Experimental Gerontology, Vol. 31, No. 4, pp. 533–544, 1996

0531-5565/96 $15.00 + .00

ELSEVIER

PII S0531-5565(96)00003-4

PHENOTYPIC ENHANCEMENT OF LONGEVITY BY ENVIRONMENTAL UREA IN *DROSOPHILA MELANOGASTER*

AMITABH JOSHI, JASON SHIOTSUGU and LAURENCE D. MUELLER

Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92717

Abstract—The phenotypic enhancement of longevity through a variety of environmental treatments, including dietary manipulations, has been observed in various species of animals, both vertebrate and invertebrate. Elucidating the mechanisms underlying such effects has provided insights into the physiological processes contributing to the determination of lifespan. Here, we report the enhancement of longevity in adult *Drosophila melanogaster* maintained on food supplemented with urea, a metabolic waste product occurring naturally in *Drosophila* cultures, especially at high larval densities. The impact of urea on longevity is shown to be through a decrease in the age-independent parameter (A) of the Gompertz equation, rather than the age-dependent parameter (α), which reflects the "rate of aging." We also present evidence suggesting that the urea-induced increase in longevity is mediated exclusively through a reduction in some aspect(s) of reproduction in adult flies maintained on urea-supplemented food.

Key Words: urea, enhanced longevity, fecundity, cost of reproduction, *Drosophila melanogaster*

INTRODUCTION

A VARIETY of environmental factors have been observed to affect longevity in diverse species spanning a range of animal taxa (Rose, 1991). Viewed from an evolutionary perspective, longevity is but one among many life-history characters that are interrelated through being rooted in the common network of physiological processes constituting metabolism (Service, 1987, 1989; Rose, 1991; Graves *et al.*, 1992; Chippindale *et al.*, 1993). Thus, understanding the mechanisms underlying observed environmental effects on longevity often provides useful insights into the nature of the constraints placed by correlations among life-history characters upon the attainment of increased lifespan under a given set of conditions.

For example, there is now considerable evidence suggesting that the enhancement of longevity through a variety of environmental treatments, including dietary manipulations, is largely mediated by a reduction in reproduction, in both insects (Service *et al.*, 1985; Partridge *et al.*, 1986, 1987; Service, 1987; Chippindale *et al.*, 1993; Tatar and Carey, 1995) and rodents

Correspondence to: L.D. Mueller
(*Received* 2 *July* 1995; *Accepted* 5 *January* 1996)

(Holehan and Merry, 1985; Masoro, 1988, 1995). Indeed, the fundamental life-history tradeoff between reproduction and survival/growth (Gadgil and Bossert, 1970; Reznick, 1985; Noordwijk and de Jong, 1986; Roff, 1992; Stearns, 1992) appears to be involved almost ubiquitously in mediating the experimental enhancement of longevity under a variety of protocols including natural selection in the laboratory, dietary restriction, and manipulations of density and temperature (reviewed in Rose, 1991).

The demographic perspective on aging suggests another level at which the nature of the effect of various factors on lifespan can be assessed, by focusing on rates of aging, rather than on changes in life-history traits correlated with longevity. In recent years, several studies have focused on "rates of aging" as reflected by the a parameter of the Gompertz (1825) equation that models age-specific mortality as an exponentially increasing function of age (Finch *et al.*, 1990; Johnson, 1990; Tatar *et al.*, 1993; Tatar and Carey, 1994; Hughes, 1995; Masoro, 1995; Mueller *et al.*, 1995). According to the Gompertz equation, the mortality rate at age x, $\mu(x)$, is given by

$$\mu(x) = Ae^{\alpha x},$$

where A is the age-independent mortality parameter, and α, the age-dependent parameter quantifies the rate of increase in mortality rate with age. Some studies on *Drosophila* suggest that environmental factors affecting longevity tend to do so through their effect on the age-independent Gompertz parameter (A), whereas genetically induced changes in longevity tend to involve changes in the Gompertz "rate of aging" (α) (T.J. Nusbaum, L.D. Mueller, and M.R. Rose, unpublished results; A. Joshi, W. Wu, and L.D. Mueller, unpublished results). There is also some evidence that, in the bruchid beetle *Callosobruchus maculatus*, mating per se may affect the Gompertz "rate of aging," whereas egg-production affects the age-independent Gompertz parameter (Tatar *et al.*, 1993).

In this article, we report results from a study investigating the effect of exposure to urea-supplemented food medium on imaginal longevity in laboratory populations of *Drosophila melanogaster.* Urea is a nitrogenous metabolic waste product normally found in laboratory cultures of *Drosophila,* especially at high larval densities, and is known to be toxic to *Drosophila* larvae (Botella *et al.*, 1985). The presence of larval metabolic wastes in the food medium has also been shown to inhibit egg-laying by *Drosophila* females, even in the absence of larvae (Aiken and Gibo, 1979). It is, therefore, not unreasonable to expect that exposure of adult flies to urea may affect their longevity, either through effects on reproduction, or through some other physiological effect that does not alter reproductive output. In the study reported here, we specifically addressed the following questions: (a) does exposure of adult flies to urea affect their longevity? (b) If so, does urea affect the age-independent (A) or age-dependent (α) Gompertz parameters, or both? (c) If urea affects longevity of adult flies, does it do so through its effect on some aspect(s) of reproduction? (d) Do populations that have evolved greater tolerance to the toxic effects of urea during their larval phase differ from their controls in the response of adult longevity to urea?

MATERIALS AND METHODS

Experimental populations

This study used five populations of *D. melanogaster* that have been subjected to selection for increased larval tolerance to the presence of toxic levels of urea in the food, as well as five control populations. The five populations selected for increased larval tolerance to urea (MX_1

. . . MX_5) are raised as larvae at low densities (50–100 eggs per 8 dram vial) on banana-molasses food with urea added to it. Adult flies are allowed to oviposit on nonnutritive agar for approximately six hours; the eggs are then placed into the food vials at the appropriate density. The following day, plastic sleeves are inserted into each vial. Once most larvae have pupated onto the plastic inserts, these are removed and placed into cages with Petri dishes containing yeasted banana-molasses food. This procedure ensures that adult flies will not be exposed to urea, so as to avoid confounding the results of adaptation to larval and adult exposure to environmental urea. Food is changed daily for five or six days after peak eclosion until egg collection for the next generation takes place. The five control populations (MC_1 . . . MC_5) are maintained on an identical regime except that they are reared on regular banana-molasses food as larvae. The generation time of both MX and MC populations is about three weeks, and all populations are maintained at 25°C on a 24-h light regime. Both sets of populations were derived from the five B populations of Rose (1984), each B population being used as the progenitor of one MX and one MC population (MX_i, MC_i derived from B_i, $i = 1 \ldots 5$). Consequently, MX and MC populations bearing the same numerical subscript are more closely related to each other, as compared to other populations subjected to the same selection regime. At the time of this study, the MX and MC populations had been maintained in the laboratory for ≈65 generations, and the MX populations had evolved a considerably higher degree of larval tolerance to the toxic effects of urea, as compared to the control MC populations (Joshi *et al.*, 1996).

Longevity assay on reproducing flies

The first longevity assay was conducted on adult flies from all 10 MX and MC populations, maintained on either regular food, or on food supplemented with 18 g/L urea (as no significant effect of selection regime on longevity was seen in this assay (see the Results section) subsequent assays of fecundity and virgin longevity were conducted only on the five MC populations). During this first longevity assay, adult males and females were kept together in vials and were, therefore, reproducing on a continuous basis. Prior to initiating the assay, all test populations were passed through two generations of identical rearing conditions, to eliminate any differences among selected lines due to environmental or maternal effects. To initiate the assay, freshly eclosed virgin flies were collected and placed into 8-dram vials with about 3 mL of food medium at a density of four males and four females per vial; 10 such vials were set up for each population × urea level combination, adding up to a total of 200 vials. The flies were transferred to fresh food vials every third day until all flies had died. All vials were checked for deaths daily; dead flies in a vial were not replaced over the course of the assay. Lifespan was measured as the time, in days, from eclosion to death.

Fecundity assay

To assess the level of reproduction in flies kept under conditions similar to those of the longevity assay described above, we examined the effect of urea-supplemented food on female fecundity, a composite measure of the consequences of a number of reproductive events in both sexes, including courtship and copulation. We focused upon fecundity because the purpose of the present study was primarily to document the effect of urea on the longevity of adult flies, and to determine, as a first step, to what degree any effect on longevity was mediated through effects on reproduction.

For assaying fecundity, freshly eclosed virgin flies from the five MC populations were collected and placed into 8-dram vials, with about 3 mL of food medium, at a density of four

males and four females per vial; five such vials were set up for each population urea level combination. Two of the vials from each population urea level combination were used to provide flies for the actual measurement of fecundity; the remaining three vials were used as backups to replace any flies that died over the course of the experiment. Female fecundity was assayed on days 5, 10, 15, 20, 25, and 30 after eclosion. Adult flies were placed in fresh vials at a density of one male and one female per vial and allowed to lay eggs for 24 h; eight such vials were set up for each population × urea level combination. At that point, the adults were removed and the number of eggs in the vials was recorded. All flies were assayed for fecundity on the same type of food (regular or urea supplemented) on which they were maintained over the 30-day duration of the experiment. As far as possible, the same individual flies were assayed at each time interval; any flies that died were replaced by others from the backup vials.

Longevity assay on virgin flies

To assay the effect of urea-supplemented food on the longevity of virgin flies, freshly eclosed flies from the five MC populations were collected, segregated by sex, and placed into 8-dram vials, with about 3 mL of food medium, at a density of either eight males or eight females per vial; four such vials were set up for each population × sex × urea level combination, adding up to a total of 80 vials. The flies were transferred to fresh food vials every third day until all flies had died. All vials were checked for deaths daily; dead flies in a vial were not replaced over the course of the assay. Lifespan was measured as the time, in days, from eclosion to death.

Statistical analyses

All analyses were performed using SAS for Windows version 6.08. Due to the pattern of relatedness among the MX and MC populations (MX_i and MC_i are more closely related than either of the pairs MX_i and MX_j or MC_i and MC_j, $i \quad j$), sets of MX and MC populations, matched by subscripted indices, were treated as random blocks in the analyses of variance (ANOVA) that were done on data gathered from the longevity assay on reproducing flies. Selection regime (MX or MC), and urea level (0 or 18 g/L) were treated as fixed factors crossed with blocks. Because the measurement of longevity was done on individual flies in each vial, the ANOVA model included vial as a random effect nested within the block × selection × urea interaction; sex was treated as a fixed factor crossed with all the rest. The appropriate *F*-ratios for testing the significance of the various effects in this ANOVA model are listed in Table 1.

From the data on longevity of reproducing flies, we also estimated the age-independent (A) and age-dependent (α) parameters of the Gompertz equation that models the age-dependence of mortality rates (Gompertz, 1825), using a maximum-likelihood method utilizing untransformed survival data (Mueller *et al.*, 1995). This method does not assume constant mortality rates, and yields more accurate and unbiased estimates of the Gompertz parameters compared to techniques based on linear and nonlinear regression, and maximum-likelihood with approximation (Mueller *et al.*, 1995). The estimates of A and α from each combination of population × urea level × sex were then used as data in analyses of variance, treating selection regime, urea level and sex as fixed factors crossed with the five replicate blocks. For each population × urea level × sex combination, the coefficient of determination, R^2, was also determined to get some indication as to whether the Gompertz model provided a reasonable explanation of the observed survivorship data.

For the ANOVA on the fecundity data, urea level and time of measurement were treated as fixed factors crossed with the five replicate blocks (populations $MC_1 \ldots MC_5$). In the ANOVA

TABLE 1. APPROPRIATE *F*-RATIOS FOR TESTING THE SIGNIFICANCE OF THE VARIOUS EFFECTS IN THE ANOVA MODEL USED TO ANALYZE DATA FROM THE LONGEVITY ASSAY ON REPRODUCING FLIES

Effect	*Appropriate* F-*ratio for hypothesis testing*
Block	MS Blk/MS Vial
Selection	MS Sel/MS Blk × Sel
Urea	MS Urea/MS Blk × Urea
Sex	MS Sex/MS Blk × Sex
Vial (Blk × Sel × Urea)	MS Vial/MS Error
Blk × Sel	MS Blk × Sel/MS Vial
Blk × Urea	MS Blk × Urea/MS Vial
Blk × Sex	MS Blk × Sex/MS Sex × Vial
Sel × Urea	MS Sel × Urea/MS Blk × Sel × Urea
Sel × Sex	MS Sel × Sex/MS Blk × Sel × Sex
Urea × Sex	MS Urea × Sex/MS Blk × Urea × Sex
Sex × Vial (Blk × Sel × Urea)	MS Sex × Vial/MS Error
Blk × Sel × Urea	MS Blk × Sel × Urea/MS Vial
Blk × Sel × Sex	MS Blk × Sel × Sex/MS Sex × Vial
Blk × Urea × Sex	MS Blk × Urea × Sex/MS Sex × Vial
Sel × Urea × Sex	MS Sel × Urea × Sex/MS Blk × Sel × Urea × Sex
Blk × Sel × Urea × Sex	MS Blk × Sel × Urea × Sex/MS Sex × Vial
Error (Blk × Sel × Urea × Sex)	

Blk: block, Sel: selection; parentheses are used to denote nested effects.

model used for the virgin longevity data, urea level and sex were treated as fixed factors crossed with the five replicate blocks (populations $MC_1 \ldots MC_5$); the model included vial as a random effect nested within the block × urea × sex interaction.

RESULTS

Longevity assay on reproducing flies

The ANOVA results for longevity of reproducing flies showed significant effects of urea level and sex; selection regime did not have a significant effect on longevity (Table 2). In general, longevity of both males and females of all populations was greater when maintained on urea-supplemented food (Figs. 1 and 2); the increase in longevity was somewhat larger in the MX populations (Fig. 3), contributing to a significant selection regime × urea level interaction effect in the ANOVA (Table 2). As expected for *D. melanogaster,* males lived longer than females; moreover, this difference was not affected by urea level.

The ANOVA results for the age-independent (A) and age-dependent (α) mortality parameters of the Gompertz equation indicated that the urea-supplemented food enhanced longevity solely through a decrease in A (Table 3). Of the fixed factors in the ANOVA model, sex [$F_{1,4} = 42.56$, $P < 0.005$], urea level [$F_{1,4} = 108.00$, $P < 0.0005$], and selection regime [$F_{1,4} = 9.48$, $P < 0.05$] were seen to have significant effects on the estimates of A. Estimates of α, on the other hand, were unaffected by any of the model terms. The fit of predicted to observed data was very good; on average, across the 40 combinations of population urea level sex, the Gompertz model accounted for about 92% of the variation in the observed survivorship data (mean R^2 across 40 plots = 0.915; 95% confidence interval = ± 0.022).

TABLE 2. ANALYSIS OF VARIANCE FOR LONGEVITY OF MX AND MC FLIES MAINTAINED ON 0 OR 18 G/L UREA IN THE FOOD MEDIUM

Source	df	*MS*	F	P
Block	4	1069.59	5.72	<0.0005
Selection	1	5118.82	4.21	n.s.
Urea	**1**	**12121.44**	**36.26**	**<0.005**
Sex	**1**	**15982.83**	**76.32**	**<0.001**
Vial (Blk × Sel × Urea)	178	187.25	1.36	<0.005
Blk × Sel	4	1215.28	6.50	<0.0005
Blk × Urea	4	334.29	1.79	n.s.
Blk × Sex	4	209.40	0.91	n.s.
Sel × Urea	**1**	**718.05**	**15.21**	**<0.025**
Sel × Sex	1	1184.53	2.48	n.s.
Urea × Sex	1	25.75	0.05	n.s.
Sex × Vial (Blk × Sel × Urea)	164	229.04	1.66	<0.005
Blk × Sel × Urea	4	47.22	0.25	n.s.
Blk × Sel × Sex	4	478.02	2.09	n.s.
Blk × Urea × Sex	4	496.70	2.17	n.s.
Sel × Urea × Sex	1	1.36	0.004	n.s.
Blk × Sel × Urea × Sex	4	234.71	1.02	n.s.
Error	912	137.67		

Longevity was assayed on flies kept in groups of four males and four females per vial. Significant fixed main effects and interactions are indicated in bold type (Blk: block, Sel: selection, n.s.: not significant; parentheses are used to denote nested effects).

Fecundity assay

Flies maintained on urea-supplemented food exhibited a consistent decline in fecundity over time, relative to those maintained on regular food (Fig. 4). This trend is reflected in the significant effects of urea level, time of measurement, and the time × urea interaction in the ANOVA (Table 4).

Longevity assay on virgin flies

In the longevity assay on virgin flies, as expected for *Drosophila,* adult males outlived adult females, regardless of urea level. The ANOVA results revealed no significant effect of urea level on longevity (Table 5). The only factors showing significant effects on longevity, other than sex, were block and vial (Table 5).

DISCUSSION

It is clear from the results that exposure to urea-supplemented food enhanced mean longevity of reproducing flies in all populations, MX and MC, and that this effect was qualitatively unaffected by the past selection history of the populations (Fig. 3, Table 2); evolved larval tolerance to urea, therefore, does not seem to affect the adult response to urea. Because the longevity of virgin flies was not affected by urea level (Table 5), it appears that the effect of urea on the longevity of reproducing flies was mediated solely through effects on some aspect(s) of

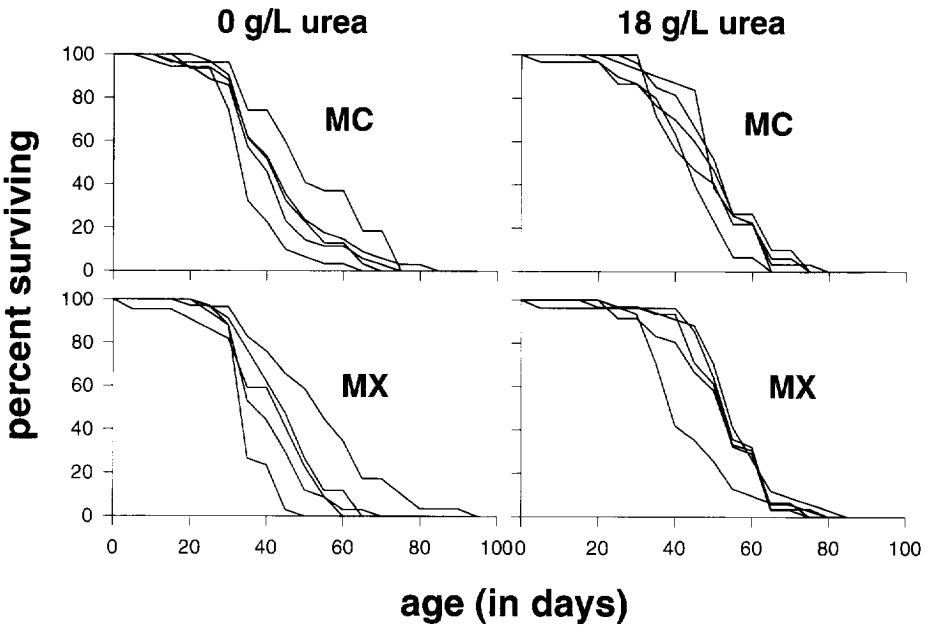

FIG. 1. Percent survival to various ages of adult males from each of the five replicate MX and MC populations, when kept on 0 or 18 g/L urea in the food medium.

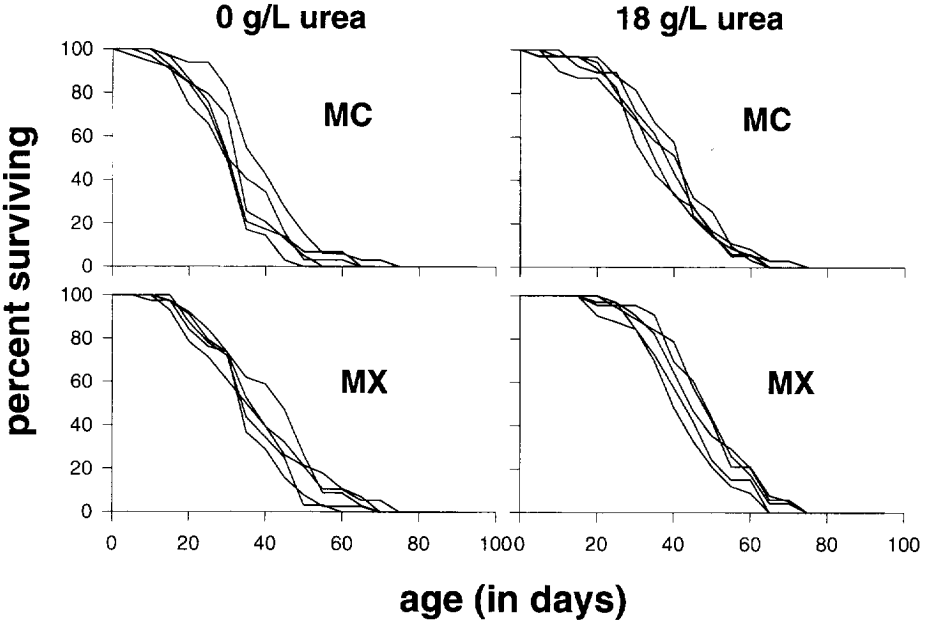

FIG. 2. Percent survival to various ages of adult females from each of the five replicate MX and MC populations, when kept on 0 or 18 g/L urea in the food medium.

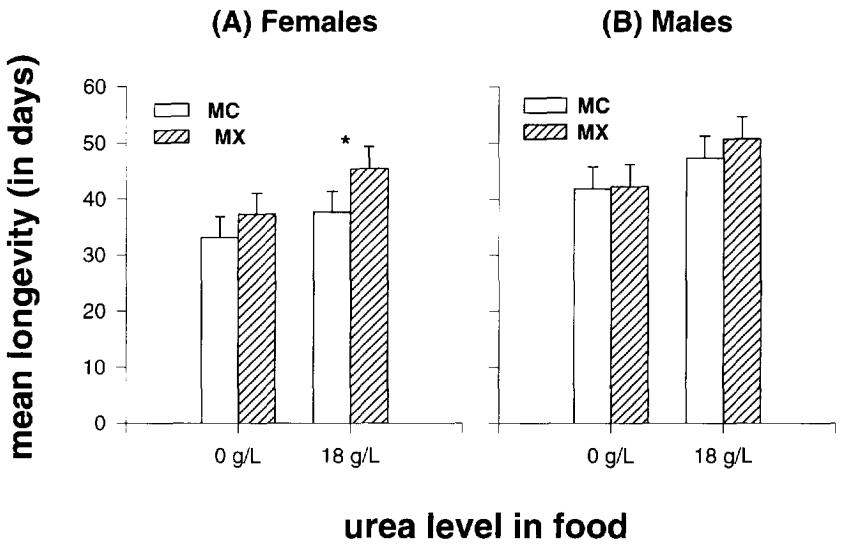

FIG. 3. Mean longevity of female (A) and male (B) flies from the MX and MC populations kept four males and four females per vial on 0 or 18 g/L urea in the food medium. The error bars depict 95% confidence intervals about the mean of the five replicate populations of each selection regime, and were calculated using least squares estimates of the standard errors of cell means in the randomized block ANOVA. At 18 g/L urea, the mean longevity of MX females is significantly greater than that of MC females ($*P < 0.05$); all other differences between MX and MC populations are not significant.

reproduction. The consistent decline in fecundity observed in females flies kept on urea-supplemented food (Fig. 4) further supports this conclusion.

We cannot, unfortunately, use these data to assess the degree to which various components of reproduction, such as courtship, copulation, and actual egg-production in females, may be affected by exposure to urea. There is some evidence, from other studies, that mating per se may exact a "cost of reproduction," independent of actual egg-production (Partridge *et al.*, 1986, 1987; Chapman *et al.*, 1995). If exposure to urea causes a reduction in mating activity, it would directly explain the increased longevity of males on urea-supplemented food. For the beetle *Callosobruchus maculatus*, it is known that the effect of mating is to increase the age-dependent Gompertz parameter (α) (Tatar *et al.*, 1993), which was unaffected by urea in our study. However, it is is not known if the effect of mating on longevity is mediated through the age-dependent Gompertz parameter in *Drosophila* species as well. Moreover, a direct effect of urea on females, resulting in lowered egg laying, could also exert an indirect influence on mating frequency as females laying less eggs will tend to mate less frequently. The question of which specific component of reproduction is directly affected by exposure to urea is certainly an interesting one that warrants further investigation.

The pattern of change over time in the fecundity of flies maintained on normal vs. urea-supplemented food suggests that continued exposure to urea has some kind of cumulative effect on fecundity (Fig. 4). The mean fecundity of flies maintained on normal food fluctuated considerably but did not exhibit any clear temporal trend. On the other hand, the mean fecundity of flies maintained on urea-supplemented food underwent a consistent, almost linear decline over time (Fig. 4).

The results from these experiments provide another example of the ubiquitous tradeoff

TABLE 3. ESTIMATES OF THE AGE-INDEPENDENT (A) AND AGE-DEPENDENT (α) MORTALITY PARAMETERS OF THE GOMPERTZ EQUATION

	0 g/L urea		*18 g/L urea*	
Selection regime	*Male*	*Female*	*Male*	*Female*
		Estimates of A		
MC	0.0019	0.0024	0.0009	0.0021
	(0.0011)	(0.0004)	(0.0002)	(0.0006)
MX	0.0012	0.0021	0.0008	0.0014
	(0.0007)	(0.0006)	(0.0002)	(0.0005)
		Estimates of α		
MC	0.0832	0.0958	0.0879	0.0844
	(0.0255)	(0.0184)	(0.0125)	(0.0111)
MX	0.0998	0.0849	0.0850	0.0825
	(0.0367)	(0.0168)	(0.0021)	(0.0081)

The entries are the means (±95% confidence intervals) of the five replicate populations in each selection regime × urea level combination. The only significant effects in the analyses of variance were those of sex, urea level, and selection regime on estimates of A.

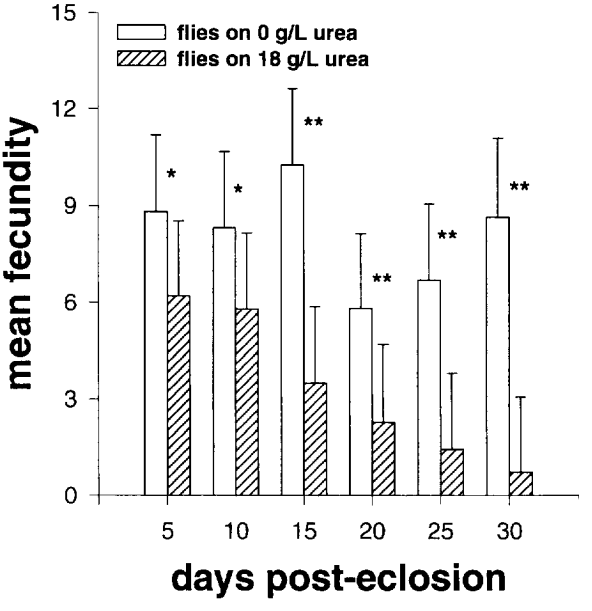

FIG. 4. Mean fecundity of MC flies maintained on 0 or 18 g/L urea in the food medium, measured at five-day intervals. Fecundities were measured on the same urea level at which the flies were maintained. The error bars depict 95% confidence intervals about the mean of the five replicate populations, and were calculated using least squares estimates of the standard errors of cell means in the randomized block ANOVA (*$P < 0.05$; **$P < 0.01$).

TABLE 4. ANALYSIS OF VARIANCE FOR FECUNDITY OF MC FLIES MAINTAINED ON 0 OR 18 G/L UREA IN THE FOOD MEDIUM, MEASURED AT DAYS 5, 10, 15, 20, 25 AND 30 AFTER ECLOSION

Source	df	*MS*	F	*P*
Block	4	49.01	1.87	n.s.
Time	**5**	**202.13**	**11.31**	**<0.0005**
Urea	**1**	**2659.98**	**26.48**	**<0.01**
Block × Time	20	17.87	0.68	n.s.
Block × Urea	4	100.44	3.84	<0.005
Time × Urea	**5**	**98.71**	**3.49**	**<0.025**
Block × Time × Urea	20	28.30	1.08	n.s.
Error	409	26.18		

Fecundities were measured on the same urea level at which the flies were maintained. Significant fixed main effects and interactions are indicated in bold type (n.s.: not significant).

between reproduction vs. growth and survival being involved in mediating an environmental modification of longevity. It would also appear that the levels of urea used in this study (18 g/L), though extremely toxic to larvae (Joshi *et al.*, 1996), are not particularly toxic to the adult flies. Were urea strongly toxic to adults, one would expect exposure to urea to cause a decline in both fecundity and longevity as part of a general syndrome of lowered fitness, as has been seen for some other severe stresses. For example, in dietary restriction experiments with *Drosophila,* longevity increases with decreasing fecundity as food-levels are reduced; below a certain level of food, however, both longevity and fecundity are depressed (Chippindale *et al.*, 1993). Similarly, severe crowding of *Drosophila* adults results in a decline of both fecundity and longevity (A. Joshi, W. Wu, and L.D. Mueller, unpublished results).

TABLE 5. ANALYSIS OF VARIANCE FOR LONGEVITY OF VIRGIN MC FLIES MAINTAINED ON 0 OR 18 G/L UREA IN THE FOOD MEDIUM

Source	df	*MS*	F	P
Block	4	2639.45	6.57	<0.001
Urea	1	252.10	0.66	n.s.
Sex	**1**	**3607.28**	**10.15**	**<0.05**
Vial (Blk × Urea × Sex)	56	402.02	1.55	<0.01
Blk × Urea	4	380.17	0.95	n.s.
Blk × Sex	4	355.42	0.88	n.s.
Urea × Sex	1	435.65	0.57	n.s.
Blk × Urea × Sex	4	760.17	1.89	n.s.
Error	515	260.15		

Longevity was assayed on flies kept in groups of either eight males or eight females per vial. Significant fixed main effects and interactions are indicated in bold type (Blk: block; parentheses are used to denote nested effects).

The effects of urea on longevity seen in this study are, at least superficially, similar to the effects of limited food on lifespan, which have been documented across a wide range of species (Robertson and Salt, 1981; Yu *et al.*, 1985; Masoro, 1988; Austad, 1989; Ernsting and Isaaks, 1991; Kaitala, 1991; Chippindale *et al.*, 1993). However, in the case of dietary restriction, it is almost certain that the effect of reduced food levels on longevity is due to a reduction in energy input, which, in turn, lowers fecundity. Exposure to urea, on the other hand, may reduce fecundity through some unknown physiological mechanism triggered by the ingestion of urea. Alternatively, individual females may simply be reducing egg output once they sense the presence of urea in the egg-laying environment; such short-term behavioral responses to the presence of larval metabolic wastes has been previously observed (Aiken and Gibo, 1979). Because we assayed flies for fecundity on the same type of food on which they were maintained, it is not possible, from our results, to discern the extent to which the effect of urea on fecundity is a short-term behavioral response to conditions at the time of egg-laying, as opposed to a longer term conditioning effect of some kind. There is also the possibility that the presence of urea in the food may induce lower levels of feeding by the adult flies, thereby leading, indirectly, to an effect similar to dietary restriction.

The observation that urea enhances longevity through lowering the age-independent Gompertz parameter (A) is concordant with other observations suggesting that, at least in *Drosophila,* environmental manipulations of longevity tend to act by altering age-independent mortality rates rather than the Gompertz "rate of aging" (α) (T.J. Nusbaum, L.D. Mueller, and M.R. Rose, unpublished results; A. Joshi, W. Wu, and L.D. Mueller, unpublished results). Some support for this view also comes from studies on the bruchid beetle *Callosobruchus maculatus,* in which increased egg-production has been seen to enhance mortality rates through increases in the age-independent Gompertz parameter (A) (Tatar *et al.*, 1993). It is, nevertheless, difficult to say how general this trend might be across taxa, in the absence of more extensive data on a variety of invertebrate and vertebrate species. Clearly, effects of various environmental treatments on longevity should be further dissected to determine longevity is affected by a retardation of the aging process, or merely through correlated responses to changes induced in other life-history traits such as component aspects of reproduction.

Acknowledgments—We thank Michael R. Rose for useful suggestions and comments during the course of this study, and two anonymous reviewers for their helpful comments on the manuscript. We also thank Langley E. Ho, Yoshinobu T. Morimoto, and Wendy A. Oshiro for excellent technical assistance in the laboratory. This study was supported by Grants AG09970 from the National Institutes of Health, and DEB-9410281 from the National Science Foundation, to L.D. Mueller.

REFERENCES

AIKEN, R.B. and GIBO, D.L. Changes in fecundity of *Drosophila melanogaster* and *D. simulans* in response to selection for competitive ability. *Oecologia* **43,** 63–77, 1979.

AUSTAD, S.N. Life extension by dietary restriction in the bowl and doily spider, *Frontinella pyramitela. Exp. Gerontol.* **24,** 83–92, 1989.

BOTELLA, L.M., MOYA, A., GONZALES, A.C., and MENSUA, J.L. Larval stop, delayed development and survival in overcrowded cultures of *Drosophila melanogaster*: Effect of urea and uric acid. *J. Insect Physiol.* **31,** 179–185, 1985.

CHAPMAN, T., LIDDLE, L.F., KALB, J.M., WOLFNER, M.F., and PARTRIDGE, L. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature* **373,** 241–244, 1995.

CHIPPINDALE, A.K., LEROI, A.M., KIM, S.B., and ROSE, M.R. Phenotypic plasticity and selection in *Drosophila* life-history evolution. I. Nutrition and the cost of reproduction. *J. Evol. Biol.* **6,** 171–193, 1993.

ERNSTING, G. and ISAAKS, J.A. Accelerated aging: A cost of reproduction in the carabid beetle *Notiophilus biguttatus* F. *Funct. Ecol.* **5,** 299–303, 1991.

FINCH, C.E., PIKE, M.C., and WITTEN, M. Slow increases of the Gompertz mortality rate during aging in certain animals approximate that of humans. *Science* **249,** 902–905, 1990.

GADGIL, M. and BOSSERT, W.H. Life historical consequences of natural selection. *Am. Nat.* **102,** 52–64, 1970.

GOMPERTZ, B. On the nature of the function expressive of the law of human mortality and on a new mode of determining life contingencies. *Philos. Trans. R. Soc. Lond. Series A* **115,** 513–585, 1825.

GRAVES, J.L., TOOLSON, E.C., JEONG, C., VU, L.N., and ROSE, M.R. Dessication, flight, glycogen, and postponed senescence in *Drosophila melanogaster. Physiol. Zool.* **65,** 268–286, 1992.

HOLEHAN, A.M. and MERRY, B.J. Lifetime breeding studies in fully fed and dietarily restricted CFY Sprague–Dawley rats. 1. Effects of age, housing conditions and diet on fecundity. *Mech. Ageing Dev.* **33,** 19–28, 1985.

HUGHES, K.A. The evolutionary genetics of male life history characteristics in *Drosophila melanogaster. Evolution* **49,** 521–537, 1995.

JOHNSON, T.E. Age-1 mutants of *Caenorhabditis elegans* prolong life by modifying the Gompertz rate of aging. *Science* **249,** 908–912, 1990.

JOSHI, A., KNIGHT, C.D., and MUELLER, L.D. Genetics of larval urea tolerance in *Drosophila melanogaster. Heredity,* (in press) 1996.

KAITALA, A. Phenotypic plasticity in reproductive behaviour of waterstriders: Trade-offs between reproduction and longevity during food stress. *Funct. Ecol.* **5,** 12–18, 1991.

MASORO, E.J. Food restriction in rodents: An evaluation of its role in the study of aging. *J. Gerontol.* **43,** 59–64, 1988.

MASORO, E.J. Dietary restriction. *Exp. Gerontol.* **30,** 291–298, 1995.

MUELLER, L.D., NUSBAUM, T.J. and ROSE, M.R. The Gompertz equation as a predictive tool in demography. *Exp. Gerontol.* **30,** 553–569, 1995.

NOORDWIJK, A.J. VAN and DE JONG, G. Acquisition and allocation of resources: Their influence on variation in life-history tactics. *Am. Nat.* **128,** 137–142, 1986.

PARTRIDGE, L., FOWLER, K., TREVITT, S., and SHARP, W. An examination of the effects of males on the survival and egg-production rates of female *Drosophila melanogaster. J. Insect. Physiol.* **32,** 925–929, 1986.

PARTRIDGE, L., GREEN, A., and FOWLER, K. Effects of egg-production and exposure to males on female survival in *Drosophila melanogaster. J. Insect Physiol.* **33,** 745–749, 1987.

REZNICK, D. Costs of reproduction: An evaluation of the empirical evidence. *Oikos* **44,** 257–267, 1985.

ROBERTSON, J.R. and SALT, G.W. Responses in growth, mortality, and reproduction to variable food levels by the rotifer, *Asplanchia girodi. Ecology* **62,** 1585–1596, 1981.

ROFF, D.A. *The Evolution of Life Histories,* Chapman and Hall, New York, 1992.

ROSE, M.R. Laboratory evolution of postponed senescence in *Drosophila melanogaster. Evolution* **38,** 1004–1010, 1984.

ROSE, M.R. *Evolutionary Biology of Aging,* Oxford University Press, New York, 1991.

SERVICE, P.M. Physiological mechanisms of increased stress resistance in *Drosophila melanogaster* selected for postponed senescence. *Physiol. Zool.* **60,** 321–326, 1987.

SERVICE, P.M. The effect of mating status on lifespan, egg laying, and starvation resistance in *Drosophila melanogaster* in relation to selection on longevity. *J. Insect Physiol.* **35,** 447–452, 1989.

SERVICE, P.M., HUTCHINSON, E.W., MACKINLEY, M.D., and ROSE, M.R. Resistance to environmental stress in *Drosophila melanogaster* selected for postponed senescence. *Physiol. Zool.* **58,** 380–389, 1985.

STEARNS, S.C. *The Evolution of Life Histories,* Oxford University Press, Oxford, 1992.

TATAR, M. and CAREY, J.R. Genetics of mortality in the bean beetle *Callosubruchus maculatus. Evolution* **48,** 1371–1376, 1994.

TATAR, M. and CAREY, J.R. Nutrition mediates reproductive trade-offs with age- specific mortality in the beetle *Callosobruchus maculatus. Ecology* **76,** 2066–2073, 1995.

TATAR, M., CAREY, J.R., and VAUPEL, J.W. Long-term cost of reproduction with and without accelerated senescence in *Callosobruchus maculatus*: Analysis of age-specific mortality. *Evolution 47,* 1302–1312, 1993.

YU, B.P., MASORO, E.J., and MCMAHAN, C.A. Nutritional influences on aging of Fischer 344 rats. I. Physical, metabolic and longevity characteristics. *J. Gerontol. 40,* 657–670, 1985.