Targeting environmental and genetic aspects affecting life history traits

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Chapter 4

The interaction between food condition and life span in two sets of *D. melanogaster* lines selected for increased longevity and increased starvation resistance

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Abstract

Lines with divergent phenotypes of interest can be acquired by experimental evolution and artificial selection. The knowledge gained from these selection lines can be substantial, especially for complex quantitative traits. Starvation resistance and longevity are such traits. They have often been found to be positively correlated in selection lines. Yet, such correlations are usually only tested in one (laboratory) environment. The universal nature of the genetic correlations that is often assumed has been questioned in earlier work. Therefore, we tested lines selected for increased starvation resistance and increased longevity over a range of environments differing in caloric food levels. The analysis showed that the lifespan profiles over the food gradient differed among lines. These interactions were consistent throughout multiple levels of analysis of the individual lines. This implies that though longevity and starvation share common mechanisms, they are also in part determined by different mechanisms.

Keywords

genotype-by-environment interactions, starvation resistance, longevity, shortened life span, food conditions, affluence, adversity

Introduction

Adaptation to a specific environment proceeds through natural selection. This process of adaptation results in a phenotype capable of surviving and reproducing in this environment. In experimental evolution designs of selection, one produces a population with the phenotype of interest through a selection environment, such as starvation. In artificial selection, a specific phenotype, such as longevity, is selected for by the experimenter and does not necessarily involve adaptation to a specific environment. Selection also produces correlated responses, either through pleiotropy and linkage, or indirect selection. Starvation resistance and longevity are often found as correlated responses (e.g. Zwaan et al. 1991; Rose et al. 1992; Zwaan et al. 1995b; Harshman et al. 1999b) . They are therefore thought to be underpinned by the same mechanism. In a number of studies, the traits were found to have become uncoupled over time (Archer et al. 2003; Phelan et al. 2003). Longevity and starvation resistance are both traits that are not only linked to lifespan, but also to specific food conditions. These traits are essential for life history and adaptation to the environment throughout distant taxa such as fungi, protostomes and deuterostomes (Longo and Fabrizio 2002; Partridge and Gems 2002; Longo and Finch 2003). Life span and starvation resistance are determined by resource acquisition and allocation, and trade off with reproduction. To elucidate part of the complexity of the relationship between longevity and starvation resistance, we examine lines artificially selected either for increased starvation resistance or for increased or decreased life span, together with their respective controls under starved, adverse and affluent conditions. There are several possible outcomes of this experiment: a. the reaction norms of the lines do not cross - both traits are determined by a shared mechanism; or b. the reaction norms of the lines cross, but the outcome is condition specific – the traits share a mechanism, but are in part independently regulated; or c. a muddled picture becomes apparent where no clear patterns can be identified – the mechanisms underlying the traits are not shared. We expect to find crossing reaction norms here and have a clear hypothesis of how these lines should interact (figure 1). In short, we hypothesise the following: Starvation resistant lines will outperform all other lines under adverse (starvation and half medium) conditions, but not under affluent (double medium) conditions. Longevity will outperform all lines under affluent conditions, but not under adverse ones. We thus think that the life span advantage of each of the selection directions (e.g. longevity) is environment specific. As has been shown in the *chico* mutant (Clancy et al. 2002), we think that the different selection lines have a relative life span optimum under different food conditions. Put in a more general context, we think that the lines selected for allocation to the soma will generally live longer than the other lines, but will interact over a food gradient because of their specific environmental adaptation.

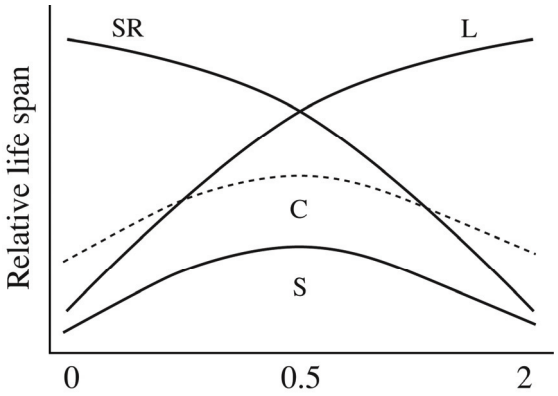

Figure 1. The hypothetical relationship between food condition and relative lifespan (cf. Chapman and Partridge 1996; Clancy et al. 2002). The horizontal axis represents the food gradient with from left to right starvation (SR), half times standard food medium (0.5) and two times standard food medium (2). On the vertical axis the relative life span among the lines is depicted. SR are the starvation resistant lines, L, the long-lived ones, S the short-lived ones and C are the control lines.

Materials and methods

Flies

We used flies from two selection experiments. Zwaan et al. (1995b) artificially selected *D. melanogaster* lines for increased and for decreased life span. After an extended period of relaxed selection the lines were re-selected in the same direction (Vermeulen 2005). This resulted in two long-lived lines (La and Lb), two short-lived lines (Sa and Sb) and two control lines (Ca and Cb). Re-selection restored most of the original life span, but failed to reveal all features found by Zwaan et al. (1995b). The most striking example of which was the decoupling of virgin and mated life span. All of these lines and their controls were disinterestedly donated by the Groningen Evolutionary Genetics laboratory for experimentation in the Leiden laboratory.

The other lines used are those where flies were selected for increased starvation resistance (SR1, SR2, SR3 and SR4) by means of experimental evolution and their controls (C1 and C2). All starvation selected lines showed a 60 to 80% increase in their starvation resistance over 20 generations of selection. After these 20 generations, the lines have been maintained under a regime of relaxed selection. Lines SR1 and SR2 showed an increase in relative fat content, longevity and paraquat resistance as correlated responses to increased starvation resistance. Lines SR3 and SR4 had only acquired an increased fat content, but showed the strongest response to selection. Metabolic rate in feeding situations was not affected in either line, whereas in starved conditions it was higher (Baldal et al. 2006). A total of 3558 flies was assayed for this experiment.

Experimental design

To prevent a bias in the comparison of the lines, we performed a 'blind' experiment. The lines were randomly coded by other members of the laboratory. The code was availed to us only after all experiments had ended. The life span selected lines from the Groningen laboratory were given three generations to adapt to the Leiden laboratory environment before the experiments began. Eggs were collected from young flies (3-5 days old) and in groups of 100 put in glass vials containing 6 ml of standard medium. Standard medium consisted of 20 g agar, 9 g kalmus (10 parts acidum tartaricum, 4 parts ammonium sulphate, 1 part magnesium sulphate and 3 parts potassium phosphate), 10 ml nipagin (100 g 4-methyl hydroxy benzoate per liter ethanol), 50 g saccharose and 35 g granulated yeast per litre water.

The resulting flies were collected within 8 hours post-eclosion to prevent the flies from mating. Adult males and females were kept separately in vials containing 5 flies throughout life, with a total of 50 flies per sex of each line for each treatment. Life span measurements were performed in glass vials containing 6 ml double medium, half medium or starvation medium. In double medium, the amounts of yeast and sugar are double that of standard medium. In half medium, the amounts of sugar and yeast are half that of standard medium. In both these media the concentrations of the other ingredients were maintained as in standard medium. Starvation medium consists of 20 gr. Agar, 9 gr. kalmus, 5 ml. propionic acid, and 5 ml. nipagin per liter water.

The vials were checked daily for living flies, dead individuals were removed immediately to prevent the living flies from feeding on corpses or body fluids and to prevent disease to spread. Immobile flies were checked for death by physical stimulation, while vials were replaced weekly. The flies were then redistributed to a density of 5 individuals per vial.

All maintenance, rearing, and experimentation took place in a 25°C cell with a 12/12hr dark/light regime and a relative humidity of 50%.

Statistics

Cox proportional hazard analysis could not be used to analyse life span because its requirement for non-crossing reaction norms was not met. The lines crossed within treatment and generally the shape of the survival curves differed. Therefore we used a full factorial ANOVA analysis. Normality was checked for each sex on each medium and did not show a significant deviation from a fitted normal distribution (Shapiro Wilkinson W test, all $P<0.0001$), while variances were equal among sexes of each medium. Because of the continuous re-distribution to 5 flies per vial, no effect of vial number could be taken into account. Redistributing the flies in this way ensures that the vial specific effects that are usually taken into account in the vial number factor are minimized and that the flies in the experiment can be regarded as a single

population. Animals that died by non-natural causes or that escaped were excluded from the analysis.

Categories of selection direction

We categorised the lines as follows; SR for the starvation resistant lines, L for the long lived lines, S for the short lived lines, CG for the Groningen control lines and CL for the Leiden control lines. When the factor line was nested in category and treated as a random factor, it was significant. The aim of this exercise was to reduce the information in the analysis and reveal a pattern that could be compared to our model. Despite the sometimes significant differences between lines within a category, we chose to treat the category of a particular line type as a single variable in category analysis.

Because the lines from Groningen and Leiden have a different genetic background for the larger part, we corrected the life span data of the selection lines by subtracting the average of the corresponding control lines for each sex and medium. In this way the distribution and variance were maintained. All tests were performed using JMP 5.0.1.

The variability of the lines was kept in mind in the interpretation of the data. We performed three analyses with increasing generalisation, so as to be able to examine whether abstracting the data changed the interpretation of the general patterns.

Results

ANOVA analysis

The overall ANOVA revealed a significant effect of medium ($F_{2,3445}$=9803, P<0.0001). On the different media, we found significant sex*line interactions (starved $F_{11,1155}$=2.7, P=0.0021; half $F_{11,1166}$=6.9, P<0.0001; double $F_{11,1124}$=6.3, P<0.0001). When analysed per sex, a significant medium*line interaction was found in both males ($F_{22,1726}$=17.8, P<0.0001) and females ($F_{22,1719}$=14.7, P<0.0001). Further analyses were performed per medium and sex, which indicated that the sex differences vary per line. Therefore, we analysed sexes separately throughout further analyses. In every further analysis the factor line was a significant factor (all P<0.0001). In appendix 1, the *post hoc* Tukey test results for the lines are listed per medium and sex. For the double medium no consistent pattern for the selection direction could be observed. However, SR2 and La have the longest life span in both sexes. The longevity of SR2 on double medium is in sharp contrast with its low ranking on half medium. There we see that the control lines ranking is scattered only in females. This can be explained by the low number of Tukey hierarchies (3) in half males. Under starvation, we observe that the lines not selected for increased starvation resistance are scattered throughout the ranking. In figure 2, we showed the average life span of the categories of different selection and control lines.

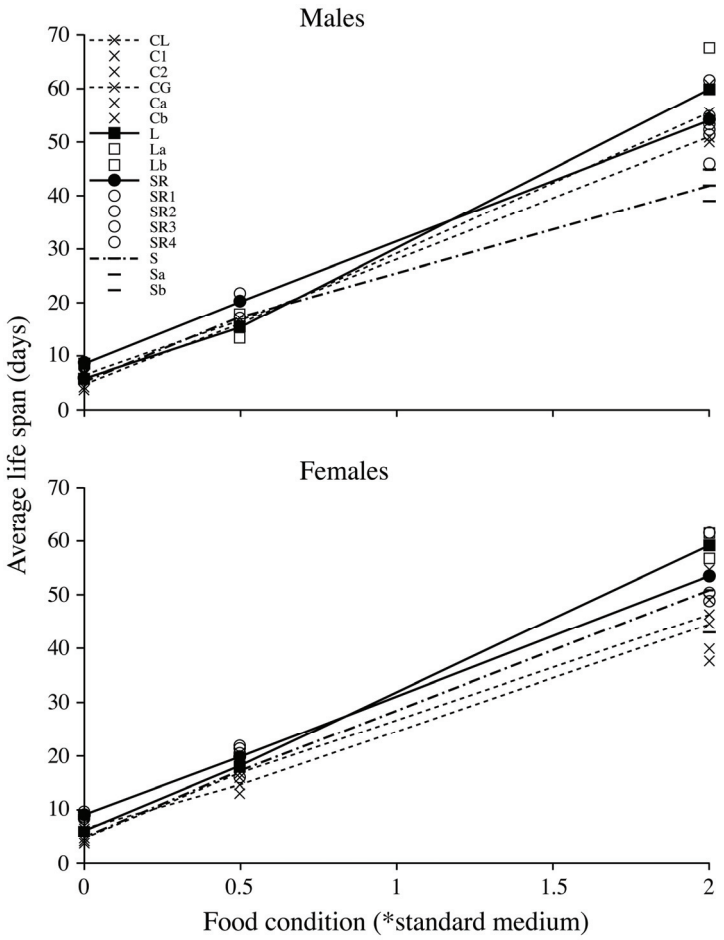

Figure 2. Average life span of individual lines for each medium and sex. Also, the grand averages are given for each selection direction. These have been connected by lines. Straight lines are starvation resistant and long-lived lines and show interactions in between the affluent and adverse media. The short lived lines average has a dash-strip-dash pattern, and control lines averages have been connected by a dotted line.

ANOVA analysis after correction for control lines

As before, for each medium significant sex*line interactions (starved $F_{7,765}$=5.0, P<0.0001; half $F_{7,781}$=12.7, P<0.0001; double $F_{7,753}$=3.8, P=0.0004) were found. Consequently, the sexes were analysed separately again. Analysis per sex revealed a significant medium*line interaction in males ($F_{14,1146}$=24.1, P<0.0001) and females

($F_{14,1153}$=13.6, P<0.0001) again. In all analyses per medium and sex the factor line was highly significant (P<0.0001). In appendix 2 we have listed the outcome of the Tukey analysis. The correction for the control line data did not result in a different hierarchical pattern in Tukey testing of the double medium data. We could see though, that in both males and females the long lived lines are among the longest lived, the short lived lines are among the shortest lived and the starvation resistant lines' life span lies between them. On half medium the starvation resistant lines generally rank highest. They are followed by the long lived lines, except for line Lb in males and eventually the short lived lines. On the starved medium, we again observe a clear pattern where the starvation resistant lines rank highest, followed by the long lived ones and then the short lived lines.

Analysis per selection direction category; two types of starvation resistance

In our earlier work (Baldal et al. 2006) we found that the starvation resistant lines could be split into two groups. We therefore designed special categories for the long lived starvation resistant lines (SR1 and SR2) and non-long lived starvation resistant lines (SR3 and SR4). In Tukey analysis (data not shown) both groups did not differ from analysis as a single group, with the exception that the SR1 and SR2 group lived significantly longer at the double medium than the SR3 and SR4 category. This is consistent with the findings of Baldal et al. (2006). However, because both groups were still neighbouring in the Tukey analysis and we wanted to examine patterns between starvation resistant and long- and short-lived lines we have excluded this factor from further analyses.

Category analysis

Because of their complexity, the data were also analysed per selection direction category, in order to obtain insight into the general patterns among the line types. Overall full factorial ANOVA analysis revealed highly significant for each factor (medium, category and sex) and their interactions. Analyses per sex revealed significant medium*line interactions for males ($F_{4,1161}$=43.1, P<0.0001) and females ($F_{4,1168}$=15.3, P<0.0001) again. Analysis per medium revealed the same for the factors sex and line and their interaction. Therefore, the analyses of category ranking were performed for each medium and sex separately. In appendix 3, the ANOVA and Tukey results are listed per medium and sex. Categories were; L for the long lived lines, S for the short lived lines, and SR for the starvation resistant lines. The double medium revealed that the long lived lines live longest, followed by the starvation resistant lines and that the short lived lines live shortest. This pattern is visible in both sexes, though in females the SR and S lines cannot be distinguished statistically. In the female data at half medium no pattern can be identified because the groups do not differ significantly. In males the long lived lines suddenly become shorter lived than the short lived lines. Life span under starvation reveals the expected ranking appears with the starvation resist lines longest lived under adversity, followed by the long lived lines and then the short lived lines.

Discussion

Here, work is presented on a set of 12 *D. melanogaster* lines, analysed for life span of both sexes under three different adult conditions. These data were analysed in three different ways: on the line level, corrected for genetic background and per selection direction. All analyses revealed similar patterns. All showed significant genotype-by-environment interactions, where lines selected for extended life span under a certain condition did not show this extension in non-selected environments. Lines selected for increased starvation resistance outperformed all other line types under adverse conditions, whereas life span of long-lived lines was highest under affluent conditions.

Control lines and the short lived lines

In general, the control lines turned out to do exactly as expected (cf. figure 1). The Groningen controls showed that they lived longer than long lived lines under adverse conditions, but not under affluent conditions. Relative to the control line, the long lived line is thus affluence-skewed in its increase in life span. The same holds for the starvation resistant lines that overlapped with the control lines on the double medium (appendix 1). There, the starvation resistant lines are adversity-skewed in their life span advantage relative to their control lines. Short lived lines turned out to be generally short-lived. If the short life span mechanism had been similar to the life span increasing mechanism then it would have been expected that under adversity they would not differ from one another and the controls. Because they differ in life span under adversity, we propose that those lines have been selected on different mechanisms than the long-lived lines.

Longevity and starvation resistance

We collected the data to test our hypothesis that the starvation resistant and long lived lines would show a genotype-by-environment interaction when examined under adverse and affluent conditions (see figure 1). There is considerable genotype-by-environment interaction for lifespan of long-lived and starvation resistant lines under affluence and adversity. This indicates that though longevity and starvation resistance are often found as correlated traits, the strength and direction of the correlation is dependent on the environment they are tested in. Starvation resistance is different from longevity because its relative peak life span is under adversity, whereas that of longevity is under affluence. This resulted in a consistent genotype-by-environment interaction in each type of analysis performed here.

Half medium, a transition between affluence and adversity

The differences among lines for life span are not clear, if present at all, at the half medium. Half medium is not as adverse as starvation, but also not as affluent as the double medium. This condition represents the transition between affluence and

adversity and neither of the lines used here is adapted to that. Here, the model that was proposed (figure 1) needs to be adjusted. The starvation resistant lines appear to be slightly longer-lived than the other lines at the half medium.

Integration

The genotype-by-environment interactions between medium and selection direction were repeatedly found in different analyses. This was irrespective of whether the life span data had been corrected for their genetic background by subtracting the average life span of corresponding control lines from the data of selected lines. In the environments where the long-lived and starvation resistant lines have been selected they outperform their control lines. The long-lived and starvation resistant lines interact and show an environment specific life span advantage. This is visible even in the intermediate condition of the half medium, where few differences could be identified. These data therefore strongly suggest that starvation resistance and longevity are not exactly two sides of the same coin (cf. Baldal et al. 2005; Baldal et al. 2006).

On the basis of the literature and of these results, we hypothesise that the phenotypes represent two mechanisms that result from changed energy/food related metabolism, that may involve insulin signalling (e.g. Partridge and Gems, 2002). As a result of selection, in both longevity and starvation resistance selection the animals will have allocation shifted to the soma instead of to the germ-line (yet, cf. Vermeulen and Bijlsma 2006), as is hinted in preliminary micro-array studies on one starvation resistant line. The starvation resistant lines will have been selected as a thrifty genotype (cf. Neel, 1962) with high storage (Baldal et al. 2006), whereas the long-lived lines will have been selected for high somatic maintenance (cf. high paraquat resistance in Vermeulen et al. 2006), which is wasteful under starvation conditions, reducing survival probability. The model we proposed in figure 1 has been proven to be largely correct. Only the low distinction that could be made at the half medium did not follow our expectations.

Appendix 1. The mean and standard error (S.E.) values of life span in days for both sexes of each line for each medium (double, half or starved). Tukey test results are given per medium and sex. Lines not represented in the same column are significantly different.

Females on double medium

	Mean	S.E.	A	B	C	D	E	F
SR2	61.7	1.68	A					
La	61.4	1.75	A					
Sa	58.7	2.1	A	B				
Lb	56.8	2.13	A	B	C			
C1	54.7	1.69	A	B	C			
SR4	53.6	2.13	A	B	C			
SR1	50.3	2.61		B	C	D		
Ca	49.4	2.09		B	C	D	E	
SR3	48.9	2.8			C	D	E	
Sb	43.1	1.51				D	E	F
Cb	40	1.99					E	F
C2	37.8	2.01						F

Males on double medium

	Mean	S.E.	A	B	C	D	E	F	G
La	67.4	2.13	A						
SR2	61.4	1.42	A	B					
C1	60.9	2.11	A	B	C				
SR4	55	2.42		B	C	D			
Ca	53.7	1.57		B	C	D	E		
SR1	53.4	1.84		B	C	D	E	F	
Lb	52.6	2.39			C	D	E	F	
C2	50.3	1.88				D	E	F	
Cb	48.6	1.15				D	E	F	
SR3	46.1	2.38					E	F	G
Sa	44.9	1.31						F	G
Sb	39	1.1							G

Females on half medium

	Mean	S.E.	A	B	C	D	E	F
SR3	21.9	0.54	A					
SR4	21.4	0.85	A	B				
SR1	20.5	0.57	A	B	C			
La	18.8	0.5		B	C	D		
Lb	18	0.48			C	D	E	
C2	17.7	0.36				D	E	
Sa	17.2	0.54				D	E	
Sb	17	0.34				D	E	
C1	16.1	0.46					E	
Cb	16	0.51					E	
SR2	16	0.81					E	
Ca	13.1	0.6						F

Males on half medium

	Mean	S.E.	A	B	C
SR4	21.7	0.52	A		
SR3	21.6	0.67	A		
SR1	20.3	0.53	A		
La	17.7	0.48		B	
Sa	17.2	0.58		B	
Sb	17.1	0.48		B	
SR2	17	0.31		B	
Ca	17	0.58		B	
C1	16.6	0.31		B	
Cb	16.3	0.52		B	
C2	15.6	0.52		B	
Lb	13.3	0.63			C

Starved females

	Mean	S.E.	A	B	C	D	E	F	G
SR1	9.36	0.16	A						
SR3	9.22	0.17	A	B					
SR4	8.58	0.19		B	C				
SR2	8.34	0.27			C				
Cb	6.76	0.14				D			
La	6.22	0.15				D	E		
Ca	6.02	0.14					E		
Lb	5.84	0.12					E		
Sa	4.94	0.12						F	
C2	4.7	0.11						F	
Sb	4.62	0.12						F	G
C1	3.92	0.11							G

Starved males

	Mean	S.E.	A	B	C	D	E	F
SR3	8.88	0.19	A					
SR1	8.5	0.15	A	B				
SR2	8.38	0.22	A	B				
SR4	8	0.16		B				
Cb	6.62	0.12			C			
La	6.14	0.12			C	D		
Ca	5.86	0.12				D		
Lb	5.72	0.1				D		
Sb	5.04	0.08					E	
Sa	5	0.13					E	
C2	4.74	0.12					E	
C1	4.06	0.12						F

Appendix 2. Tukey results for lines, for each sex and medium, corrected for controls. Lines not represented in the same column are significantly different.

Females on double medium

	A	B	C	D
La	A			
SR2	A	B		
Sa	A	B		
Lb	A	B	C	
SR4		B	C	D
SR1			C	D
SR3				D
Sb				D

Males on double medium

	A	B	C	D	E
La	A				
SR2		B			
Lb		B	C		
SR4		B	C		
SR1		B	C	D	
Sa			C	D	E
SR3				D	E
Sb					E

Females on half medium

	A	B	C
SR3	A		
SR4	A	B	
La	A	B	
SR1	A	B	
Lb	A	B	
Sa	A	B	
Sb		B	
SR2			C

Males on half medium

	A	B	C
SR4	A		
SR3	A		
SR1	A		
La		B	
SR2		B	
Sa		B	
Sb		B	
Lb			C

Starved females

	A	B	C	D	E
SR1	A				
SR3	A	B			
SR4		B	C		
SR2			C		
La				D	
Lb				D	
Sa					E
Sb					E

Starved males

	A	B	C	D
SR3	A			
SR1	A	B		
SR2	A	B		
SR4		B		
La			C	
Lb			C	
Sb				D
Sa				D

Appendix 3. Tukey results of the analysis of data per selection direction (L for long-lived; SR for starvation resistant; S for short-lived) corrected for the corresponding controls (subtraction of control average from selection line data), F and P values are also listed.

Females on double medium

$F_{2,383}$=8.4; P=0.0003

	A	B
L	A	
SR		B
S		B

Males on double medium

$F_{2,380}$=36.7; P<0.0001

	A	B	C
L	A		
SR		B	
S			C

Females on half medium

$F_{2,392}$=2.29; P=0.1029

	A
L	A
SR	A
S	A

Males on half medium

$F_{2,399}$=61; P<0.0001

	A	B	C
SR	A		
S		B	
L			C

Starved

$F_{2,393}$=1043; P<0.0001

	A	B	C
SR	A		
L		B	
S			C

Starved males

$F_{2,382}$=1046; P<0.0001

	A	B	C
SR	A		
L		B	
S			C