accumulation theory (Medawar 1952), according to which ageing is caused by the accumulation of late-acting deleterious mutations. These mutations

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would tend to accumulate because of their small individual effects on early fitness. This theory predicts increased additive genetic variation with age.

In a study of laying hens, Liljedahl et al. (1984) found increased genetic variation in egg production traits with increased age. Increased additive genetic variation for fitness traits in *D. melanogaster* was also found by Tucic et al. (1988) and Engström et al. (1989). However, Rose and Charlesworth (1981 a) found no trend in genetic variation with age in the same species.

Early and late fecundity were compared in populations of *D. melanogaster* which had been selected for either early or late reproduction (Mueller 1987). Early-age fecundity did not differ between the different lines, but lines that reproduced early were less fecund at a lage age. The accelerated senescence exhibited by the lines that reproduced early could be caused by an accumulation of deleterious alleles acting late in life.

Service et al. (1988) found evidence for both the mutation accumulation theory and the pleiotropy theory of ageing, when applying reverse selection to *D. melanogaster* lines previously selected for late fitness. The lines originally exhibited reduced early fecundity and increased resistance to the stresses of starvation, desiccation and ethanol. Later, when these lines were selected for early fecundity, there was a selection response for this trait and resistance to starvation decreased in agreement with a negative genetic correlation between early and late fitness. However, resistance against the stresses of ethanol and desiccation were unaffected, which suggests that mutation accumulation also plays a role.

Apart from the mutation accumulation theory of ageing, Liljedahl et al. (1984) suggested that formerly inactive genes are turned on during ageing to counteract the negative effects of increased sensitivity to environmental stress. Another theory (Hart and Setlow 1974) argues that genetic damage accumulates with age, causing different phenotypic effects, which reflect individual capacity for DNA repair. These additional theories also postulate increased genetic variation with age.

Attempts to select for increased longevity or higher reproductive capacity at a later age have had mixed results. In the experiment by Lints et al. (1979) selection for increased life-span was unsuccessful, but in another experiment by Rose and Charlesworth (1981 b) life-span increased in the line selected for fecundity a late age. Luckinbill and Clare (1985) produced long-lived strains of *D. melanogaster* after more than 20 generations of artificial selection. The selection response only ocurred when larvae developed in crowded conditions. Clare and Luckinbill (1985) suggest that this indicates that there are genotype-environment interactions for longevity during larval development, which may explain the different results reported by Lints et al. (1979) and Rose and Charlesworth (1981 b).

To further investigate the effect of selection for increased lifespan and the effect of ageing on fitness variation, the present study compares genetic correlations, as well as variance components, for progeny production at different ages.

Materials and methods

The base population of *D. melanogaster* used in this investigation was a cross between four wild-type non-inbred laboratory strains, from four different continents (Florida, Algeria, Taiwan and Sweden), which contributed equally to the four-way hybrid strain. The hybrid strain was supplied by Dr. M. Rasmuson at the *Drosophila* Stock Center in Umeå, Sweden. The strain was allowed to undergo more than ten generations of random mating to attain linkage equilibrium.

Selection procedure

Three lines were derived from the base population: one randomly mated control and two lines selected for fitness at later age. The control line consisted of 500 males and 500 females kept in vials to maximize effective population size (expected rate of inbreeding less than 0.05% per generation), thus minimizing genetic drift. After eclosion, this line was kept for 1 week before egg-laying to produce progeny for the next generation. A generation interval of about 16 days with discrete generations was chosen to prevent selection for either increased lifespan, or early or late fitness.

In each selection line 500 males and 500 females were isolated as virgins and transferred to new vials twice a week until approximately 50% of the flies died. Surviving males and females were then mated and the progeny collected from successful matings (approximately 30% of all matings). The rate of inbreeding for the flies in the selection lines was estimated to be 0.3–0.4% per generation. This selection procedure also enabled us to select separately on both sexes. Twelve generations of selection were applied.

Test of reproductive fitness at different ages

To test for progeny production by females of different ages, random samples of sires and dams from the two replicate lines and the control were collected after eclosion at generations 4, 8 and 12. On average each sire was mated with three dams, and the resulting daughters (approximately seven per mating) were collected. Each daughter was placed in a vial with one randomly chosen, unrelated, male of the same age from the same line. These pairs were transferred to new vials twice a week: the first time to a vial for 2 days of egg-laying and the second time for 5 days awaiting another egg-laying period. The total test, of nine egg-laying periods, covered slightly more than 2 months. The pairs were kept together except when the male died, in which case he was replaced by another randomly chosen male.

The fitness trait studied in this investigation was the 'number of adult progeny' produced by each daughter, measured from nine consecutive egg-laying periods throughout their reproductive life. Progeny were counted 15 days after the start of each egg-laying period. For the three generations tested, approximately 1 800 individuals were scored in each line on day 3, and 11 on day 59.

Test of longevity

At generation 12 a test for male survival was performed. Approximately 100 males from each line were individually placed

in vials, transferred to new vials twice a week, and the number of survivors counted. At an early age, and at a late age, each male was allowed to mate with three 'attached-X' females to see if there were any differences in sex ratio among the offspring at different ages. A different sex ratio at an early compared to a late age could indicate different amounts of mutations on the sire's X-chromosome (Björklund et al. 1988). No particular test for survival was performed for females from the different lines. However, survival data from the test of reproductive fitness at different ages at generation 12 were used to create female survival curves.

Survival curves were analyzed using the Log Rank Test (Kalbfleisch and Prentice 1980). The test statistic in this analysis compares the difference between the observed number of deaths in each line during each sampling interval and the corresponding expected number of deaths.

In all experiments the flies were kept in vials and fed a standard medium containing 10 g agar, 60 g syrup, 50 g baker's yeast, 40 g powdered mashed potatoes, 0.75 g ascorbic acid and 2 ml propionic acid per litre of water. The flies were kept in the same incubator at 25 °C and 50% relative humidity. Carbon dioxide was used for anaesthetizing and all handling was performed at room temperature.

Statistical analysis

When calculating variance and covariance components for the control and the two replicate lines, data from the first five age periods were used in order to obtain large enough numbers of observations for keeping the sampling variance reasonably small. The number of observations in the test population sampled from each line was approximately 5500, distributed between the five different age periods analysed. To avoid selection bias caused by mortality, only daughters surviving the fifth egg-laying period were used in the statistical analysis. Daughters producing no adult offspring in any egg-laying period were omitted from the analysis.

Because the mean number of progeny for consecutive egg-laying periods decreased as the females aged, data were transformed to logarithms (base ten) to eliminate the dependence between the mean and the standard deviation in order to make the different variance components comparable. Before transformation, the correlation between the mean and the standard deviation was on average 0.78 for the different generation and line combinations. Most correlations were significant. After transformation, the correlation was on average − 0.43, and only some were significant.

The method used to calculate variance and covariance components within line and generation for the five different egg-laying periods was Multivariate Restricted Maximum Likelihood under an individual animal model (Meyer 1986). Therefore, the only factor in the model was the random effect of daughters. A complete relationship matrix was used in order to take into account the covariance structure among daughters.

In the multivariate analyses, a canonical tranformation to new uncorrelated variates was used to simplify the calculations (Meyer 1985). Estimates from univariate analyses were used as starting values in the multivariate analyses, and the iterations were continued until the difference between two successive rounds was less than 0.01%. The variance component between daughters estimates the additive genetic variation, whereas the residual variance component estimates the environmental variation, ignoring other effects.

Results

Figure 1 shows the selection response for male longvity in generation 12. The maximum life-span (5% survival)

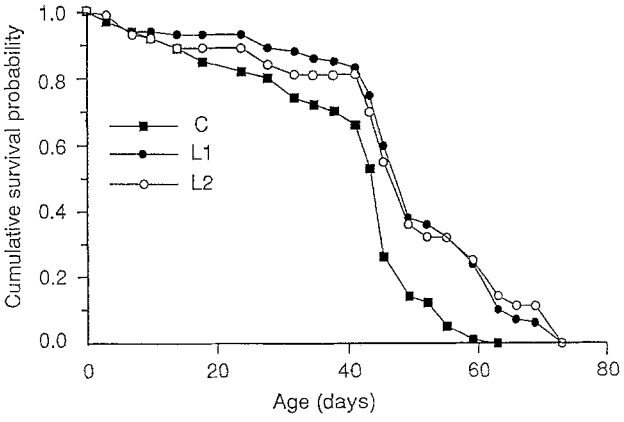

Fig. 1. Male survival curves for two lines selected for fitness at late age (*L1*, *L2*) and one randomly mated control after 12 generations of selection. Cumulative survival probability is the proportion of flies alive at the end of each sampling period

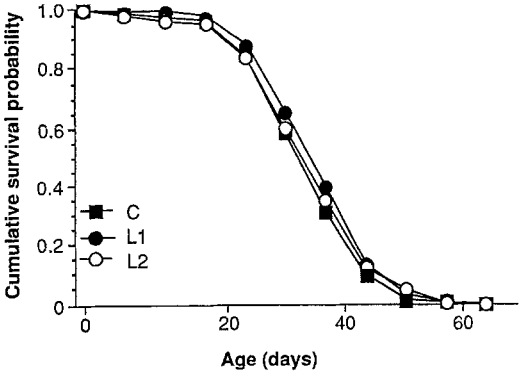

Fig. 2. Female survival curves for two lines selected for fitness at late age (*L1*, *L2*) and one randomly mated control after 12 generations of selection

increased by about 9 days (15%) in line L1 and by 11 days (18%) in line L2. The median lifespan (50% survival) increased by approximately 5 days (11%) in line L1 and by 4 days (9%) in line L2. The survival curves for L1 as well as for L2 were both significantly different from that of the control line ($P < 0.001$). No difference in sex ratio among the offspring from young compared to old males was found, indicating that there was no, or at least an undetectable, accumulation of lethal mutations in the germ cells of the males.

The female survival curves are shown in Fig. 2. The maximum lifespan (5% survival) increased by 2 days (4%) in both selection lines. The mean lifespan (50% survival) increased by 2 days (6%) in line L1 and by 1 day (3%) in line L2. The survival curve for L1 was significantly different from the control line, ($P < 0.02$) whereas that of L2 bordered on significance ($P < 0.1$). The mean number of progeny produced decreased with age, but

Table 1. Genetic correlations ± SE (below diagonal), phenotypic correlations ± SE (above diagonal) and heritabilities ± SE (diagonal) for the trait number of adult offspring at different age periods in the control line (data log transformed)

Age (days)	Age (days)				
	3–4	10–11	17–18	24–25	31–32
3–4	**0.27 ± 0.12**	0.27 ± 0.07	0.18 ± 0.04	0.15 ± 0.09	0.07 ± 0.02
10–11	0.71 ± 0.19	**0.48 ± 0.08**	0.54 ± 0.10	0.39 ± 0.02	0.12 ± 0.09
17–18	0.21 ± 0.12	0.71 ± 0.20	**0.37 ± 0.20**	0.49 ± 0.06	0.24 ± 0.06
24–25	0.28 ± 0.12	0.75 ± 0.09	0.86 ± 0.03	**0.33 ± 0.03**	0.37 ± 0.01
31–32	− 0.13 ± 0.31	0.13 ± 0.30	0.49 ± 0.18	0.48 ± 0.06	**0.27 ± 0.07**

Table 2. Genetic correlations ± SE (below diagonal), phenotypic correlations ± SE (above diagonal) and heritabilities ± SE (diagonal) for the trait number of adult offspring at different age periods in line L1 (data log transformed)

Age (days)	Age (days)				
	3–4	10–11	17–18	24–25	31–32
3–4	**0.58 ± 0.26**	0.41 ± 0.07	0.33 ± 0.03	0.21 ± 0.08	0.11 ± 0.02
10–11	0.38 ± 0.18	**0.56 ± 0.14**	0.66 ± 0.03	0.38 ± 0.07	0.18 ± 0.04
17–18	0.21 ± 0.25	0.98 ± 0.02	**0.31 ± 0.06**	0.49 ± 0.03	0.26 ± 0.05
24–25	0.04 ± 0.33	0.63 ± 0.14	0.67 ± 0.18	**0.21 ± 0.03**	0.36 ± 0.01
31–32	0.51 ± 0.16	0.47 ± 0.08	0.43 ± 0.03	0.16 ± 0.30	**0.15 ± 0.04**

Table 3. Genetic correlations ± SE (below diagonal), phenotypic correlations ± SE (above diagonal) and heritabilities ± SE (diagonal) for the trait number of adult offspring at different age periods in line L2 (data log transformed)

Age (days)	Age (days)				
	3–4	10–11	17–18	24–25	31–32
3–4	**0.48 ± 0.11**	0.34 ± 0.03	0.24 ± 0.05	0.17 ± 0.06	0.09 ± 0.05
10–11	0.47 ± 0.26	**0.46 ± 0.16**	0.49 ± 0.10	0.38 ± 0.08	0.22 ± 0.01
17–18	0.25 ± 0.16	0.82 ± 0.14	**0.37 ± 0.08**	0.48 ± 0.04	0.23 ± 0.01
24–25	0.29 ± 0.11	0.80 ± 0.09	0.92 ± 0.04	**0.25 ± 0.12**	0.32 ± 0.04
31–32	0.28 ± 0.09	0.71 ± 0.11	0.67 ± 0.06	0.61 ± 0.17	**0.12 ± 0.06**

there was no obvious response in progeny production at generation 12 for the selection lines as compared to the control (Fig. 3). One of the selection lines (L2) had a lower early progeny production for up to 24 days of age as compared to its replicate line and to the control. Up to 11 days of age early progeny production was slightly higher in the other selection line (L1) as compared to the control. From 24 days to the end of the experimental period there were no differences in progeny production between the different lines.

Because there was no change in the different variance and covariance components for progeny production at different ages during selection, the estimates presented in Tables 1–3 and Figs. 4 and 5 are means over generations. After four generations of selection 40% of the genetic correlations turned more positive in the control line, while 35% turned more positive in the selection lines. For the assay at generation 8, 42% in the control turned more positive and the corresponding percentage for the selection lines was 47%. At generation 12, the percentages for the control and the selection lines were 50% and 45% respectively. Heritabilities, genetic and phenotypic correlations are presented in Tables 1–3. The heritabilities decreased with age, indicating a change in the contributing variance components. Most genetic and phenotypic correlations between the various age periods were significantly different from zero and all but one were positive.

Figures 4 and 5 show a clear increase in both additive genetic and environmental variance with age. Significant linear regression lines were fitted for the additive genetic components of variance for the three lines ($P < 0.05$). Polynomial regression lines of the second degree were fitted for the environmental components of variance ($P < 0.003$).

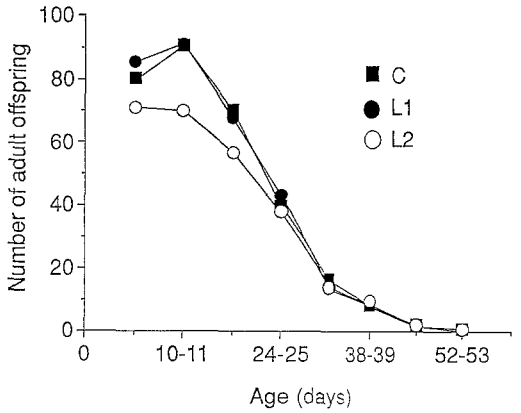

Fig. 3. The relationship between "number of adult offspring" at different age periods (means, standard error approximately 1.5) for the lines selected for fitness at late age (*L1*, *L2*) and the random mated control after 12 generations of selection

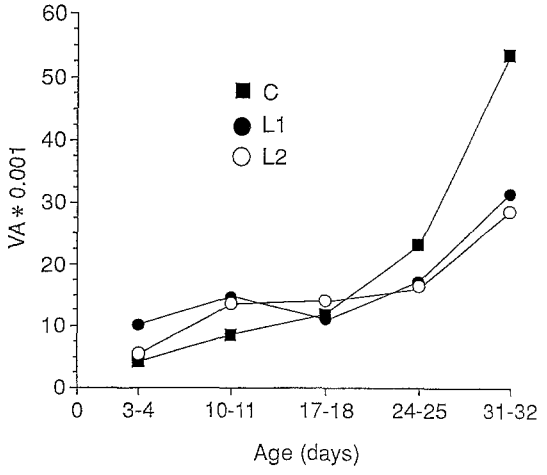

Fig. 4. Additive genetic variance (*VA*) of the trait "number of adult offspring" at different ages for the two selection lines and the control

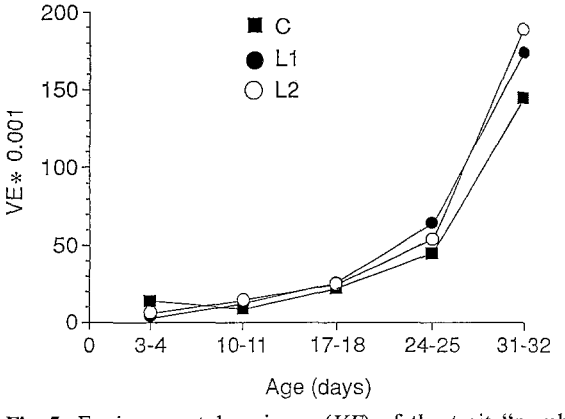

Fig. 5. Environmental variance (*VE*) of the trait "number of adult offspring" at different ages for the different lines

Discussion

The results presented here describe genetic parameters for reproductive fitness throughout the whole reproductive life in *D. melanogaster*. Genetic parameters are, strictly speaking, only valid for a particular population with its genotypic frequencies in the environment where they were estimated. However, due to large sample size, the sampling errors for the estimated variance components and genetic correlations were much smaller here than in previous investigations. The flies used had undergone more than ten generations of random mating, were well adapted to laboratory conditions, and had a minimal level of inbreeding. Therefore, the results should be unaffected by linkage disequilibrium arising from crossing the original lines, by genotype-environment interaction, and by inbreeding effects.

The selection procedure used in this experiment was different from that employed by Rose and Charlesworth (1981 b) who selected flies with the highest reproductive output late in life. In our experiment, the flies were kept virgin and only those which survived up to the highest age were allowed to reproduce. This difference in selection procedure might explain the different results achieved for genetic variance relative to age and for the different genetic correlations between different age periods.

The successful selection for increased lifespan, both in terms of mean and maximum life-span, shows that longevity is a trait under genetic control. Although most earlier selection experiments produced similar results, e.g., Rose and Charlesworth (1981 b) and Luckinbill et al. (1984), some failed to obtain a selection response (Lints et al. 1979). Unsuccessful selection for longevity might be due to low larval density (Clare and Luckinbill 1985).

In the present experiment progeny production throughout life did not differ between one of the lines selected for longevity and the control. The other line had a decreased early progeny production. A decrease in early progeny production and an increase in late progeny production for the selection lines are predicted by the pleiotropy theory. An earlier decrease in late progeny production for the control would be expected according to the mutation accumulation theory (Mueller 1987).

The positive genetic correlations between early and late progeny production in this study are contrary to the negative correlation between age periods predicted by the pleiotropy theory. However, there was a trend towards less positive values for more widely separated age periods. The genetic correlations between the age periods 3–4 days and 31–32 days were significantly positive for L1 and L2, whereas the estimate for the control line was not significantly different from zero (Tables 1–3). This study covers a comparatively long period of the animals' life compared to, e.g., the study by Rose and

Charlesworth (1981 a) which only covered the interval from 1 to 25 days of age. Most previous investigations found negative genetic correlations (Rose 1984), but positive correlations have also been reported (Giesel 1986). However, Rose (1984) argued that such positive genetic correlations could be caused by inbreeding. Inbreeding would result in a population that is homozygous for deleterious alleles, and its poor performance in both early and late fecundity would induce positive correlations. The population used in this experiment was a cross between four strains of different origin, which means that the positive correlations found could not have been caused by inbreeding.

The two estimates of variance, additive genetic and environmental, showed an obvious increase with age. Increases in phenotypic or environmental variation with age or in stressful environments has been found in many investigations (Clayton and Robertson 1966; Flock 1977; Liljedahl et al. 1984, in laying hens; Rose and Charlesworth 1981 a; Burla and Taylor 1982; Engström et al. 1989, in *Drosophila*). The explanation usually put forward is that older animals are less able to cope with environmental stress due to physiological deterioration.

The estimated increase in genetic variance could possibly be explained by effects, such as sex linkage, epistasis, and dominance, which contribute more to the total genetic variance at later age periods than at earlier periods. Those effects were not considered in this investigation but will be dealt with in a later study.

Common environmental effects arising from daughters coming from the same vial may have affected the estimated variances. If there is a common environmental effect, the additive genetic component would be overestimated at early age with the overestimation decreasing with age as the common environmental effect becomes smaller and smaller. The trend due to the influence of a common environment would be decreasing additive genetic variance with age. The trend in this investigation was toward increasing additive genetic variance, which may have been even more pronounced if common environmental effects had been dealt with in another way. Regarding the environmental variance, the trend may have been overestimated.

Increasing additive genetic variation with age was reported by Liljedahl et al. (1984) for laying hens and in *D. melanogaster* by Tucic et al. (1988) and Engström et al. (1989). However, Rose and Charlesworth (1981 a), calculating variance components and genetic correlations on transformed data in a way similar to that used in this study, did not find any trend in additive genetic variance with age, possibly due to small sizes. The total number of records in their experiment fell from approximately 1 200 on day 1 to 600 on day 25. In our experiment the total number of individuals scored for each of the three lines falls from about 1 800 on day 3 to 1 100 on day 32. Increased additive genetic variance with age can be explained by the accumulation of late-acting deleterious mutations, it can also be explained by more genes being turned on with age, or by differences in DNA repair capacity.

We conclude that longevity is a trait under genetic control because there was a significant selection response for life-span. Furthermore, we discovered no negative genetic correlations between early and late fitness. Finally we conclude that additive genetic variation increases with age for a fitness trait such as the 'number of adult offspring'. The results from this experiment do not support the pleiotropy theory. They do, however, lend some support to the mutation accumulation theory and other theories postulating increased additive genetic variation with age.

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