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LIFE-HISTORY CORRELATES OF EVOLUTION UNDER HIGH AND LOW ADULT MORTALITY

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Abstract.—Life-history theory predicts evolutionary changes in reproductive traits and intrinsic mortality rates in response to differences in extrinsic mortality rates. Trade-offs between life- history traits play a pivotal role in these predictions, and such trade-offs are mediated, at least in part, by physiological allocations. To gain insight into these trade-offs, we have been performing a long-term experiment in which we allow fruitflies, *Drosophila melanogaster,* to evolve in response to high (HAM) and low (LAM) adult mortality rates. Here we analyze the physiological correlates of the life-history trade-offs. In addition to changing development time and early fecundity in the direction predicted, high adult mortality affected three traits expressed early in life—body size, growth rate, and ovariole number—but had little or no effect on body composition (relative fat content), viability, metabolic rate, activity, starvation resistance, or desiccation resistance. Correlations among lines revealed trade-offs between early fecundity, late fecundity, and starvation resistance, which appear to be mediated by differential allocation of lipids.

Key words.—*Drosophila melanogaster,* life-history trait, reproductive effort model, trade-off.

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When extrinsic mortality rates increase, older age classes contribute less to fitness because fewer individuals survive to a given age. The strength of selection then declines faster with age and intrinsic mortality rates in the older age classes increase, which is a central prediction of the evolutionary theory of aging (Rose 1991). Extrinsic mortality rates also affect the evolution of the whole reproductive schedule. When mortality rates increase in one age class, then the optimal reproductive effort increases before that age and decreases after it (Michod 1979). When adult mortality rates increase in all age classes, then optimal reproductive effort increases early in life and optimal age at maturity decreases (Charlesworth 1980). These are some predictions of the reproductive effort model (Gadgil and Bossert 1970; Schaffer 1974; Michod 1979; Charlesworth 1980), in which it is assumed that trade-offs connect decisions made early in life with effects on reproduction and survival late in life. Knowledge of the nature and causes of such trade-offs is thus essential to test predictions of the reproductive effort model.

There is considerable evidence that trade-offs exist and have complex causes (Williams 1966; Partridge and Harvey 1985; Reznick 1985; Bell and Koufopanou 1986; Roff 1992; Stearns 1992). Trade-offs can be caused by genes that act on several life-history traits at once, for example, through a shared developmental program, and they have physiological causes when several traits share material resources (Gadgil and Bossert 1970; Bell and Koufopanou 1986; Sibly and Calow 1986). Physiological trade-offs are interesting because they cause alleles to have negative pleiotropic effects and give rise to negative genetic correlations among life-history traits (Rose 1982, 1985) that can influence both the evolutionary trajectories of populations and the plastic response of an organism to environmental variation (Reznick 1985; Bell and Koufopanou 1986; Partridge and Harvey 1988; Stearns et al. 1991). In a multitrait system, both positive and negative genetic correlations can occur despite underlying physiological trade-offs between pairs of traits (Charnov 1989; Charlesworth 1990). Charnov (1989) concluded that a simple relationship between genetic correlations and allocation functions only exists in very simple cases. The only way to decide how frequent such simple cases are is to analyze the evolutionary change of several traits in a replicated experiment. Pairs of traits that evolve in concert repeatedly in independent replicates are plausible candidates for involvement in simple trade-offs.

A key life-history trade-off exists between reproduction and survival. Consider early fecundity. In *Drosophila melanogaster,* selection experiments have shown that early fecundity can be negatively correlated with life span (Taylor and Condra 1980; Zwaan et al. 1995b), late fecundity (Taylor and Condra 1980; Rose 1984; Mueller 1987; Luckinbill et al. 1988), developmental rate (Zwaan et al. 1995a), starvation (Service and Rose 1985; Service et al. 1988; Service 1989; Rose et al. 1992), and desiccation resistance (Service et al. 1985). Thus, early fecundity trades off with other traits that influence survival and reproduction, but which trade-offs are detected depends on the experimental conditions. This is not surprising, because trade-offs have a complex structure. Evolutionary trade-offs can only be detected if there is genetic variation for the focal traits (Leroi et al. 1994a), which depends on the history of the population, and the expression of genetic variation depends on the environment (Travis 1984; Leroi et al. 1994b).

Our group started a long-term evolution experiment on *D. melanogaster* in November 1993 (Stearns et al. 1996). In contrast to artificial selection, in which a focal trait is selected directly, in experimental evolution the populations are free to evolve under the conditions defined by the experimenter. Our flies are subjected to one of two treatments: high adult

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mortality (HAM) or low adult mortality (LAM). The experiment aims to test the reproductive effort model of life-history evolution and to determine the nature and understand the causes of trade-offs. The traits measured were developmental time, body size, fecundity, viability, fat fraction, ovariole number, starvation and desiccation resistance, metabolic rate, and activity. This paper focuses on the physiological, morphological, and life-history correlates of evolution under high and low adult mortality regimes in mated females.

Material and Methods

Strains and Culture Conditions

We studied six replicate populations, three from a high adult mortality regime (HAM) and three from a low adult mortality regime (LAM). The founder stock of 820 flies consisted of 10 virgin males and 10 virgin females from each of 41 isofemale lines collected in and near Basel, held as isofemale lines for several years, then bred together in a single cage for about five generations to yield a genetically variable population. The six lines were drawn randomly from this population and maintained separately throughout the experiment without any mixing. The measurements were made after 175 weeks, which amounted to 63 generations for HAM and 39 generations for LAM. By that point, each line had experienced about 500–1000 independent mutations. The experiment started 15 November 1993, is continuing, and is maintained on a day-night cycle of 12 h light at 25°C, 12 h dark at 20°C, both at 70% humidity.

Eggs for recruits are collected from the population cages on Tuesdays and Fridays, when two bottles for each line are established, each with 250 eggs in a fixed amount of larval medium. The adults that hatch from those eggs are used 14 days later to replace flies that die or are killed in the population. Each line is readjusted to a density of 100 male and 100 female adults with 14-day-old flies twice per week when adult mortality rates are imposed by hand. Extrinsic mortality was adjusted so that intrinsic plus extrinsic mortality reached the target level. Larvae are maintained at the same density in the same medium for both treatments. We could maintain constant larval densities because of excess production of eggs and constant adult densities because we reared more flies than were needed.

The mortality rates imposed and the rearing conditions have changed twice since the start of the experiment. In the first 13.5 months in the HAM treatment, 90% of the flies in the cage were killed and replaced twice per week; the probability of surviving one week as an adult was 0.01; in the LAM treatment, the probability of surviving one week as an adult was 0.64. In January 1995 (after 20 generations in the HAM and 12 generations in the LAM treatment), larval density was raised from 6.25 to 10.40 larvae/ml and food quality was lowered from 1% to 0.75% yeast in the larval medium; in January 1996 (after 30 generations in the HAM and 17 generations in the LAM treatment), larval density was raised to 12.50 larvae/ml and the adult mortality rate in the LAM treatment was lowered by raising the probability of surviving one week as an adult to 0.81. This increased physiological stress, the difference in selection pressures, and the response to selection in weight at eclosion, developmental time, and early fecundity.

Measurements of responses to these selection regimes were performed in April 1997 at 25°C and 70% relative humidity (except for desiccation resistance) with a 12:12 light-dark cycle. Extra individuals from the larvae reared to replace adults in the population cages provided the parents of the experimental flies. After eclosion, the flies were transferred to 10- ml vials containing 20 females and 20 males each and allowed to mate for five days. This standardization should have removed parental effects that could arise in the two mortality regimes. Only mated females were measured. After eclosion each female was put into a new vial together with two males and randomly assigned to one of the experiments to determine fat content, desiccation or starvation resistance, and the number of ovarioles.

Age and Size at Eclosion

To measure differences in developmental time between females from the HAM and the LAM regimes, 21 vials per replicate were established containing 25 larvae each. The eggs from which the larvae hatched had been collected within a 3-h interval. Once all the larvae had pupated, the vials were placed in a hatching machine in which flies were collected at 2-h intervals. The newly eclosed females were weighed to 0.01 mg 12–24 h after eclosion.

Fecundity

Vials were established with one female and two males. The number of eggs laid was counted daily from the first day after eclosion until the female died in an experiment that also measured fat content, ovariole number, or desiccation/starvation resistance. The sample size of each replicate was thus reduced from about 150 vials at day 15 to about 30 at day 30 when the last females were killed. The laying surface was replaced daily by a new one with a drop of fresh yeast. Dead males were removed and replaced by a new male of the same age.

Number of Ovarioles

Between 15 and 20 females were haphazardly chosen for dissection from the fecundity assay twice, at day 17 and at day 30. The ovaries were dissected in 10% ringer solution and stained in a saturated solution of potassium dichromate for at least one day, followed by careful dissection of the ovarioles (Thomas-Orillard 1984). We counted the ovarioles of both ovaries, the number of functional ovarioles, judged by the presence of developed eggs, and the number of completely developed eggs.

Starvation Resistance

At day 16 and at day 29, 15 females were transferred from the fecundity assay together with two males. Starvation resistance was measured as total survival time in vials containing only agar to prevent desiccation. The vials were scored for dead flies twice a day, dead males were replaced by reserve males, and survival time was measured as the midpoint between the last two observations.

Desiccation Resistance

Fifteen females per population were transferred twice, at day 16 and at day 29, into vials that contained silica gel, which reduced the relative humidity to about 30%. The vials were scored every hour until the last female died. Desiccation resistance was measured as the midpoint between the last two observations.

Relative Fat Content

Fat content was measured at day 17 and day 30 on females from the fecundity assay. Fat content of females was also measured after desiccation and after starvation in the day 16 sample. Each replicate consisted of five flies in an Eppendorf tube, and for each of the 30 experimental units (experiment × age class × line[treatment]) five replicates were measured (sample size was smaller for some groups late in life). Samples were dried at 50°C for 12 h and weighed to 0.01 mg to obtain the dry weight. Fat was extracted overnight with diethyl ether at room temperature, and the samples were redried for 4 h at 50°C. The adult fat weight was determined as the difference in weight before and after ether extraction. The relative fat content is given by the ratio of fat weight to dry weight.

Respirometry and Activity

Metabolic rate and activity of the HAM and LAM populations were measured about three months later, in July 1997. The flies were transported from Basel to Seattle and cultivated for one generation on standard Basel medium before measurements were taken. Methods for measuring metabolic rate in small insects are given in Lighton (1993) and Berrigan and Lighton (1994). We used a Sable Systems (Henderson, NV) TR-3 respirometry apparatus consisting of a Licor 6262 infrared CO_2 detector together with a computer controlled baseline device, flow controller, and A/D board. Flies were measured in groups of 20, larvae in groups of 15. Before measuring the flies, the empty vial containing food medium without fresh yeast was measured to give a baseline metabolic rate of the vials (CO_2 production of empty vials was very near zero in all cases). Flies or larvae were transferred to the vials and allowed to equilibrate for 2 h, after which the CO_2 output was recorded for 40 min. CO_2 outputs were calculated according to Lighton (1993). Metabolic rates are reported as ml CO_2 $g^{-1}hr^{-1}$. After the metabolic rate had been recorded, the flies were dried overnight at 60°C and weighed to the nearest 0.001 mg.

To measure the metabolic rate of larvae, 25 larvae were collected 28 h after egg laying. Two days later, 15 of these larvae were transferred to a new vial whose CO_2 output had been measured before. After equilibrating for 2 h, the CO_2 output was recorded. Six replicate vials from each of the six selection lines were measured. To measure the metabolic rate of adult flies, three vials per line were established containing 75 larvae and 6 ml of medium each. Twelve days later, the flies were sexed and six replicates per line and sex were established containing 20 flies each. At day 13 from birth CO_2, output was recorded. To measure the CO_2 production of older flies, the flies were grown in the same way as the young flies. After eclosion the flies were transferred to new vials every third day. One day before the measurements, the sexes were separated, and the CO_2 output was recorded on six replicate vials per line and sex at day 29 from egg laying.

The activity of adult flies was measured on flies that were the same age as the adult flies in the respirometry experiments. Flies were put individually into 5.5-cm- long glass tubes and the number of times the fly tripped an infrared sensor in 17.4 min was recorded for 10 replicate individuals per line, age, and sex.

Correlations between Traits

We used two criteria to detect broad-sense genetic correlations between life- history traits. The first method relies on differences between the two different selection regimes. Two traits were correlated if the two treatments differed significantly in both traits. This method may fail to detect a linkage between traits if differences are stronger between lines than between treatments. The second method relies on differences between lines. Line means of trait values were taken as independent observations, and broad-sense genetic correlations between pairs of traits were calculated as the correlation coefficient. With six independent selection lines, an $r^2 > 0.66$ is required in a Fisher r to Z significance test for significance at the $P < 0.05$ level.

Statistical Analysis

The responses to selection on adult mortality rate were tested for significance in analyses of variance (ANOVAs) and analyses of covariance (ANCOVAs). Significance of treatment effects was calculated with the mean square for replicates nested within treatments as the denominator for the F-ratio. Data were transformed to the natural logarithm prior to the analyses; percentages were arcsine transformed. To correct for differences in traits that were due to differences rearing densities, viability was taken as a covariate. All statistical work was done using SuperANOVA (Abacus Concepts, Berkeley, CA) and StatView (SAS Institute, Cary, NC) on Macintosh computers.

RESULTS

Table 1 summarizes the responses to experimental evolution under high (HAM) and low (LAM) adult mortality regimes.

Life-History Traits

The differences in adult mortality had no consistent effects on viability (survival from first-instar larvae to adult). Large differences were detected among lines ($P < 0.0001$), with viability ranging from 60 % to 84% (Table 2, Appendix). To correct for the confounding effects of different rearing densities, viability of the larvae was used as a covariate in analyses of other traits. Treatments differed significantly in age and size at eclosion in an ANCOVA with viability of larvae as a covariate. Females from the low adult mortality regime eclosed later ($P < 0.05$) and were heavier ($P < 0.05$) than HAM females (Fig. 1, Table 2, Table 3).

Fecundity was measured during the first two weeks of adult life (days 14 to 28 from egg). Fecundity data of females that

TABLE 1. Genetic correlations between traits. Correlations were calculated with the six lines as independent observations. Only traits that were significantly correlated to at least one other trait are displayed. Different measurements of body size (e.g., fresh weight, dry weight, fat weight) were generally positively correlated, which allowed us to simplify the table, using dry weight at eclosion as the sole measure of body size. Correlations that were significant at the $P < 0.05$ level are in bold letters, the critical values for significance are $|r| > 0.9$ for $P < 0.01$, $|r| > 0.81$ for $P < 0.05$, and $|r| > 0.74$ for $P < 0.1$. In traits 2–9 significant differences between the HAM and LAM treatments were detected. MSM, mass-specific metabolic rate.

Trait	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Viability	1	**−0.9**				**−0.82**										
Body size (dry weight)	2	**−0.9**	−0.77			**0.92**	**0.88**	**0.93**	**−0.97**							
Larval size (after 48 h)	3	−0.77		**−0.92**			**−0.96**									
Development time	4		**−0.92**				**0.87**									
Early fecundity	5									**0.85**	**−0.91**		**0.94**			
No. ovarioles, early	6	**−0.82**	**0.92**					0.76	**−0.83**							
No. ovarioles, late	7		**0.88**	**−0.96**	**0.87**											
Desiccation time, early	8		**0.93**			0.76										
Rel. fat content after starvation, early	9		**−0.97**			**−0.83**								−0.78	**−0.92**	**−0.86**
Starvation time, late	10				**0.85**						−0.77		**0.92**		**0.85**	**0.86**
Late fecundity	11				**−0.91**					−0.77		−0.8	**−0.85**			
Desiccation time, late	12										−0.8					
Rel. fat content, late	13				**0.94**					**0.92**	**−0.85**					
Rel. fat content after desiccation, early	14								−0.78						**0.81**	**−0.9**
Rel. fat content after desiccation, late	15								**−0.92**	**0.85**				**0.81**		**−0.97**
MSM, late	16								**0.86**	**−0.86**				**−0.9**	**−0.97**	

had eclosed from the same vial were pooled over three-day intervals and analyzed in an ANCOVA with viability of larvae as covariate. HAM females laid significantly more eggs than LAM females during the first three-day interval ($P < 0.05$); line effects were not significant (Table 1, Appendix). Differences between treatments in early fecundity were partly compensated by differences in larval viability. When the covariate viability was removed, there was no longer a significant difference in fecundity. Part of the difference between treatments was also caused by the faster development time of HAM females. At day 14 from egg, most LAM females had not yet started to lay eggs. When early fecundity was defined as the mean number of eggs laid during the first three days of adult life, which was a different age class for LAM than HAM, there was no longer a significant difference, but HAM females still had a higher early fecundity per mg fresh weight. No significant fecundity differences between treatments were detected in older females, but there were weak line effects ($P = 0.085$; Table 2, Fig. 2, Appendix)

Stress Resistance Traits

Evolutionary responses to adult mortality were not reflected in starvation resistance. Survival time under starvation declined significantly from about 98 h in young females (16 days from eggs) to 88 h in older females (29 days from egg; $P < 0.05$). Differences in starvation resistance between lines were weak for young females ($P = 0.106$), but stronger in older flies ($P < 0.05$), with replicate populations differing by up to 33 h (Table 2, Appendix).

Females from the LAM regime had higher desiccation resistance early in life (Table 2, Fig. 3, $P < 0.005$). Two weeks later, flies from the two treatments no longer differed in desiccation resistance; both had lower resistance than younger flies. The reduction in desiccation resistance in older flies was stronger in LAM females, and whereas the treatment effect was stronger than differences between replicate populations early in life, the opposite was true for older flies. The greater desiccation resistance of young LAM females was only partly attributable to their larger body size; incorporation of fresh weight into the analysis as a covariate did not change the results (LAM > HAM; $P < 0.05$).

Physiological and Morphological Traits

Ovariole number was determined as the total number of ovarioles in both ovaries. Empty ovarioles that apparently did not produce any mature eggs were scored as nonfunctional (Fig. 4). HAM females produced 20% more eggs per ovariole early in life ($P < 0.01$) and 16% more late in life ($P < 0.05$) than LAM females. LAM females had more ovarioles both at day 3 ($P < 0.1$) and day 17 of adult life ($P < 0.05$; Table 2) because of their larger size; when fresh weight was used as a covariate, the difference between HAM and LAM flies disappeared. The proportion of reduced ovarioles was higher in young LAM females ($P < 0.05$; Table 2), an effect that did not depend on fresh weight. Whereas effects of treatment on ovariole number were generally stronger than line effects, line effects on the number of reduced ovarioles were strong, especially in older flies (Table 2, Appendix). Compared to younger flies, older flies had more reduced ovar-

TABLE 2. Summary of the analyses and mean values with standard errors of the traits from the two treatments. Data were transformed to natural logarithms prior to analysis; percentages were arcsine transformed. One degree of freedom for treatment, four for line effects. Only females were considered. Early refers to traits measured during the first five days of adult life (days 14–17 after hatching from the egg); late traits were measured at days 15–18 of adult life (days 27–30 after hatching). MSM, mass-specific metabolic rate.

Trait	*n*	HAM	LAM	MS MS treatment	MS MS lines	MS MS residual	*P*-value: Treatment/lines MS	*P*-value: Lines/residuals MS
Life-history traits								
Viability (%)	1087	80 ± 0.5	74 ± 0.7	0.26	0.31	0.029	0.4117	0.0001
Development time (h)	1087	294 ± 0.5	315 ± 0.8	0.113	0.006	0.0004	0.0112	0.0001
Fresh-weight (μg)	1087	720 ± 10	820 ± 10	0.427	0.047	0.004	0.0391	0.0001
Early fecundity (no./eggs)	931	28.4 ± 0.6	26.4 ± 0.8	1.939	0.151	0.109	0.0232	0.2437
Late fecundity (no./eggs)	312	28.5 ± 1.5	30.9 ± 1.5	0.161	0.388	0.184	0.5541	0.0853
Stress resistance traits								
Starvation time, early (h)	108	99 ± 3.8	97 ± 3.4	0.031	0.156	0.08	0.6802	0.1057
Starvation time, late (h)	96	91 ± 4.0	85 ± 4.5	0.254	0.399	0.13	0.4695	0.0202
Desiccation time, early (min)	105	595 ± 18	721 ± 19	1.066	0.03	0.054	0.004	0.6955
Desiccation time, late (min)	105	514 ± 19	506 ± 18	0.001	0.288	0.064	0.9482	0.0023
Morphological and physiological traits								
No. ovarioles, early	100	33.6 ± 0.5	35.0 ± 0.6	0.033	0.006	0.012	0.0839	0.7065
No. ovarioles, late	93	32.8 ± 0.5	34.3 ± 0.5	0.037	0.004	0.011	0.0338	0.845
No. functional, early	100	31.4 ± 0.7	29.3 ± 0.8	0.116	0.093	0.046	0.326	0.097
No. functional, late	93	28.5 ± 1.0	25.6 ± 1.2	0.381	0.19	0.165	0.2291	0.3376
No. nonfunctional, early	100	2.2 ± 0.6	5.7 ± 1.0	378.2	54.66	23.23	0.0582	0.0596
No. nonfunctional, late	93	4.3 ± 1.1	8.7 ± 1.1	16.082	4.197	1.104	0.1219	0.0068
Dry weight, larvae (μg)	42	166 ± 9.0	128 ± 9.0	0.819	0.087	0.08	0.0029	0.3817
Dry weight, early (μg)	150	360 ± 5.0	393 ± 8.0	0.054	0.005	0.004	0.0294	0.3694
Dry weight, late (μg)	106	284 ± 9.0	345 ± 6.0	0.061	0.026	0.004	0.203	0.0009
Dry weight desiccation, early (μg)	105	319 ± 4.0	357 ± 8.0	0.202	0.01	0.005	0.0115	0.104
Dry weight desiccation, late (μg)	105	242 ± 5.0	285 ± 6.0	0.206	0.016	0.005	0.0235	0.0258
Dry weight starvation, early (μg)	108	256 ± 6.0	254 ± 6.0	0.001	0.027	0.007	0.8581	0.0141
Residual weight, early (μg)	150	298 ± 4.0	321 ± 8.0	0.04	0.006	0.006	0.0594	0.4206
Residual weight, late (μg)	106	222 ± 6.0	276 ± 5.0	0.087	0.01	0.005	0.0445	0.1238
Residual weight desiccation, early (μg)	105	277 ± 4.0	314 ± 7.0	0.206	0.012	0.005	0.0141	0.0865
Residual weight desiccation, late (μg)	105	202 ± 5.0	236 ± 5.0	0.18	0.008	0.008	0.009	0.4191
Residual weight starvation, early (μg)	108	235 ± 6.0	234 ± 5.0	0.0002	0.03	0.007	0.9399	0.0067
Fat weight, early (μg)	150	62 ± 2.0	72 ± 2.0	0.151	0.017	0.011	0.0412	0.2031
Fat weight, late (μg)	106	59 ± 3.0	68 ± 4.0	0.031	0.091	0.032	0.5931	0.0518
Fat weight desiccation, early (μg)	105	28.0 ± 1.0	32.0 ± 2.0	0.155	0.01	0.049	0.0184	0.9284
Fat weight desiccation, late (μg)	105	40 ± 5.0	50 ± 5.0	0.543	0.458	0.183	0.3373	0.0693
Fat weight starvation, early (μg)	108	21.0 ± 0.5	20.0 ± 1.0	0.064	0.036	0.027	0.2537	0.2821
Relative fat content, early	150	17.3 ± 0.5	18.3 ± 0.5	0.001	0.001	0.001	0.25	0.287
Relative fat content, late	106	20.9 ± 1.0	19.6 ± 0.9	0.0003	0.003	0.002	0.7482	0.1949
Relative fat content, desiccation, early	105	9.3 ± 0.4	9.1 ± 0.5	0.0001	0.0004	0.001	0.7048	0.8272
Relative fat content, desiccation, late	105	16.2 ± 1.9	17.3 ± 1.5	0.002	0.014	0.007	0.709	0.1278
Relative fat content, starvation, early	108	8.3 ± 0.2	7.7 ± 0.3	0.001	0.001	0.0003	0.3324	0.0556
MSM	42	5.1 ± 0.3	4.1 ± 0.3	0.004	0.035	0.016	0.7638	0.0878
MSM, early	36	16.5 ± 0.5	15.4 ± 0.6	0.046	0.041	0.017	0.3519	0.069
MSM, late	36	11.6 ± 0.2	11.5 ± 0.2	0.001	0.01	0.007	0.7643	0.2152
Activity, early (counts)	60	42.6 ± 4.3	42.3 ± 4.3	0.06	2.203	0.413	0.8765	0.0011
Activity, late (counts)	58	22.4 ± 2.5	24.2 ± 3.0	0.282	1.426	0.773	0.6797	0.1345

ioles and fewer functional ovarioles ($P < 0.005$ for both traits). Older females had significantly fewer eggs stored in their ovaries than young females. There was a weak positive correlation across individuals between the number of functional ovarioles and early fecundity ($r = 0.24$; $P < 0.05$).

Selection did not influence the pattern of fat allocation. Young LAM females had higher dry weights ($P < 0.05$), fat weights ($P < 0.05$), and residual weights (dry weight after fat extraction; $P = 0.059$) than young HAM females, but there was no difference in relative fat content (Table 2). Compared to flies three days from eclosion, flies two weeks older had lower dry weight and residual dry weight ($P < 0.01$ for both traits), but the weight of the fat fraction remained constant and relative fat content increased from 18% to 20%. No differences in dry weight and fat weight were detected between treatments in old flies, but line effects were significant for both traits (Table 2, Appendix).

The composition of mated females was also determined after desiccation and starvation (Fig. 5). Desiccation reduced both the fat weight and the residual weight after fat extraction. The weight of the fat fraction was reduced by almost 60% in young and 30% in older flies; the residual weight was less affected, with only 2% weight loss in young and 16% weight loss in older flies. Both HAM and LAM flies responded similarly; after desiccation LAM flies still had greater fat and residual weight. Starvation yielded different results. Starved LAM flies lost more fat weight (72% vs. 63%) and more residual weight (27% vs. 21%) than did HAM flies. After starvation mated females from the two selection regimes no longer differed in their weight components, but there were

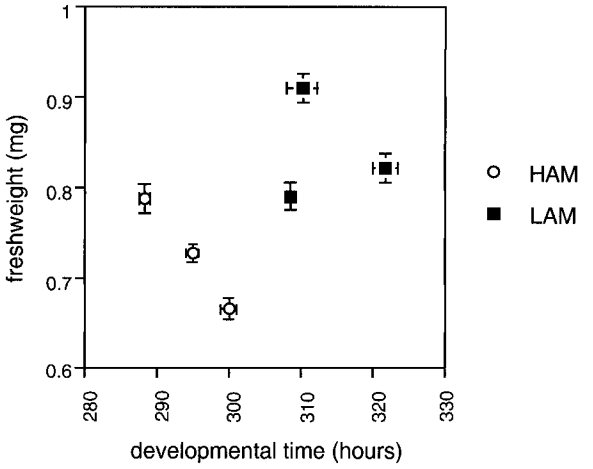


FIG. 1. Age and size at eclosion of the three replicate populations of the two treatments. Bars indicate standard errors.

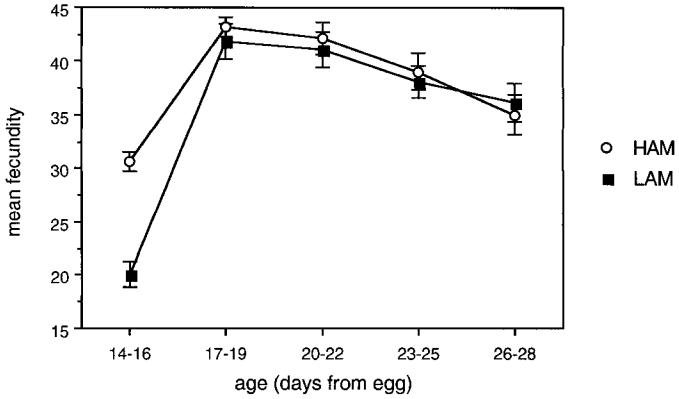


FIG. 2. Female fecundity calculated as the mean number of eggs laid during three consecutive days for the first two weeks of adult life. Bars indicate standard errors.

significant line effects for dry weight, residual weight, and relative fat content (Table 2, Appendix).

The mortality treatments had no consistent effect on metabolic rates and activity (Fig. 6). In larvae the mass-specific metabolic (MSM) rates did not differ between treatments, but there was weak evidence for line effects ($P = 0.088$). In young adults the MSM rates also did not differ between treatments. Males had a higher MSM than females, and there was some indication of a treatment × sex effect ($P < 0.1$), with LAM females showing lower MSM rates than HAM females; the males were virtually identical. In older adults the MSM rates were lower than in young flies; there was no evidence of a treatment effect. As in young flies, the MSM rate was greater in males than in females.

The treatments had no effects on the activity of either young or old adult flies. Young flies were more active than old flies, and males were more active than females. In young flies, line effects were significant ($P < 0.05$). In old flies there was a weakly significant treatment × sex effect ($P < 0.1$): LAM males were more active than HAM males; females were similar.

The dry weights of adult flies measured in Seattle were similar to the dry weights measured in Basel three months earlier. In young and old flies females were heavier than males, and LAM flies were heavier than HAM flies. The correlation between Basel and Seattle of the female dry weight of the six lines was 0.917 ($P < 0.05$) for young and 0.950 ($P < 0.01$) for old females. At least for dry weight, genotype × laboratory interactions were not important. HAM larvae were larger than LAM larvae 48 h after hatching. This result is surprising because LAM adults are larger than HAM adults. HAM flies seem to have evolved more rapid development, albeit to smaller size, by changing their growth trajectories (Table 3).

Table 3 summarizes the effects of differences in adult mortality rates. HAM and LAM females diverged in many traits,

TABLE 3. Summary overview of the effects of different adult mortality regimes on life-history, physiological, and stress resistance traits. Results for mated females only (except for body size of larvae). In some cases several traits were condensed, e.g., body size, where different measures, fresh weight, dry weight, fat weight, and residual weight gave very similar results, and ovariole number, where HAM females had fewer ovarioles than LAM females both early and late in life. The symbols < and > stand for significant differences at the $P < 0.05$ level; · means no significant difference between treatments. MSM, mass-specific metabolic rate.

Trait	Effect of experimental evolution	Difference HAM vs. LAM young females/ old females
Viability	HAM · LAM	+9%
Body size (dry weight)	HAM < LAM	−13%
Larval size (after 48 h)	HAM > LAM	+23%
Development time	HAM < LAM	−7%
Early fecundity	HAM > LAM	+8%
Late fecundity	HAM · LAM	−9%
No. ovarioles	HAM < LAM	−4%
No. functional ovarioles	HAM · LAM	+6%/+10%
No. reduced ovarioles	HAM < LAM	−160%/−102%
Starvation time	HAM · LAM	+4%/+8%
Desiccation time, early	HAM < LAM	−21%
Desiccation time, late	HAM · LAM	+1%
Relative fat content	HAM · LAM	−6%/+9%
MSM, early	HAM > LAM	+7%
MSM, late	HAM · LAM	+1%
Activity	HAM · LAM	−1%/+7%

* Significant at the $P < 0.1$ level.

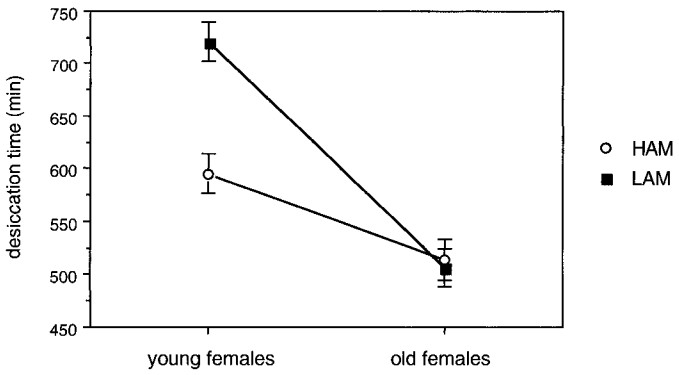


FIG. 3. Desiccation resistance of young (16 days from egg) and old (29 days from egg) mated females from the two selection regimes. The strong difference between treatments in young flies disappears completely as the flies age. Bars indicate standard errors.

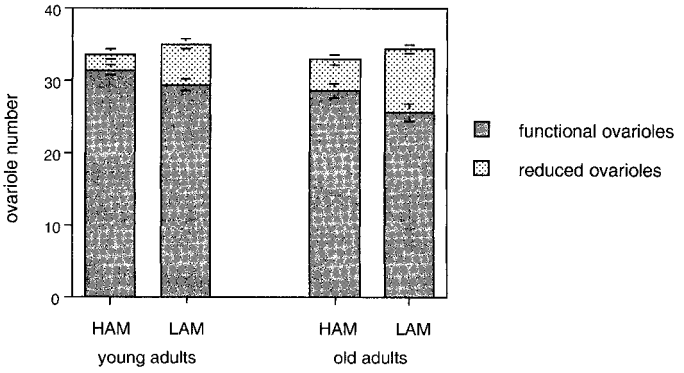


FIG. 4. Ovariole number and the number of functional ovarioles in young (17 days from egg) and old (30 days from egg) adult females. Bars indicate standard errors.

especially in those expressed early in life, from egg deposition until about day 17 from egg, corresponding to days 3 to 6 of adult life, but not in viability, starvation resistance, fat content, and activity. The strongest divergence was detected in body size. Two-day-old HAM larvae were 23% larger than LAM larvae. In young adult females the size difference was reversed: HAM flies were lighter than LAM flies by about 13%.

Broad-Sense Genetic Correlations between Traits

Broad-sense genetic correlations between traits were calculated as the pairwise correlation coefficient between two traits with the six populations as independent observations (Table 1). Therefore part of the correlations reported reflects the responses of the two traits to the mortality regimes. This was the case for correlations among traits 2 to 9 (see Table 1) where HAM and LAM populations differed significantly in both individual traits; 12 of the 27 correlations reported fell into this category.

Most significant correlations were with body size. The two other morphological traits, ovariole number and size of 48-h-old larvae, were both linked to adult body size, but body size of adults and growth rates of larvae were inversely correlated. Desiccation resistance of young adults was positively correlated with body size, as expected because heavier flies have more stored water and a smaller surface:volume ratio. In older females there was no correlation between body size and desiccation resistance.

Significant correlations were also detected between traits in which the treatments did not differ but lines did (traits 1 and 10 to 16, see Table 1). Most of these involved late-life traits that connected stress resistance to reproduction and fat allocation. A strong trade-off was detected between early and late fecundity. Females that had high fecundity early in life had low fecundity late in life, lower metabolic rates, and a better resistance to starvation. Flies that laid fewer eggs late in life had higher desiccation and starvation resistance. The amount of stored fat largely explained time to starvation, but the physiological basis for tolerating starvation and desiccation appeared to differ. Lines that mobilized much fat during starvation used little fat during desiccation, as can be seen by the inverse relation of the relative fat contents after starvation and after desiccation.

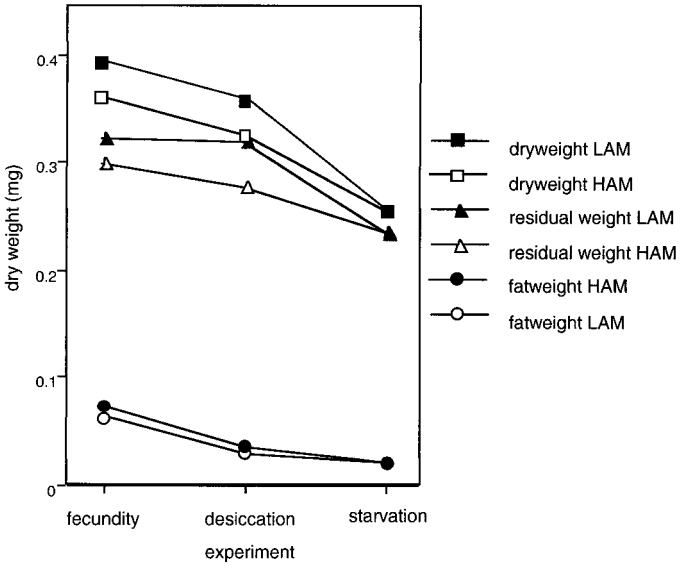


FIG. 5. Weight components of young mated females (16–17 days from egg) in the original population and after desiccation and starvation. Bars indicate standard errors.

DISCUSSION

To predict evolutionary changes in response to differences in age specific mortality rates, life-history theory needs details about the trade-offs that constrain evolutionary change. Despite much work on *D. melanogaster* in evolutionary research, we do not yet know what trade-offs to expect under which environmental conditions. To understand trade-offs, we must learn more about their genetic, physiological, and developmental causes. This study makes three main points. First, it confirms the predictions that increased adult mortality leads to higher fecundity early in life and to the faster development time that makes higher early fecundity possible in this experiment. Among those who have gotten similar results are Reznick et al. (1990, 1996), working with guppies; Sparkes (1996) and Wellborn (1994), working with isopods; and Stibor (1992), working with the

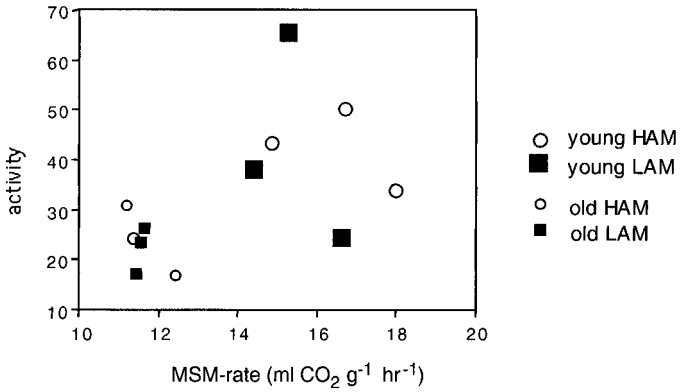


FIG. 6. Relation between dry mass and mass-specific metabolic rates (MSMs) of young (13 days from egg) and old (29 days from egg) adult females.

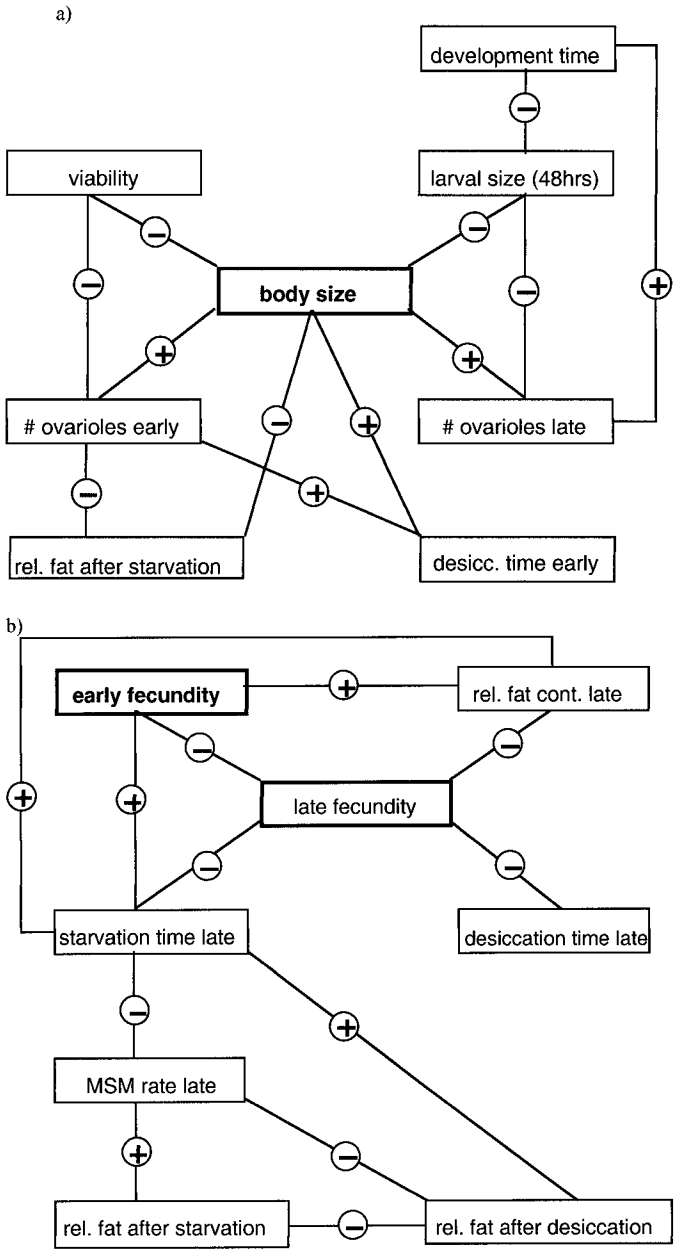


FIG. 7. Overview of significant broad-sense genetic correlations detected among traits. Traits that show a significant correlation are connected by lines with a sign representing the sign of the correlation. Correlations fall into two distinct classes: (a) traits that are correlated with body size/development time or (b) traits that depend on the reproductive schedule.

plastic life-history responses of *Daphnia* to predators. Second, we describe the traits that evolved in response to changes in adult mortality, thereby suggesting where trade-offs are, and are not, to be found. Differences in adult mortality rates had little or no impact on body composition, viability, metabolic rate, activity, starvation resistance, or desiccation resistance, but did affect body weight, development time, growth rate, early fecundity, and the number of ovarioles. Third, we suggest possible causes for trade-offs. Trade-offs between early fecundity, late fecundity, and starvation resistance appear to be mediated by the differential allocation of lipids, whereas positive correlations between body size, ovariole number, and desiccation resistance can be explained by allometric relations.

Confirmation of Theoretical Predictions

Differences in adult mortality agree with predictions of the reproductive effort model. Flies that evolved under high adult mortality had a 7% shorter development time and laid 8% more eggs early in life. No significant fecundity difference was detected in older flies. Because fitness is more sensitive to early than to late fecundity, a stronger response in early fecundity is expected (Hamilton 1966). Old HAM females produced 8% fewer eggs than old LAM females, but this difference was obscured by strong line effects. Late fecundity also depended strongly on other traits, as discussed below.

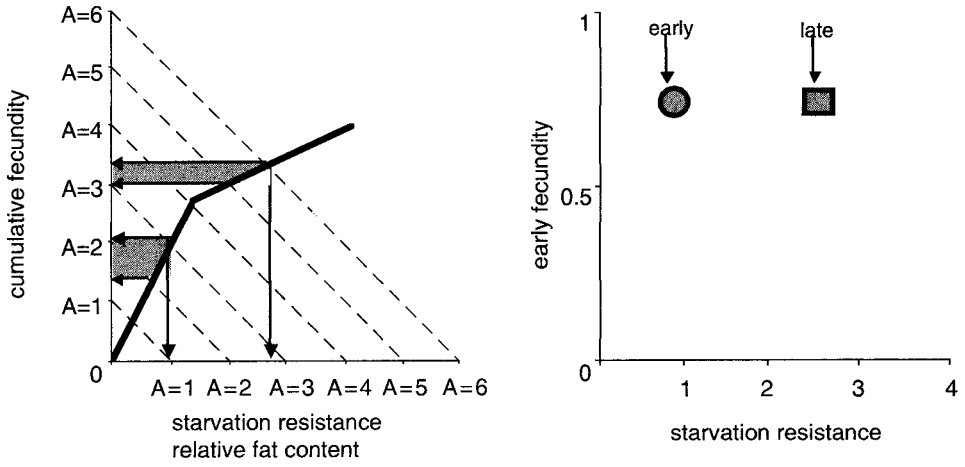
Mortality selection can be imposed in two ways. Here we imposed age-dependent selection. In a related experiment, Barclay and Gregory (1982) imposed stage-dependent selection, that is, conditions changed at eclosion, at whatever age eclosion might occur, rather than on day 14 of life. As predicted by a modification of the reproductive effort model (Roff 1986), Barclay and Gregory found that higher adult mortality significantly increased development time, rather than decreasing it, as we found. This suggests that increased mortality leads to a greater acquisition of resources by larvae under stage-dependent than age-dependent mortality selection.

Responses to Experimental Evolution: the Nature of Trade-Offs

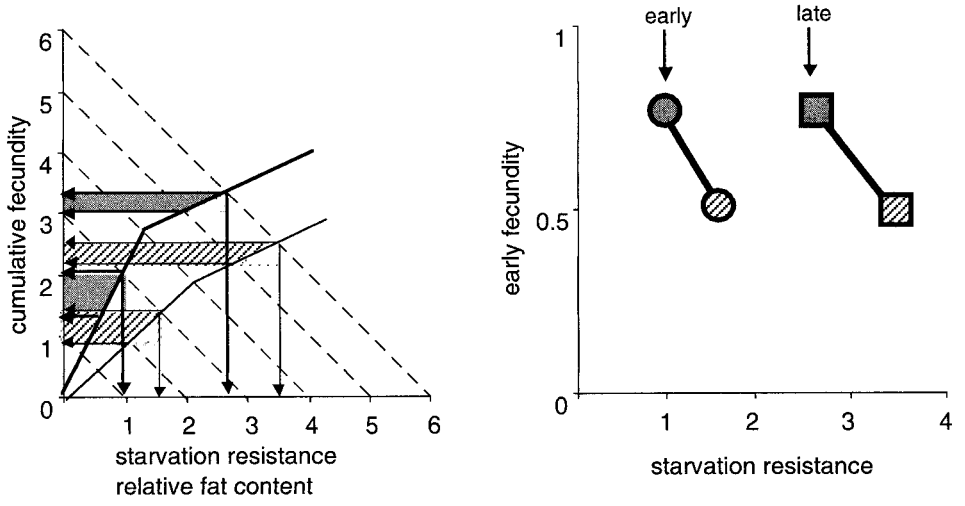
In addition to direct effects on development time and early fecundity, differences in adult mortality affected many other traits expressed early in life (Table 2). Effects on body size and developmental time were strong. Positive genetic correlations between body size and development time have been found in several selection experiments (Hillesheim and Stearns 1991; Partridge and Fowler 1993; Zwaan et al. 1995a), and these two traits may be so tightly connected by physiology and development that it is impossible to select one independent of the other (Lints and Gruwez 1972). Thus, it is surprising that HAM larvae grow faster, as seen by their larger size two days after egg deposition. We have not weighed the contributions to this size difference of a faster growth rate, earlier hatching, or difference in egg size.

The ovarioles in *D. melanogaster* are a permanent morphological structure that are costly to produce, may mediate the trade-off between fecundity and survival, show considerable genetic variation (Thomas-Orillard and Jeune 1984), are sometimes positively associated with female fecundity (David 1970; Boulétreau-Merle et al. 1982), and have been used to study the maintenance of quantitative genetic variation (Wayne et al. 1997). In this study LAM females had 4% more ovarioles than HAM females; line effects were not significant. In contrast to David (1970) and Boulétreau-Merle et al. (1982), but in agreement with Wayne et al. (1997), no association was found between ovariole number and fecundity. Ovariole number depended strongly on body size, as in some studies (Lemeunier et al. 1986; Berrigan 1991), but not others (Robertson 1957; Wayne et al. 1997). One factor that confounds associations of ovariole number with fecundity

a)


A=6
A=5
A=4
A=3
A=2
A=1
0
cumulative fecundity
A=1 A=2 A=3 A=4 A=5 A=6
starvation resistance
relative fat content
1
0.5
0
early fecundity
early
late
1 2 3 4
starvation resistance

b)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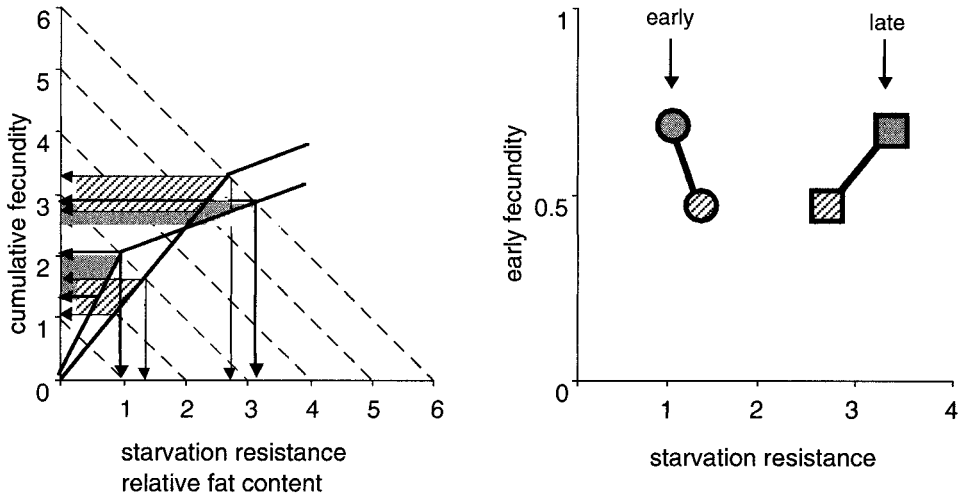
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and body size is the variation depending on line and age of the females in proportion of supernumerary ovarioles: between 4% and 34% of the ovarioles were reduced to an empty tube and clearly produced no eggs (Fig. 3). Although the number of ovarioles might set an upper limit for egg production under optimal conditions, this limit is not be reached in many situations. Further studies would be needed to determine the costs of supernumerary ovarioles and the conditions under which they are produced.

Because treatments affected dry weight, fat weight, and fat-free weight in similar ways, we conclude that the body composition of the flies was not changed by the treatments. These results corroborate those of Zwaan et al. (1995a) and suggest that selection worked to a large extent by shifting development time.

Broad-Sense Genetic Correlations

Figure 7 gives an overview of genetic correlations between traits detected among lines by lumping treatments. Traits that showed a significant correlation are connected by lines with a sign representing the sign of the correlation (cf. Table 1). Traits can be divided into two classes characterized by significant correlations of traits within one class and the absence of significant correlations with traits in the other class. One class contains traits that are correlated with body size; the other contains traits that depend on the reproductive schedule. Treatments differed significantly in most traits correlated with body size (Table 3).

Positive genetic correlations between body size, development time, and desiccation resistance have been found in several selection experiments (e.g., Zwaan et al. 1995b). More intriguing was the observation of negative genetic correlations of body size and development time with growth rate (larval size after 48 h) and viability. Negative genetic correlations between development time/body size and viability in *Drosophila* were reported by Partridge and Fowler (1993). In contrast, Chippindale et al. (1994) reported a positive correlation between development time and viability.

Traits that describe the reproductive schedule, the allocation of resources, and resistance to stress showed strong interdependence (Fig. 7a). Most models of life-history theory assume trade-offs between current and future reproduction, and the trade-off among lines between early and late fecundity found here corroborates earlier findings (Taylor and Condra 1980; Luckinbill et al. 1984; Rose 1984; Mueller 1987) and confirms the assumptions of theoretical models.

Causes of Trade-Offs

Figure 7a lists traits that are correlated with body size. The smaller body size of HAM flies followed from their shorter development time, the trait most directly influenced by the mortality regime. At day 15 from birth, HAM flies have their first—and for 90% of the population their only—chance to reproduce in the selection regime. The only way to achieve high fertility this early in life was to shorten development time, which could be partly but not fully compensated with faster growth, leading to a smaller body size (Zwaan et al. 1995b). Two traits, ovariole number and desiccation resistance, were positively correlated with body size, as in other studies (e.g., Zwaan et al. 1995b).

Desiccation and starvation are very important environmental stresses for small insects like *D. melanogaster*. Did experimental evolution affect these stress resistance traits, and if so, did we find a parallel response in both traits? The answer is no. The amount of stored fat had a strong impact on the time a fly could survive starving conditions (Fig. 7b), as Service (1987) also found. Two other traits, late fecundity and metabolic rate, reduced starvation resistance. This is not surprising because metabolically or reproductively more active flies use up their reserves faster. Additionally, the amount of fat that could be mobilized to survive starvation seemed to be reduced in more active flies, as suggested by the positive correlation between MSM rate and relative fat content after starvation.

Desiccation resistance depended on body size early in life and traded off with fecundity in old females. Whereas the first effect can be explained by the smaller surface:volume ratio of larger flies, the latter effect could be partly mediated by a higher mass-specific metabolic (MSM) rate in reproductively active females. Evidence for a positive correlation of the two traits is indirect: Young HAM flies had both a higher MSM rate and a higher fecundity, but a lower desiccation time than did LAM flies. In spite of the negative correlations of both starvation and desiccation resistance with

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FIG. 8. Illustration of trade-offs between early fecundity and starvation resistance that can arise due to differential allocation of lipids across six age classes (A1–A6). Our assumptions are: (1) a fixed amount of lipids (A) can be metabolised in each age class; (2) fecundity and starvation resistance are connected through a physiological trade-off such that A = fecundity + starvation resistance; the fraction of fat allocated to reproduction is determined by the slope of the allocation function (bold line); and (3) lipids that are allocated to reproduction are completely metabolized; lipids that are allocated to starvation resistance are stored, thereby increasing the relative fat content, and are no longer available for reproduction. (a) Illustration of how trait values for early fecundity and starvation resistance (right part of the figure) can be deduced from the allocation function (left). Cumulative fecundity at a certain age is given by projecting the allocation function at that age to the y-axis (horizontal arrows). Early fecundity is defined here as fecundity from age class 2 to 3 (early fecundity = cumulative fecundity [3] − cumulative fecundity [2]). Late fecundity (age class 5 to 6) is calculated by the same method. Starvation resistance is given by projecting the allocation function at a given age to the x-axis (vertical arrows).

Genetic variation in the functions that determine the allocation of lipids to reproduction or starvation resistance lead to genetic correlations between the traits.

(b) Two genotypes differ in the initial slope of the allocation function. This leads to trade-offs between early fecundity and starvation resistance both early and late in life (right). (c) The allocation functions of two genotypes intersect each other. The correlation between early fecundity and starvation resistance switches from negative to positive at the age where the two allocation functions intersect. This results in trade-offs between early fecundity and starvation resistance (right), between early fecundity and late fecundity, and in a positive correlation of early fecundity with starvation resistance late in life.

late fecundity, the physiological mechanisms that confer resistance seem to be different. Whereas starvation time depends both on the amount of fat that is stored and on the amount that can be mobilized, fat reserves were not important for determining resistance to desiccation, a result consistent with that of Gibbs et al. (1997), who concluded that metabolic water has no strong impact on resistance against desiccation.

We found positive correlations between early fecundity, relative fat content, and starvation resistance, and trade-offs between relative fat content/starvation resistance and late fecundity. Both results seem to contradict earlier findings. Trade-offs between early fecundity and starvation resistance/relative fat content early in life have been found in selection experiments on late fecundity (Service and Rose 1985; Service et al. 1985, 1988) and on starvation resistance (Rose et al. 1992; Leroi et al. 1994c). Here we present a model to explain why the contradiction between our results and previous work is only apparent.

An Allocation Model

We suggest that the physiological mechanisms that allocate lipids between storage and reproduction correspond to a simple Y model (Sheridan and Barker 1974; van Nordwijk and de Jong 1986). This model can predict the contrasting results of our study and previous work. That such mechanisms of allocation exist is supported by the observation that the female fat body is a source of yolk protein synthesis (Postlethwait and Sirk 1981). In the model, we make the following assumptions: (1) at each age a fixed maximal amount of lipids (A) can be metabolized; (2) fecundity and starvation resistance are connected through a physiological trade-off such that A = fecundity + starvation resistance; and (3) lipids allocated to reproduction are completely metabolized; lipids allocated to starvation resistance are stored, increasing relative fat content, and are no longer available for reproduction. This third assumption simplifies the model and is not strictly necessary. Whenever stored lipids can be more efficiently reinvested to increase starvation resistance than to produce more eggs, trade-offs may evolve. This appears realistic because stored fat does not limit egg production under some environmental conditions, but is always limiting under starvation.

Figure 8 shows how trait values for early fecundity and starvation resistance (right part of the figure) can be deduced from the function that determines the allocation of lipids to starvation resistance or reproduction (left). Variations in the functions that determine the allocation of lipids lead to genetic correlations between the traits. If allocation functions intersect each other (Fig. 8c), the correlation between early fecundity and starvation resistance switches from negative to positive at the age where the functions intersect. Two processes can lead to intersecting allocation functions: genetic variation in alleles that mediate the allocation of lipids and variation in the temporal expression of such alleles, mediated by differences in the timing of the reproductive schedule. In Figure 8 this corresponds to the flattening of allocation functions at older ages.

If the assumptions hold, the model allows us to predict the effects of variations in alleles that mediate the allocation of lipids to reproduction or starvation resistance on the genetic correlation between the traits. Large genetic variation in such alleles combined with little variation in temporal expression will produce trade-offs between early fecundity and starvation resistance during the whole life, for the allocation functions do not intersect (Fig. 8b). In contrast, large variation in temporal expression combined with less genetic variation leads to intersecting allocation functions if flies that have high early fecundity shift their allocation of resources earlier in life, for example, if there is a trade-off between early and late fecundity (Fig. 8c). The result will be high fat reserves that increase resistance to starvation later in life; the correlation between early fecundity and starvation resistance will then become positive at older ages.

Therefore, the apparent contradiction between earlier results (Service and Rose 1985; Service et al. 1985, 1988; Rose et al. 1992; Leroi et al. 1994c) and our findings of a positive correlation between early fecundity and starvation resistance may have resulted from differences in the age at which the flies were sampled. As in the earlier experiments, in our study both starvation resistance and relative fat content early in life were negatively, though not significantly, correlated with early fecundity, whereas later in life the correlation between early fecundity and starvation resistance became positive.

Our results do agree with phenotypic manipulation experiments that documented a nongenetic trade-off between fecundity and starvation over gradients of food quality (Chippindale et al. 1993) and larval rearing densities (Zwaan et al. 1991). Our interpretation is that selection on adult mortality had little effect on alleles that affect the allocation of lipids or the rate of food intake. Differences between lines in starvation resistance and fat content can be explained by a physiological integration of the traits with fecundity through a shared lipid metabolism and by differences between lines in the timing of the reproductive schedule, which is partly due to differences in development time.

In summary, differences in adult mortality between the two treatments affected growth rate, development time, body size, early fecundity, ovariole number, and the desiccation resistance of young flies—all traits expressed early in life. Most of these effects can be explained by a shift to faster development in HAM flies, which leads to their smaller body size and causes changes in other early traits because of developmental integration. No clear differences between treatments were detected in traits expressed late in life, in activity, metabolic rates, and relative fat content. The analysis of correlations among lines uncovered trade-offs between these traits mediated by differential allocation of lipids.

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Appendix

Line means with standard errors of the traits measured.

Trait	*n*	HAM			LAM		
		Line 7	Line 8	Line 9	Line 10	Line 11	Line 12
Life-history traits							
Viability (%)	1087	67 ± 1.0	84 ± 0.6	84 ± 0.7	76 ± 0.8	78 ± 1.0	60 ± 1.0
Development time (h)	1087	288 ± 0.7	295 ± 1.0	299 ± 0.9	309 ± 1.3	323 ± 1.2	310 ± 1.4
Fresh weight (μg)	1087	780 ± 10	720 ± 10	670 ± 10	780 ± 10	810 ± 10	890 ± 10
Early fecundity (no./eggs)	931	33.6 ± 1.0	28.3 ± 0.9	24.8 ± 0.9	26.4 ± 1.2	23.9 ± 1.6	29.2 ± 1.5
Late fecundity (no./eggs)	312	23.0 ± 2.5	29.2 ± 2.7	32.3 ± 2.4	29.4 ± 2.3	34.1 ± 2.7	28.6 ± 3.0
Stress resistance traits							
Starvation time, early (h)	108	99 ± 8.5	107 ± 6.8	93 ± 4.4	101 ± 4.8	101 ± 4.6	86 ± 8.2
Starvation time, late (h)	96	105 ± 9.0	98 ± 5.3	72 ± 4.4	80 ± 9.1	84 ± 7.3	91 ± 6.9
Desiccation time, early (min)	105	578 ± 28	620 ± 35	583 ± 31	752 ± 36	681 ± 26	729 ± 37
Desiccation time, late (min)	105	463 ± 28	600 ± 25	467 ± 33	506 ± 34	454 ± 28	562 ± 27
Morphological and physiological traits							
No. ovarioles, early	100	34.5 ± 0.8	33.7 ± 1.0	32.7 ± 0.5	34.9 ± 1.2	34.6 ± 1.0	35.5 ± 1.1
No. ovarioles, late	93	33.1 ± 0.4	32.6 ± 1.0	32.9 ± 1.0	33.5 ± 0.9	34.8 ± 0.9	34.5 ± 1.0
No. functional, early	100	32.4 ± 1.5	32.4 ± 0.9	29.5 ± 1.3	26.8 ± 1.2	31.5 ± 0.9	30.1 ± 1.8
No. functional, late	93	29.9 ± 1.2	29.6 ± 2.0	25.6 ± 1.7	21.9 ± 1.8	27.9 ± 1.6	26.7 ± 2.4
No. nonfunctional, early	100	2.1 ± 1.0	1.4 ± 0.6	3.2 ± 1.3	8.1 ± 1.9	3.1 ± 1.1	5.4 ± 1.8
No. nonfunctional, late	93	3.1 ± 1.1	2.9 ± 2.4	7.4 ± 1.6	11.5 ± 1.9	6.9 ± 1.7	7.8 ± 2.1
Dry weight, larvae (μg)	42	161 ± 14	172 ± 19	166 ± 17	146 ± 21	104 ± 8.0	133 ± 13
Dry weight, early (μg)	150	367 ± 9.0	366 ± 6.0	347 ± 7.0	401 ± 13	379 ± 11	399 ± 18
Dry weight, late (μg)	106	351	301 ± 10	263 ± 5.0	339 ± 9.0	351 ± 8.0	349
Dry weight desiccation, early (μg)	105	317 ± 3.0	307 ± 10	295 ± 4.0	350 ± 9.0	346 ± 17	377 ± 8.0
Dry weight desiccation, late (μg)	105	256 ± 9.0	240 ± 9.0	230 ± 7.0	270 ± 8.0	281 ± 9.0	304 ± 4.0
Dry weight starvation, early (μg)	108	272 ± 16	260 ± 5.0	241 ± 8.0	244 ± 10	243 ± 6.0	278 ± 8.0
Residual weight, early (μg)	150	305 ± 8.0	299 ± 4.0	289 ± 7.0	328 ± 12	306 ± 10	329 ± 17
Residual weight, late (μg)	106	254	229 ± 8.0	213 ± 6.0	273 ± 7.0	281 ± 7.0	261
Residual weight desiccation, early (μg)	105	290 ± 4.0	277 ± 8.0	267 ± 3.0	319 ± 9.0	314 ± 16	344 ± 5.0
Residual weight desiccation, late (μg)	105	203 ± 13	197 ± 6.0	205 ± 6.0	229 ± 10	228 ± 8.0	250 ± 2.0
Residual weight starvation, early (μg)	108	252 ± 15	238 ± 4.0	220 ± 8.0	223 ± 8.0	226 ± 5.0	257 ± 6.0
Fat weight, early (μg)	150	62 ± 2.0	67 ± 5.0	57 ± 2.0	72 ± 3.0	73 ± 2.0	69 ± 4.0
Fat weight, late (μg)	106	61	71 ± 3.0	51 ± 3.0	66 ± 5.0	71 ± 6.0	65
Fat weight desiccation, early (μg)	105	27.0 ± 3.0	30.0 ± 4.0	28.0 ± 1.0	31.0 ± 3.0	32.0 ± 2.0	34.0 ± 4.0
Fat weight desiccation, late (μg)	105	52 ± 10	43 ± 6.0	25 ± 4.0	42 ± 8.0	53 ± 11	54 ± 4.0
Fat weight starvation, early (μg)	108	20.0 ± 1.0	22.0 ± 1.0	21.0 ± 0.4	20.0 ± 2.0	18.0 ± 1.0	21.0 ± 2.0
Relative fat content, early	150	17.0 ± 0.4	18.3 ± 1.1	16.5 ± 0.6	18.1 ± 0.7	19.3 ± 0.7	17.5 ± 1.0
Relative fat content, late	106	17.4	23.8 ± 0.9	19.3 ± 1.2	19.4 ± 1.4	20.0 ± 1.6	18.6
Relative fat content desiccation, early	105	8.5 ± 0.8	9.8 ± 1.1	9.4 ± 0.2	9.0 ± 1.0	9.3 ± 0.7	8.9 ± 0.9
Relative fat content desiccation, late	105	20.5 ± 4.1	17.6 ± 2.0	10.6 ± 1.7	15.5 ± 2.8	18.5 ± 3.5	17.9 ± 1.3
Relative fat content starvation, early	108	7.3 ± 0.2	8.6 ± 0.3	8.7 ± 0.2	8.2 ± 0.6	7.2 ± 0.4	7.6 ± 0.5
MSM	42	5.3 ± 0.4	4.7 ± 0.4	5.3 ± 0.6	4.6 ± 0.5	3.3 ± 0.3	4.3 ± 0.4
MSM, early	36	16.7 ± 0.6	14.8 ± 0.8	18.0 ± 0.8	16.6 ± 1.4	15.2 ± 0.7	14.4 ± 0.5
MSM, late	36	11.2 ± 0.3	11.3 ± 0.4	12.4 ± 0.4	11.6 ± 0.5	11.4 ± 0.3	11.4 ± 0.4
Activity, early (counts)	60	50 ± 5.4	43.3 ± 7.4	33.7 ± 9.1	24.2 ± 3.5	65.3 ± 6.1	38.2 ± 5.8
Activity, late (counts)	58	31.0 ± 4.0	24.2 ± 5.8	16.8 ± 5.4	26.3 ± 4.1	23.7 ± 5.6	17.3 ± 3.4